UNIVERSIDADE FEDERAL DE UBERLÂNDIA
FACULDADE DE ENGENHARIA QUÍMICA
PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA DE ALIMENTOS

EFEITO DA FERMENTAÇÃO NA QUALIDADE FÍSICO-QUÍMICA E SENSORIAL DO CAFÉ ARÁBICA VARIEDADE CATUAÍ AMARELO (*Coffea arabica)*

Carlos Johnantan Tolentino Vaz

Patos de Minas/MG
Janeiro de 2021

UNIVERSIDADE FEDERAL DE UBERLÂNDIA

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Carlos Johnantan Tolentino Vaz

Orientadora: Carla Zanella Guidini

Coorientadoras: Michelli Andriati Sentanin

Marta Fernanda Zotarelli

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UNIVERSIDADE FEDERAL DE UBERLÂNDIA
Coordenação do Programa de Pós-Graduação em Engenharia de Alimentos - Patos de Minas
Av. Getúlio Vargas, 230 - Bairro Centro, Patos de Minas-MG, CEP 38700-103
Telefone: (34) 3823-3714 - www.ppgea.feq.ufu.br - coordppgea@feq.ufu.br

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Iniciando os trabalhos a presidente da mesa, Carla Zanella Guidini, apresentou a Comissão Examinadora e o discente, agradeceu a participação do público, e concedeu ao discente a palavra para a exposição do seu trabalho. A duração da apresentação do mesmo se deu em conformidade às normas do Programa.

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O competente diploma será expedido após cumprimento dos demais requisitos, conforme as normas do Programa, a legislação pertinente e a regulamentação interna da UFU.

Nada mais havendo a tratar foram encerrados os trabalhos. Foi lavrada a presente ata que após lida e achada conforme será assinada pela Banca Examinadora.

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“A única coisa que não muda é que tudo muda. Nada fica estático no mundo, tudo muda, tudo se move. O rio muda a cada segundo, do mesmo modo que as pessoas mudam a cada segundo, sendo assim, ninguém entra em um mesmo rio uma segunda vez, pois quando isso acontece já não se é o mesmo, assim como as águas do rio já são outras”

“Sabedoria consiste em falar e agir de verdade. Aprendizagem muita não ensina compreensão. Todas as coisas vêm a seu tempo devido. O sol é novo a cada dia”

Heráclito de Éfeso

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RESUMO

O café arábica é um dos produtos agrícolas de maior expressão dentro do mercado nacional de commodities e possui grande importância para a balança comercial nacional. É um produto no qual a qualidade sensorial está diretamente relacionada ao valor comercial no mercado final, então, aprimorar tal qualidade pode significar aumento da renda do cafeicultor e de toda a região na qual este produto foi produzido. O tipo de processamento desempenha papel fundamental nas características sensoriais finais do produto, e nesse contexto a etapa de fermentação se destaca na alteração da qualidade. Neste trabalho foi estudado a qualidade sensorial final e as características físico-químicas do café arábica, da cultivar Catuaí Amarelo IAC-62, produzido na região de Carmo do Paranaíba – Minas Gerais. O café colhido manualmente, no estágio de cerejas amarelas foi inoculado com a levedura *Saccharomyces cerevisiae*, para a fermentação. Foi realizado um Planejamento Composto Central Rotacional (PCCR), para avaliar a etapa de fermentação com relação às variáveis tempo e temperatura de fermentação, e obtendo como respostas pH da massa fermentativa, umidade do fruto (durante o processo), teor de açúcares e ácidos orgânicos (*HPLC*) e qualidade sensorial avaliada pela metodologia da Associação de Cafés Especiais (*Specialty Coffee Association* – SCA), em dois níveis de torra diferentes (média clara e média). Foram determinadas as equações de ajuste à nota final dos cafés, em ambos os níveis de torra proporcionando um primeiro passo para a otimização da etapa da fermentação em campo. Independente do ensaio realizado em $+\alpha$ ou $-\alpha$, observou-se que os cafés, mesmo em níveis extremos de tempo e temperatura, se comportaram sensorialmente similares com relação às notas finais (no geral entre 81 e 85), sendo que todos foram classificados como cafés especiais. A concentração de frutose variou apenas com o tempo de fermentação e não com a temperatura, enquanto a sacarose e glicose decresceram com o avanço da temperatura e do tempo. O glicerol foi formado dependendo de ambas as variáveis, e sua concentração aumentou com o tempo e com o aumento da temperatura. O pH se comportou conforme foi descrito na literatura, decrescendo durante o avanço do tempo, porém mais intensamente com as maiores temperaturas. Alguns ácidos foram medidos ao final da fermentação como o acético, propiônico, succínico e lático. Na avaliação sensorial, os atributos aroma, uniformidade, ausência de defeitos e doçura não foram afetados pelas diferentes condições de fermentação, nem pelo tipo de torra. A acidez da bebida foi influenciada pela fermentação, nos experimentos de torra clara, e maiores extensões da etapa de fermentação parecem contribuir positivamente para este atributo. Além disso, o maior grau de torra parece provocar redução na acidez da bebida. O atributo corpo foi influenciado pela fermentação, mas não pela torra, enquanto o sabor residual foi influenciado por ambos. O equilíbrio da bebida também foi afetado pela fermentação, e graus mais elevados de torra parecem influenciá-la de forma negativa. Quanto à avaliação global, as temperaturas de fermentação mais elevadas aumentaram as notas deste atributo, mas o efeito de torra não pôde ser bem estabelecido. Por meio da Análise de Componentes Principais foi possível correlacionar aroma, sabor, acidez, corpo, sabor residual, equilíbrio e nota geral como os atributos que tiveram maior influência e alta contribuição para o perfil sensorial do café.

Palavras-chave: cafés especiais, fermentação de café, *Saccharomyces cerevisiae,* análise sensorial.

ABSTRACT

Arabica coffee is one of most expression agricultural products on national commodities market and has a great importance on national trade balance and it's a product which sensorial quality is directly related to final commercial price, and so, increase such quality may mean an increase on coffee producer final income and such of entire coffee production region. Processing type plays an important role on final sensorial quality, and on this context, fermentation step stands out on quality changes. On this work, were studied the arabica coffee's final sensorial quality and physicochemical characteristics, of a Yellow Catuai IAC 62, produced on Carmo do Paranaiba (Minas Gerais State) Region. Coffee fruits were handpicked, only ripe yellow cherries were selected, inoculated with *Saccharomyces cerevisiae* for fermentation. An experimental design were done by a Composite Central Rotational Design (CCRD) to evaluate fermentation process related to fermentation time and temperature and getting as responses fermentative mass pH, fruit moisture (just as process were conducted), sugars and organic acids content (HPLC) and sensorial quality by Specialty Coffee Association (SCA) on two different roasts levels (light medium roast and medium roast). There were determined fit equations to final coffee scores, on both roasts' levels providing a first step for field process optimization. Independent of test (from – α to + α), it was observed that coffees, even in extreme time and temperature, behave sensorially similarly related to final scores (in general from 81 to 85 in SCA score table), being all of them classified as specialty coffees. Fructose content vary only with fermentation time and not with temperature, while sucrose and glucose contents decreased while time and temperature advances. Glycerol was formed depending on both variables, and its content increase with time and temperature increase. pH behaved just as described by literature, decreasing with time advance, and more intensely as temperature increases. Organic acids such as acetic, propionic, succinic and lactic were measured at final fermentation. Sensorial evaluation attributes such aroma, uniformity, clean cup were not affected by different fermentation conditions not by roast level. Coffee drink's acidity was affected by fermentation, mainly on light roasts tests, and increase of time seams to positively contribute for this attribute. Besides, more intense roasts levels seem to provoke acidity attribute score decrease. Attribute body were influenced by fermentation, but not by roast level, while residual taste was influenced by both. Drink balance was also affected by fermentation, and on more intense roast levels seems to influence it negatively. As for the global evaluation, higher fermentation temperatures increased this attribute scores, but roast influence couldn't be determinate. By Main Component Analysis were possible to correlate aroma, flavor, body, residual taste, balance and final score as those ones with greater influence on contribution for coffee's sensorial profile.

Keywords: Specialty coffee, coffee fermentation, *Saccharomyces cerevisiae,* sensorial analysis.

CAPÍTULO 1- INTRODUÇÃO

O Café arábica (*Coffea arábica*), dentre as commodities agrícolas, fica em quinto lugar no faturamento total das exportações do Brasil (BRASIL, 2020), representando no ano de 2019 um total de 6,1% das exportações de produtos agrícolas e 2,3% do faturamento total das exportações, ficando atrás apenas da soja, carne, papel e celulose, álcool e açúcar. No entanto, considerando a área plantada, o faturamento foi maior em 3,85 vezes que a soja, conforme dados do Instituto Brasileiro de Geografia e Estatística (IBGE) (IBGE, 2020). Segundo Lee *et al*. (2015) o café é a segunda maior commodity comercializada mundialmente em termos financeiros, depois apenas do petróleo. O Brasil é o maior produtor mundial de café e contribuiu, na safra 2018/19 com quase 63 milhões de sacas de café, representando aproximadamente 37% do total produzido no mundo (OIC, 2020a).

De acordo com a Organização Internacional do Café (OIC) (OIC, 2020b), o consumo de café na safra 2019/2020 foi de 169,3 milhões de sacas, sendo que o maior consumidor mundial foi a União Europeia, seguido dos Estados Unidos, com um total de 45,6 milhões e 27,9 milhões de sacas respectivamente (representando 27% e 16,5% respectivamente) e em terceiro lugar o Brasil, com um consumo de 22,3 milhões de sacas (13,2% do consumo mundial).

O Brasil também se destaca mundialmente no quesito de produção. De uma forma geral, o país apresenta características de clima, topografia e solo favoráveis para a produção do café de boa qualidade sensorial, o que faz com que o país possua a maior área apta à cafeicultura no mundo. Essa característica contribui, portanto, para que o Brasil seja o maior produtor mundial deste produto com um total de 62,9 milhões de sacas na safra colhida em 2019 (OIC, 2020a) e uma estimativa de produção de 61,9 milhões de sacas para a safra a ser colhida em 2020 (CONAB, 2020).

A qualidade do café, suas diferentes nuances e características sensoriais são um dos motivos pelos quais o café é tão apreciado no mercado. Illy e Viani (2005) mostraram que o efeito do consumo do café é muito mais que o efeito estimulante da cafeína, que é o componente químico até então mais estudado do café. De acordo com os autores, a bebida melhora a atividade cerebral e comportamental, aumenta a atividade metabólica, podendo ser um grande aliado na perda de peso, além da presença de produtos antioxidantes que podem prevenir doenças degenerativas e câncer.

Diversos fatores que atuam nas etapas de pré e pós-colheita podem ocasionar modificações à qualidade do café. Dentre as características que afetam destaca-se o local de plantio, no qual se caracterizam as condições climáticas, flora microbiana e solo, que são fundamentais para uma boa qualidade sensorial do fruto (PIMENTA, 2003).

O processamento na etapa de pós-colheita, por sua vez, é um dos principais fatores que influenciam na qualidade sensorial do café (BORÉM, 2008), sendo que tradicionalmente é processado de três formas: processo seco, semi-seco e via úmida, e em todos os três processos a fermentação atua ativamente na degradação dos açúcares presentes no fruto.

No processo via seca os frutos de café, na forma cereja, são fermentados e secos simultaneamente logo após a colheita (BRANDO & BRANDO, 2014; SILVA, 2014). A característica química dos cafés naturais, que é a denominação para os cafés processados na via seca, é a de apresentar um maior teor de sólidos solúveis e também açúcares totais (RIBEIRO *et al.*, 2011), desta forma a bebida tem um corpo superior e também uma maior doçura, dois atributos que são considerados para a classificação sensorial do café.

Segundo Evangelista *et al.* (2014a) o processamento via úmida é aquele em que os frutos são descascados, parte da polpa e da mucilagem são removidas mecanicamente e os grãos são encaminhados para tanques de fermentação antes da secagem, de forma a remover o excesso de mucilagem. A mucilagem é uma camada do fruto basicamente composta de açúcares e um substrato pectinoso (GARCIA *et al.*, 1991) a qual está firmemente aderida ao firmemente aderia aos grãos (DE BRUYN *et al.,* 2016). A polpa geralmente é a camada mais externa do mesocarpo, geralmente removida juntamente com a casca no descascamento (DE BRUYN, *et al.*, 2016*)*

Por outro lado, o processamento via semi-seca é uma variação do processo via úmida, no qual os cafés são encaminhados descascados para a fermentação que ocorre simultaneamente a secagem, em tanques, plataforma ou terreiros, diretamente sob a radiação do sol.

Conforme descrito, a qualidade intrínseca do café é estabelecida ao nível de fazenda, pelas características regionais e pelo processamento, que é a etapa mais importante para a determinação da qualidade final do produto. Ou seja, dos diversos passos que interferem na qualidade sensorial do café a fermentação é o mais importante deles (VELMOUROGANE, 2013). A fermentação do café é uma etapa que atua diretamente para a formação dos atributos sensoriais, principalmente para o aroma. Lee *et al.* (2017) citaram que extratos de café fermentados, com leveduras, apresentaram notas florais e frutadas devido à transformação de aldeídos em álcoois e cetonas pelo processo fermentativo.

Como a qualidade do produto como o café agrega valor, as tecnologias que melhoram a qualidade do café podem ser implementadas em diferentes estágios da produção, desde a plantação até o armazenamento, mas estas especialmente, devem ocorrer na etapa do processamento (fermentação e secagem), de todas as operações que ocorrem do café, da colheita à extração, as etapas de fermentação e secagem são as que mais influenciam na qualidade final do produto (RIBEIRO *et al.*, 2017b).

O objetivo deste trabalho foi desenvolver um sistema fermentativo em processamento via seca com café cereja *(Coffea arabica*), da linhagem Catuaí Amarelo, produzido na Região do Cerrado Mineiro utilizando a levedura *Saccharomyces cerevisiae*, de forma a melhorar a características físico-químicas e sensoriais do produto. Os objetivos específicos foram:

- Avaliar as variáveis tempo e temperatura de fermentação, por meio de um planejamento composto central rotacional (PCCR) na fermentação do café tendo como resposta os açúcares consumidos e ácidos formados, que implicam diretamente na qualidade sensorial;
- Avaliar o pH da massa de fermentação para os experimentos do PCCR;
- Avaliar o teor final de ácidos orgânicos *versus* atributo acidez da bebida;
- Avaliar teor de açúcares totais *versus* atributo doçura da bebida;

ESTRUTURA DO TRABALHO

Este trabalho foi estruturado para que o mesmo tenha seus conteúdos abordados de maneira lógica e sequencial. Desta forma os capítulos foram divididos da seguinte forma:

Capítulo 1: Introdução;

Capítulo 2: Revisão Bibliográfica;

Capítulo 3: Efeito da fermentação do café arábica com levedura *Saccharomyces cerevisiae* nas características físico-químicas e análise sensorial.

Capítulo 4: Perfil sensorial de bebidas produzidas a partir de café fermentado em diferentes condições de tempo e temperatura.

Capítulo 5: Propostas para trabalhos futuros;

Capítulo 6: Referências Bibliográficas;

Capítulo 7: Apêndice.

Os capítulos 3 e 4 foram escritos na forma de artigo, em língua inglesa, de forma a melhorar a eficiência no momento da realização da formatação e submissão dos mesmos O capitulo 3 realizou uma abordagem dos resultados das avalições sensoriais e com os resultados das análises físico-químicas, com base em um planejamento Central Composto de forma a buscar ajustes adequados para as respostas sensoriais.

CAPÍTULO 2 – REVISÃO BIBLIOGRÁFICA

Neste capítulo são abordados os conceitos que sustentam este trabalho por meio de uma revisão sobre o tema a partir da literatura, assim, são apresentados os preceitos, produção, mercado e processamento do café arábica.

2.1 Produção de Café no Cerrado Mineiro

Segundo a Fundaccer (2019), a região do Cerrado Mineiro é composta por 55 municípios, com aproximadamente 210 mil hectares em produção de café, produzindo 5 milhões de sacas por ano, sendo delimitada com base na Portaria do Instituto Mineiro de Agropecuária (IMA), nº 165/95 de 27 de abril de 1995 (IMA, 1995).

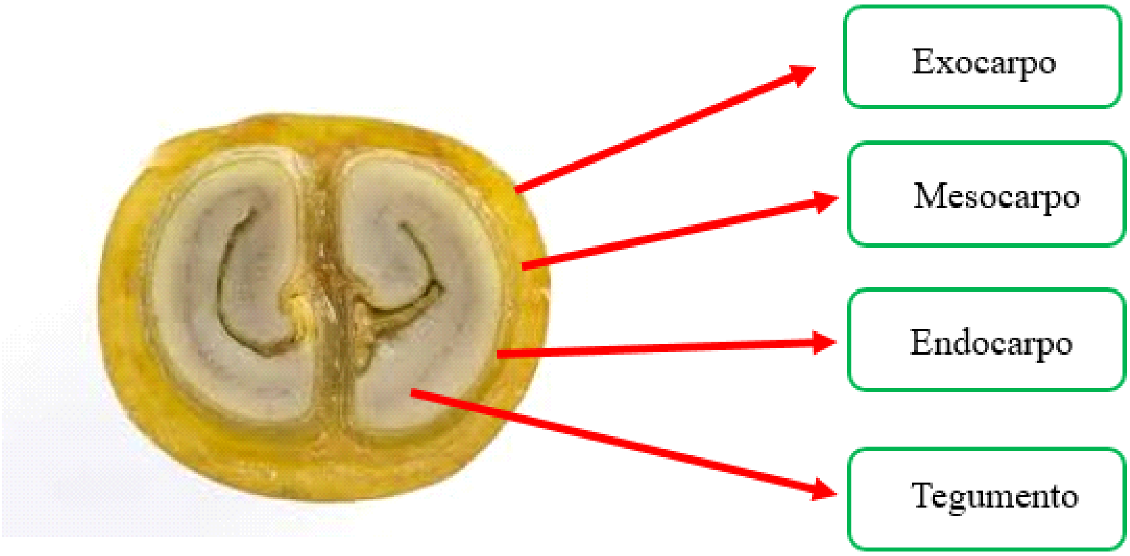
A região do Cerrado Mineiro é um território demarcado por produzir um produto que possui características únicas e que não pode ser encontrado em nenhum outro lugar, sendo esta denominação registada no Instituto Nacional de Propriedade Intelectual (INPI) com o código IG201011 (BRASIL, 2019). Os cafés produzidos na região do Cerrado Mineiro tendem a apresentar características naturais de aroma intenso, com nuances variando de caramelo a nozes, acidez delicadamente cítrica, corpo moderado à encorpado, sabor adocicado com aspecto de chocolate e finalização de longa duração (FUNDACCER, 2019).

O município de Carmo do Paranaíba é integrante da região do Cerrado Mineiro, localizado na mesorregião do Alto Paranaíba, com uma área territorial de 1.307,862 km². O município é um grande produtor de café e, segundo o Instituto Brasileiro de Geografia e Estatística (IBGE), produziu quase 275.000 sacas de café em uma área produtiva de quase 9.500 ha em 2017 (IBGE, 2019).

2.1. Café

O fruto de café geralmente consiste em uma camada de pele (casca), denominada exocarpo, sob esta existe uma camada de polpa seguida por um mesocarpo mucilaginoso (mucilagem) e firmemente aderido à camada rígida chamada pergaminho (endocarpo). O pergaminho protege individualmente duas sementes envoltas em uma fina membrana, denominada película prateada (EVANGELISTA *et al.*, 2015). Na Figura 2.1 está representado um corte do fruto de café, mostrando os principais constituintes do mesmo.

Figura 2.1 - Representação do fruto do café em corte transversal.



Fonte: Adaptado de BORÉM, 2008.

Borém (2008) definiu o exocarpo, mais comumente chamado de casca, como o tecido externo do fruto, o qual consiste em uma única camada de células compactas, as quais, após o amadurecimento tendem a possuir coloração vermelha ou amarela (SALAZAR *et al*., 1994; BORÉM, 2008). A polpa possui de 6 a 8% de mucilagem, e é constituída basicamente de carboidratos, proteína bruta, fibra bruta e cinzas (BORÉM, 2008).

O mesocarpo ou mucilagem é um tecido formado por cerca de 20 camadas de células de tamanho variável, composta em média de 88,34% de água em base úmida (b.u.) e 7,17% de carboidratos totais. O endocarpo ou pergaminho é a estrutura mais interna do pericarpo e é formado de três a sete camadas de células, envolvendo completamente a semente, de forma a proteger a mesma e também limitar seu crescimento (SALAZAR *et al*., 1994; BORÉM, 2008). O tegumento ou semente é formada pela película prateada, endosperma e embrião; com formato plano convexo, elíptica e sulcada na parte plana. O endosperma é o principal tecido de reserva, constituindo de maior volume da semente madura de café (BORÉM, 2008).

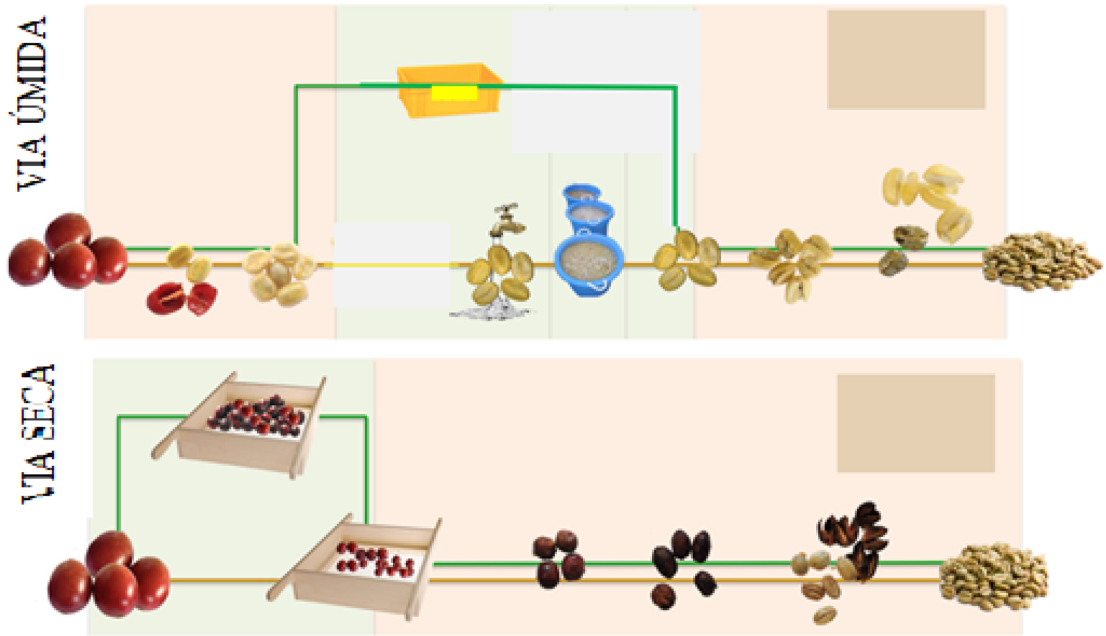
2.3. Processamento do café

Os frutos de café, após a etapa de colheita, podem ser submetidos a diversos métodos para transformá-los de grãos frescos e úmidos para grãos secos, os quais são comercializados nos mercados nacionais e internacionais (RIBEIRO *et al*., 2017a). A escolha do método (seco, semi-seco e via úmida) influencia diretamente na composição química dos grãos e nas características sensoriais da bebida do café (SILVA, 2014; RIBEIRO *et al*., 2017a).

Tradicionalmente são utilizados dois diferentes métodos para o processamento e secagem do café, a via seca e via úmida. A via seca, apresentada na Figura 2.2 é um processo no qual os frutos são processados de forma integral, ou seja, com a casca, produzindo frutos secos, denominado cocos, sendo este o processo mais simples e rústico de processamento do café. O café, nestas condições, fermenta durante a etapa de secagem. No processamento via úmida, apresentado na Figura 2.2, a polpa e a mucilagem (exocarpo e mesocarpo) é removida mecanicamente e o restante da mucilagem é removida de forma mecânica ou biológica. Neste processo são produzidos frutos em pergaminho (BORÉM, 2008, EVANGELISTA *et al*., 2014a).

Com relação à qualidade na maturação, os frutos secos geralmente apresentam densidade inferior a da água, e assim flutuam sobre a mesma, sendo por isso chamados de 'boia'. Esses frutos são separados no lavador, uma vez que geralmente passam por processos fermentativos indesejados. Os frutos totalmente maduros são denominados cerejas e têm o melhor ponto da maturação, estando integralmente maduros e com isso apresentando melhores teores dos componentes necessários à qualidade sensorial. Os frutos imaturos são denominados verdes e geralmente apresentam adstringência e qualidade inferior (BORÉM, 2008).

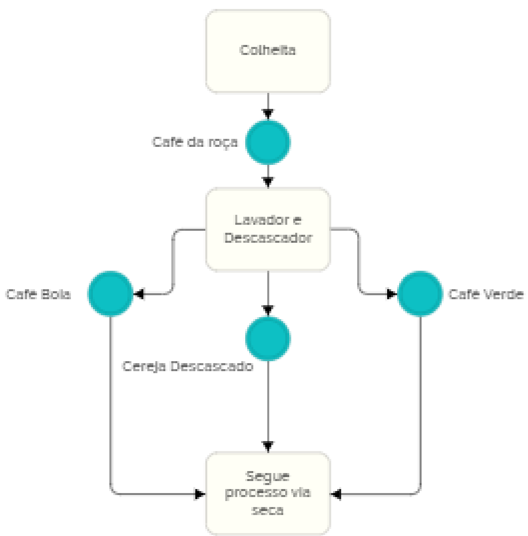
Figura 2.2- Etapas do processamento via seca, via úmida e via semi-umida do café



Fonte: Adaptado de De Bruyn, 2016.

O método semi-seco, conforme Figura 2.3, trata de uma variação do processo via úmida que tem os frutos descascados, sendo removidos o exocarpo e parte do mesocarpo e a secagem é acompanhada de fermentação, tal como ocorre no processo via seca (EVANGELISTA *et al*., 2014a; RIBEIRO *et al*., 2017a).

Figura 2.3 - Processamento semi-seco do café.



Fonte: Adaptado de BORÉM, 2008.

2.4. Qualidade do Café

No cenário mercadológico liberal, a qualidade assume uma importância primordial entre as commodities de exportação (VELMOROUGANE, 2013), e com relação ao café não é diferente. Dentre as espécies de café, a arábica (*Coffea arabica*) é considerada de qualidade superior devido às suas propriedades sensoriais, assim sendo é uma das bebidas mais preparadas no mundo (LASHERMES *et al*., 2009; RIBEIRO *et al*., 2017b).

Entretanto, mesmo no café arábica, a definição de bebida de qualidade é uma questão complexa. Assim a qualidade do café, tanto visual quanto sensorial é uma contribuição cumulativa de vários parâmetros físicos e sensoriais, como umidade, defeitos (grãos ardidos, pretos, verdes, quebrados ou com problemas de formação), tamanho dos grãos, compostos químicos que resultarão na doçura, acidez, limpeza de xícara, ausência de defeitos, e inclusive o preparo de amostra para a degustação. Dessa maneira, a junção desses aspectos reflete na maior aceitação e valor agregado do produto no mercado (LEROY *et al*., 2006; VELMOROUGANE, 2013). Existem, segundo Esquivel e Jiménez (2012), diferentes tipos de bebidas, caracterizadas por diferentes nuances em termos de corpo, aroma, acidez e adstringência da bebida. Taveira *et al*. (2014), escreveu que a qualidade do café tem sido valorizada nos últimos anos, dando credibilidade aos cafés especiais, que são caracterizados por um conjunto de aromas e sabores memoráveis e equilibrados, além da ausência de defeitos.

A geração dos atributos sensoriais (*flavor*) começa ainda na planta, onde os percursores do sabor se formam simultaneamente ao desenvolvimento da cereja. A complexidade do sabor ainda se desenvolve conforme o processamento do café e o tipo de preparo na xícara (SUNARHARUM *et al*., 2014. LEE *et al*. 2015) afirmaram que os perfis aromáticos e voláteis do café torrado são muito dependentes da composição de percursores do aroma presentes nos grãos verdes.

Compostos não voláteis presentes no café torrado podem ser importantes para o sabor, incluindo alcaloides (cafeína, trigonelina), ácidos clorogênicos, ácidos carboxílicos, carboidratos, polissacarídeos poliméricos, lipídeos, proteínas, melanoidinas e minerais (SUNARHARUM *et al*., 2014). Açúcares, aminoácidos, proteínas, compostos fenólicos são importantes percursores do aroma presentes nos grãos de café verde, os quais realizam uma importante função na formação do aroma do café. O mesmo acontece com vários ácidos orgânicos não voláteis, que passam por transformações químicas formando potentes compostos fenólicos voláteis (LEE *et al.*, 2015).

A acidez percebida no café é um atributo importante para a análise sensorial do produto e sua intensidade é influenciada por diversos fatores como: condições climáticas durante a secagem e colheita, local de origem, tipo de processamento e estágio de maturação (ABREU E SIQUEIRA, 2006), esta tem grande influência devido à presença de ácidos orgânicos tais como os ácidos succínico, acético, cítrico, lático e málico (SILVA *et al.*, 2013; EVANGELISTA *et al.*, 2014a, PEREIRA *et al.*, 2015). Estes ácidos melhoram a acidez final do produto, proporcionando diferentes nuances, os ácidos como lático, succínico, málico, cítrico e acético podem influenciar positivamente nas características sensoriais do café, e sua presença é desejada, no entanto, a presença de ácidos tais como butílico e propiônico podem prejudicar a qualidade final do café, uma vez que podem proporcional sabores desagradáveis (SILVA *et al.*, 2013; EVANGELISTA, *et al.*, 2014a).

A análise química de cafés processados por diferentes métodos mostraram que os açúcares de baixo peso molecular, como frutose, glicose, arabinose e galactose são significantemente menores nos cafés processados na via úmida que nos processado na via seca, enquanto os cafés processados via úmida têm maiores teores de ácidos glutâmicos e aspártico, justificando desta forma, o que acontece de maneira geral, melhores notas em doçura e menores notas de acidez nos cafés naturais que nos cafés descascados (BYTOF *et al.*, 2005; KNOPP *et al.*, 2005). A presença de açúcares de baixo peso molecular (glicose e frutose, por exemplo) favorece o crescimento microbiano, servindo de substrato para a fermentação, tal como encontrado por Lee *et al.* (2016a), no qual utilizaram a fermentação do café verde com o fungo *Rhizopus oligosporus* e encontraram uma redução nestes açúcares, com a formação de ácido lático como metabólito.

Como a questão de qualidade é subjetiva, foram criados então, para a definição dos padrões de qualidade existentes, nos diversos países, várias normas específicas voltadas para a classificação da qualidade do café. De acordo com a OIC (2020c), o café é geralmente classificado com base em um ou mais critérios como altitude e região, tipo botânico, tipo de preparo, tamanho, formato e cor do grão, número de defeitos, aparência da torra e qualidade na prova de xícara (resultado da classificação e qualidade da bebida).

No Brasil, a norma utilizada para a classificação do café é definida pelo Regulamento Técnico de Identidade e Qualidade para a Classificação do Grão Beneficiado Cru e a Instrução Normativa do Ministério da Agricultura Pecuária e Abastecimento (MAPA), nº 08 de 11 de junho de 2003 (BRASIL, 2003) que classifica a bebida como Estritamente Mole, Mole, Apenas Mole, Dura, Riada, Rio e Rio Zona, da melhor para a pior bebida, respectivamente.

Entretanto, devido à lacuna da mesma na caracterização sensorial dos cafés, uma vez que a metodologia adotada pelo MAPA não descreve os atributos sensoriais encontrados na bebida, e sim a ausência de defeitos (iodofórmio, adstringência) e a intensidade da qualidade, é também utilizada a escala da *Specialty Coffee Association* (SCA), para definir os padrões de classificação, principalmente sensorial. Nesta avaliação, o café é pontuado em 10 atributos, com notas de 1 a 10 baseado na fragrância e aroma (pó seco e hidratado, após quebra da crosta), uniformidade das xícaras, ausência de defeitos, doçura, sabor, acidez, corpo, finalização, equilíbrio, e a avaliação geral é dada pela soma de todos os atributos (SCA, 2020a).

A avaliação desta qualidade é realizada geralmente por um Provador de Café Certificado pela SCA, também denominado "*Q Grader*". Estes têm paladar distinto, tal qual um *sommelier*, e podem profundamente identificar a qualidade do café por meio de avaliação sensorial sistemática de cafés (SCA, 2020b).

Aknésia *et al.* (2015) definiram cafés especiais como cafés de qualidade *premium*, o qual passaram por um complexo processo de plantio, cultivo e processamento pós-colheita, todos estritamente controlados para atingir uma melhor bebida, porém, de forma a definir um padrão numérico, com base na nota da SCA, conforme descrito no Quadro 2.1.

Quadro 2.1. Tipo de Qualidade Conforme nota SCA do café

NOTA TOTAL	CLASSIFICAÇÃO	ESPECIAL
90 – 100	Excepcional	Sim
85 – 89,99	Excelente	Sim
80 – 84,99	Muito Bom	Sim
< 80	Abaixo de Especial	Não

FONTE: Adaptado de SCA (2020a)

2.5. Fermentação

A fermentação é um dos mais antigos métodos de processamento de alimentos. Dentre os produtos resultantes dos processos de fermentação destacam-se a fabricação de vinhos, queijos e molho de soja (SALQUE *et al.*, 2013; CARVALHO NETO *et al.*, 2018). No café, entretanto, a fermentação é a etapa mais difícil de ser controlada de todo o processamento do café, uma vez que as unidades operam bem aquém das condições ótimas tanto em custo de operação (consumo de água e energia) quanto em qualidade final do produto (CORREA *et al.*, 2014).

Pereira *et al.* (2015) citaram diversos estudos que demonstraram que a fermentação deve ser bem controlada para garantir o desenvolvimento dos microrganismos que proporcionam uma bebida de alta qualidade e um bom aroma, mostrando que uma fermentação controlada pelo uso de 'culturas iniciadoras' pode garantir uma qualidade padronizada e reduzir as perdas econômicas do produtor, assim como ocorre em outros alimentos, tais como queijos, iogurtes, pão e cerveja.

A fermentação pode ocorrer em um meio submerso (fermentação submersa) ou em estado sólido. A tecnologia de fermentação em estado sólido, está expandindo com incremento da importância da formação de produtos com alto valor agregado (BUCK *et al.*, 2015). A fermentação em estado sólido considera o crescimento microbiano em substratos sólidos e úmidos, na ausência de quantidade expressiva de água (BEHERA e RAY, 2016).

2.5.1. Fermentação do café

A fermentação de diferentes matrizes de café, ao longo do processamento, apresenta cafés com características sensoriais únicas e desejáveis (LEE *et al.*, 2016a). Segundo Pimenta (2003), a fermentação do café ocorre a partir de sucessivas etapas, passando de desejáveis à indesejáveis, sendo favorecidas por condições de anaerobiose enquanto houver umidade suficiente no meio. A primeira fermentação é a lática ou alcoólica e é benéfica, podendo ocorrer naturalmente e ser responsável por um bom paladar e aroma, sendo caracterizada pelo cheiro de álcool etílico. Em seguida à esta ocorre a fermentação acética, e já se inicia a formação de metabólitos prejudiciais à qualidade do produto.

Durante a fermentação do café, alguns microrganismos pectinolíticos são associados com a degradação da polpa e mucilagem (ricas em polissacarídeos), produzindo álcoois, ácidos e outros compostos metabólitos, que interferem na qualidade final da bebida (EVANGELISTA *et al*., 2014b).

A fermentação do café ocorre geralmente, simultaneamente à secagem ou previamente à mesma, de forma a degradar o mesocarpo do fruto do café, facilitando desta forma a etapa de secagem (RIBEIRO *et al.* 2017a)

Segundo Lee *et al*. (2015), o maior problema envolvendo a fermentação é a falta de controle da etapa, uma vez que, havendo um controle inadequado, por mais que a fermentação possa agir melhorando a qualidade do aroma do café, existe a possibilidade da degradação desta qualidade sensorial. O ponto de finalização da fermentação é comumente baseado em observações subjetivas. Fermentações além do necessário possibilitam a produção de grãos pretos e ardidos (*Stinker*), que além de ser um defeito visual, causa um grande dano na qualidade sensorial do café. O controle biológico e de condições ambientais são fundamentais para melhoria da qualidade final.

Diversos fungos encontram-se associados aos processos de produção dos grãos de café durante todo o ciclo produtivo e podem, sob condições específicas, causar perdas de qualidade produzindo aromas e sabores desagradáveis e em alguns casos podem produzir metabólitos tóxicos (micotoxinas), comprometendo a característica de segurança do produto (BORÉM, 2008). De acordo com Velmourougane et al. (2013) atrasos durante o processo de fermentação seja para o início ou para o final podem acarretar o crescimento acelerado de fungos filamentosos, o que pode causar riscos à segurança alimentar, bem como uma perda da qualidade de bebida. Evangelista et al. (2014b) concluíram que o uso de culturas iniciais, tal como leveduras, resulta em uma bebida com sabor distinto e de boa qualidade sensorial, e Ribeiro et al. (2017a), em seu estudo concluíram que o uso de leveduras teve grande efeito na cultivar de café Ouro Amarelo produzindo um café de boa qualidade.

2.6. Microrganismos presentes na fermentação e inoculação

A atividade microbiana durante o processamento na fazenda é creditada como uma importante etapa no desenvolvimento do sabor do café (LEE *et al*., 2015). Uma grande gama de microrganismos foram isolados da fermentação do café, em diferentes regiões produtoras do mundo, entre elas diversas leveduras, bactérias láticas e produtoras de ácido acético como *Bacillus* ssp., além de fungos filamentosos (AVALLONE *et al*., 2001, PEREIRA *et al*., 2015, DE BRUYN *et al*., 2016, ELHALIS *et al*., 2020a, SILVA *et al*., 2014).

Especificamente, as leveduras promovem um significante aumento em álcoois, ésteres, aldeídos, glicerol e ácidos orgânicos no café fermentado (ELHALIS *et al*., 2020a), além de também reduzir o crescimento de fungos filamentosos e a produção de metabólitos indesejáveis tal como os ácidos butílico e acético (ELHALIS *et al*., 2020a, MARTINEZ *et al*., 2017), além de também aumentar significativamente a produção de enzimas pectinolíticas (MARTINEZ *et al*., 2017).

Os microrganismos responsáveis pela boa fermentação do café possuem gêneros específicos que têm uma maior predominância no processo e entre as leveduras, destacam-se: *Pichia, Saccharomyces, Candida, Starmerella, Hanseniaspora* e *Torulaspora* (DE BRUYN, *et al*., 2016, VILELA *et al*., 2010, ELHALIS *et al*., 2020b). Estas espécies nativas isoladas do próprio café são responsáveis pela fermentação e geralmente se comportam melhor que outras de outros substratos, conforme comparado por Ribeiro *et al*. (2017a), entre duas cepas de *S. cerevisiae*, uma isolada da cana-de-açúcar (CCMA 0200) e outra isolada do café (CCMA 0543).

A inoculação de microrganismos iniciadores, para modificar a microbiota natural da fermentação da mucilagem do café, é utilizada para melhorar não só a consistência do processo fermentativo, mas também para melhorar ou impactar certos atributos sensoriais (WANG, *et al*., 2018). Diversos autores trabalharam com a inoculação de microrganismos com os mais variados resultados.

Alguns estudos foram realizados com diferentes leveduras, utilizando cepas de *Candida parapsiloris, Pichia guillermondii Torulospora delbrueckii*, *Saccharomyces cerevisiae,* e *Pichia fermentaris* (EVANGELISTA *et al*., 2014a e 2014b; PEREIRA *et al*., 2015; MARTINEZ *et al*., 2017; RIBEIRO *et al*., 2017a; BRESSANI *et al*., 2018). Uma das cepas que apresentou grande destaque foi a cepa de *S. cerevisiae*, apresentando bons resultados (MARTINEZ *et al*., 2017, BRESSANI *et al*., 2018).

Ribeiro *et al*. (2017a) inocularam duas cepas diferentes de *S. cerevisiae* utilizando duas variedades de café, e em uma delas apresentou um incremento na pontuação sensorial da Tabela SCA em até 1,5 pontos. Em outro trabalho realizado no mesmo local mostrou que cepas diferentes podem ter características diferentes em cultivares genéticos diferentes (RIBEIRO *et al*., 2017b).

2.7. Condições e parâmetros ambientais para a fermentação do café

A mucilagem é composta por açúcares e substrato pectinoso, os quais são convertidos em álcoois e ácidos orgânicos de maneira exotérmica, e a produção destes metabólitos leva à redução do pH ao aumento da temperatura da massa de fermentação (AVALLONE *et al*., 2001).

Como os organismos fermentadores utilizam a polpa do café como fonte de carbono e nitrogênio, produzem como metabólitos uma grande quantidade de etanol, ácido acético, lático, entre outros, abaixando o pH de 5,5-6,0 para 3,5-4,0 (PEREIRA *et al*., 2015). Neste mesmo trabalho o autor mostrou que os valores de pH estão fortemente correlacionados com o crescimento bacteriano, apresentando menores valores de pH para fermentações com maior crescimento bacteriano.

Estudos realizados na Nicarágua por Jackels e Jackels (2005) sugerem que a etapa e o ponto final da fermentação são caracterizados pelo pH, como um parâmetro confiável para se estabelecer, uma vez que a redução do pH da massa de fermentação de 5,5 a 4,0 foi bem consistente com várias replicatas e tamanhos de reatores em diversos estudos citados por Lee *et al*. (2015) (VELOMOUROGANE, 2013; ROTHFOS, 1985). Jackels *et al*. (2006), avaliaram a qualidade sensorial do café no qual a fermentação foi interrompida em três diferentes valores de pH: 4,6; 4,3 e 3,9 e foi observado que, em pH menores correspondeu à uma perda na qualidade sensorial, o que foi atribuído à fermentação excessiva. Desta forma, o pH é uma importante e confiável ferramenta para poder controlar o ponto final da fermentação e evitar a super ou sub fermentação.

Devido ao crescimento microbiano liberar calor, a fermentação tanto nos mecanismos enzimáticos ou microbiológicos são favorecidos pelo aumento da temperatura, e o aumento da temperatura durante a etapa, pode ser um indicativo de uma fermentação excessiva. No experimento realizado por Jackels *et al*. (2005), os autores observaram que as temperaturas dos tanques de fermentação foram maiores que a temperatura ambiente, normalmente de 1 a 4 °C. Porém, segundo os autores, quando esta diferença de temperatura chega a 6 °C, pode indicar uma fermentação excessiva. O aumento de temperatura pode ser explicado principalmente devido à natureza exotérmica das reações bioquímicas da fermentação (VELMOROUGANE, 2013).

O tempo de fermentação é uma variável a qual depende dos demais fatores envolvidos, uma vez que sua principal função é realizar a degradação da mucilagem do café. Essa degradação depende de fatores, tais como, a composição inicial da mucilagem do café, temperatura ambiente, características e população de microrganismos, entre outros. Velomouragane (2013) obtiveram para café robusta e arábica diferentes tempos de fermentação, sendo este tempo suficiente para a degradação da camada de mucilagem. No caso do café arábica, o mesmo levou 13 horas para a completa degradação da mucilagem, enquanto no robusta o tempo foi de 98 horas, nas mesmas condições de temperatura ambiente e umidade. Este resultado foi provavelmente devido à maior concentração de taninos e polifenóis presentes na mucilagem do café robusta.

2.8. Secagem, beneficiamento, classificação e torra do café

A secagem do café é também uma fase crítica para a obtenção de grãos de qualidade, pois entre a transição do café úmido para o café seco, pode haver o crescimento de microrganismos indesejáveis, continuando a fermentação e formando mofos toxicogênicos e degradação da qualidade do café (PRADA *et al.*, 2019). Além da perda de qualidade, a secagem trata-se da mais relevante operação no que tange o consumo de energia e custo do processamento do café (BORÉM, 2008).

Na secagem mecânica o uso de temperaturas acima das toleradas pelo café pode ser observada em alguns processos, com o objetivo de acelerar a etapa e aumentar a capacidade de secagem, no entanto, práticas como esta podem comprometer a qualidade do café, devido à dano térmico nos secadores (ALVES *et al.*, 2017). Por mais que as tecnologias de secagem permitam o aumento da temperatura para aumentar a taxa de secagem dos grãos de café, temperaturas da massa de secagem superiores à 40 °C, causam danos que depreciam a qualidade do mesmo (BORÉM, *et al.*, 2013).

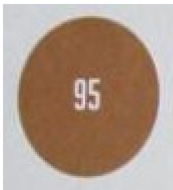
O café verde deve ser torrado e moído para obter as características desejadas em questão de aroma e sabor (DE BRUYN *et al.*, 2016). O aroma do café é geralmente formado durante a torra por uma série de reações complexas de Maillard, caramelização e outras reações térmicas envolvendo os precursores do aroma que estão presentes no grão verde, sendo a torra uma etapa que tem grande impacto no aroma do café (LEE *et al.*, 2015). Com a torra, os grãos de café se transformam da cor verde para marrom ou até mesmo preto. O grão se torna intensamente quebradiço de forma que a moagem se torna possível. Temperaturas acima de 200 °C são necessárias para a operação de torra.

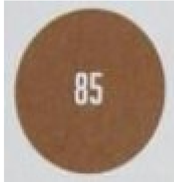
Lee *et al*. (2016b) realizaram uma avaliação do café fermentado em três diferentes níveis de torra, clara, média e escura e encontraram diferentes concentrações dos compostos voláteis em cada nível, mostrando que desta forma, os diferentes tipos de torra podem acarretar diferentes qualidade sensoriais.

Diferentes métodos são estudados para a determinação do grau de torra, sendo elas a cor, perda de massa, grau de torra, umidade do grão, degradação de ácidos clorogênicos, entre outros, porém a natureza desta etapa ainda é extremamente complexa para estabelecer-se um padrão único, e apesar de pouco precisa, o padrão colorimétrico é ainda o mais utilizado (BAGGENSTOSS *et al*., 2008; YANG *et al*., 2016).

Existem vários métodos colorimétricos de determinação do grau de torra, conforme recomendado nos padrões da SCA (SCA, 2020a), sendo eles Agtron®, Colortrack®, Probat Colorette®, entre outros, sendo o mais comum utilizado o sistema Agtron. Este sistema utiliza um espectrofotômetro de onda abaixo do infravermelho para refletir a luz da amostra de café e proporcionar uma leitura numérica, sendo que quanto menor a leitura, maior a intensidade da torra (a escala varia de 0 a 100). A SCA possui um kit de identificação colorimétrica do nível de torra, o qual contém um padrão colorimétrico físico para a comparação com o nível de torra adotado, partindo de um nível de torra de Agtron#95 que é denominada muito clara para o nível de torra Agtron#25 que é muito escura. No Quadro 2.2 estão apresentadas as classificações oficiais, com base no kit da SCA, e as respectivas características visuais do café.

Quadro 2.2. Cor da Torra conforme número Agtron e Características

Cor da torra	**Superfície do grão**	**Número Agtron**	**Nomes comuns**	**características**	**foto**
Muito Clara	Seca	#95	Café Cru	Torra muito clara, sabor de chá. Não recomendável.	

Clara	Seca	#85	Café Cru	Torra muito clara, sabor de chá. Não recomendável.	
Moderadamente Clara	Seca	#75	Canela Escandi-návia	Pode ter sabor azedo ou grãos.	
Média Clara	Seca	#65	Americana	Sabor totalmente desenvolvido, boa acidez. Utilizado na prova SCA.	
Média	Seca	#55	Média	Sabor totalmente desenvolvido, boa acidez. Utilizado na prova SCA.	
Moderadamente escura	Seca a poucas manchas oleosas	#45	Italiana e Vienna	Torra normal para boa parte dos torrefadores, começa a mudar acidez dos grãos e surge o amargor. Utilizado na norma Italiana de expresso.	
Escura	Superfície levemente brilhante	#35	Italiana e Francesa	Perda total da acidez do café e poucas	

				características do café verde ainda são perceptíveis. O amargor é predominante.	
Muito Escura	Superfície Brilhante	#25	Francesa Escura	Todas as características do café verde são perdidas, sabor de torrado e carbonizado predominam.	

FONTE: Adaptado de SCA (2020a)

A partir do exposto pode-se observar que a qualidade do café é um atributo que depende de muitos fatores desde o campo até a última etapa de processamento. E, que especialmente a fermentação, secagem e torra podem ser etapas decisivas na obtenção de cafés com características especiais, sendo a fermentação uma etapa importante para a qualidade final do café, e consequentemente seu valor de mercado.

CAPÍTULO 3 - EFFECT OF FERMENTATION OF ARABICA COFFEE WITH YEAST *Saccharomyces cerevisiae* ON PHYSICOCHEMICAL CHARACTERISTICS AND SENSORY ANALYSIS

In this chapter, the effects of fermentation on the physical-chemical and sensory characteristics of coffee are addressed, based on a central composite rotatable design (factorial design technique) with two variables, fermentation time and temperature. In this chapter, the evolution of the fermented mass's pH, moisture, sugar content, glycerol, organic acids, and sensory influence were evaluated.

3.1 Introduction

Coffee is one of the most popular and consumed drinks in the world. Brazil is the largest coffee producer in the world, and among agricultural commodities, it is in fifth place in the country's total export earnings (BRASIL, 2020). This drink has a rich and complex flavor of olfactory sensations, making it a product of more excellent added value. The taste of a freshly prepared coffee cup is a final expression and a noticeable result of a long chain of operations that transform the seed and take it to the cup (JOËT *et al*., 2010). The definition of the coffee's quality as a beverage is very broad, depending on the beans' chemical composition, determined by several factors: harvesting, processing methods, preparation, and roasting of the coffee (PIMENTA *et al*., 2018). The flavor is a decisive parameter and significantly influenced by the processing to transform the cherries into green grains (WANG *et al*., 2018). Elhalis *et al*. (2020b) believe that post-harvest processing has a significant impact on the final taste, affecting directly alcohols, sugars and acids concentration, but with still unknown effects of the entire process.

The coffee fermentation process can take place in a submerged medium or a solid state. Bioreactors can provide controlled environmental conditions for fermentation development (TANG *et al*., 2021). Solid fermentation is a fermentation process carried out on solid materials in the absence or low moisture of water that acts as physical support and a source of nutrients for microorganisms. Due to this low water availability, a limited number of microorganisms can develop for solid fermentation.

In the dry process, coffee cherries are fermented and dried simultaneously immediately after harvest, taking up to 20 days (BRANDO and BRANDO, 2014; SILVA, 2014). This process is generally totally aerobic and can retain the highest glucose and fructose concentration in the fruits (KNOPP, BYTOF and SELMAR, 2005). These coffees have a chemical characteristic of natural coffees, with a higher content of soluble solids and total sugars (RIBEIRO *et al*., 2011). Consequently, the drink has a superior body and also more remarkable sweetness.

The use of microorganisms that initiating fermentation was evidenced by several authors (EVANGELISTA *et al*., 2014a, 2014b; PEREIRA *et al*., 2015; RIBEIRO *et al*., 2017a; MARTINEZ *et al*., 2017a; ELHALIS *et al*., 2020) as conductors of an appropriate fermentative process producing special coffees, with scores above 80 points, according to Cupping Specialty Coffee protocols-SCA (SCA, 2020a). During fermentation, microorganisms produce several metabolites. The microbial activity and the extent of fermentation determine the concentration of free sugars (fructose and glucose, for example) and free amino acids, which remain in the grain and subsequently contribute to the production of the compounds of the Maillard reactions and volatile compounds during the roasting process (HAILE *et al*., 2019a).

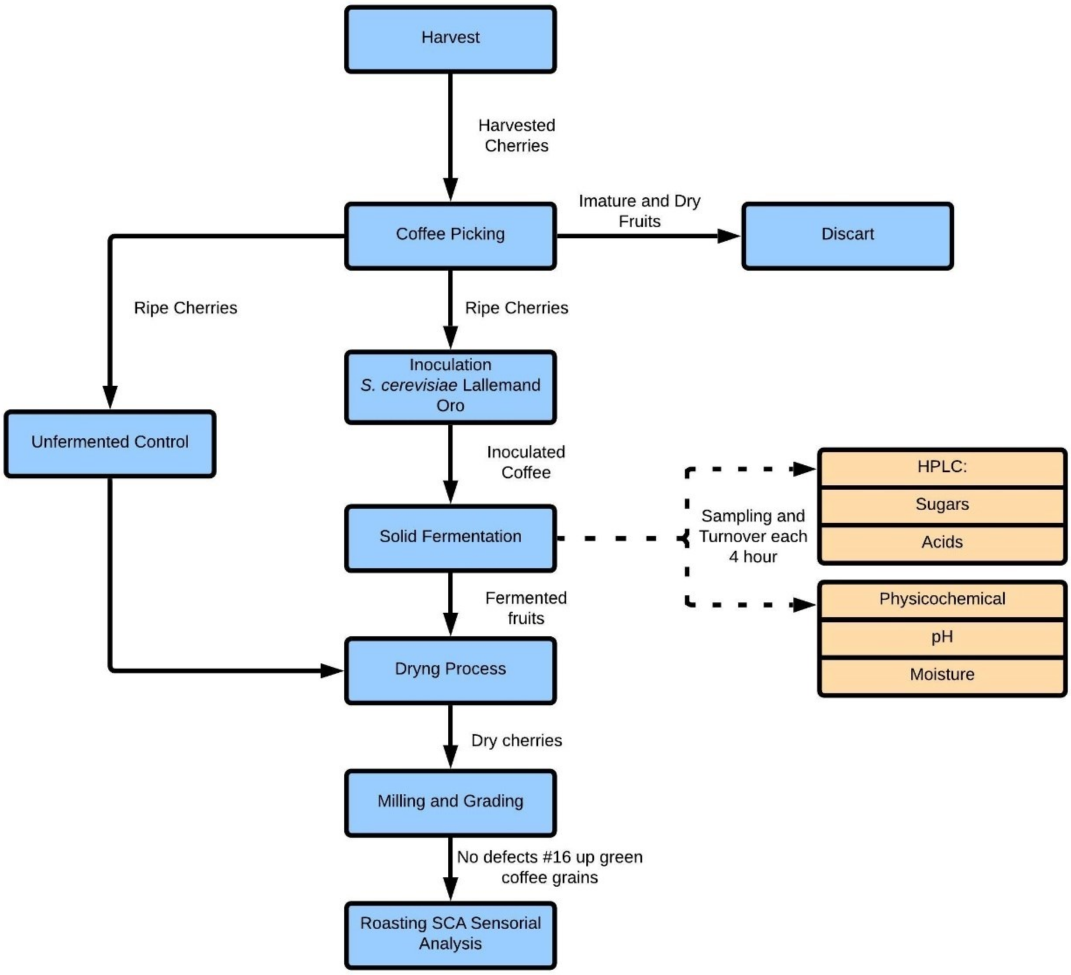
The aroma of coffee is associated with yeast metabolism. It is evident from several studies that the addition of selected yeasts to wild yeasts from the coffee cherry microbiota affects coffee aroma. *Saccharomyces cerevisiae* is an important microorganism that adapts well to solid fermentation conditions and can be used worldwide to produce food and beverages (SANTOS DA SILVEIRA et al., 2019). The yeast *Saccharomyces cerevisiae* has been reported to improve the sensory quality of coffee, contributing to coffee's fruity aroma at the end of the roast (BRESSANI et al., 2018; WANG et al., 2020).

Most coffee fermentation processes are still carried out worldwide in processes without environmental control and spontaneously, producing coffee beans with inconsistent and unpredictable quality (ELHALIS, *et al.,* 2020). This study aimed to analyze the effects of fermentation of Arabica coffee, the Catuaí Amarelo strain, on the physical-chemical characteristics and the product's sensory result.

3.2. Material and methods

The steps followed for the development of this work are presented in the flowchart of Figure 3.1. The description of the respective methodology used is described in items 3.2.1 to 3.2.9.

Figure 3.1 – Project flowchart.



Source: Luci chat – Desingned by Autor

3.2.1 Choice of cultivar and production area

The rural property chosen for the harvest of the fruits is located in the greater coffee production region in Carmo do Paranaíba, Minas Gerais - Brazil. The coffee field, taken from the samples, is in the geographical coordinates Lat.: *18 57'37"S*/ Long.:*46 36'17"O*, with an altitude of 1,080 meters. The cultivar, from the area mentioned above, is the Arabica coffee of the Catuaí Amarelo strain, IAC 62, which, according to the Coffee Research Consortium (2020), is a variety that produces the majority of fruits with beans called flat beans, medium, retained in #16 sieve, without mochas, leaving to sensorial evaluation only big flat grains. Generally, it has more than 82% of flat fruits, with excellent drink quality. The satellite image of the farm's location is shown in Appendix (A1).

3.2.2 Sample collection and preparation

The coffee was harvested manually to select only the ripe fruits, having more significant potential for quality in the cherry stage, which, yellow fruits, has brass color and no greenish color in any part of it. The harvested fruits were placed in plastic containers, and after harvesting, a new classification was carried out to separate fruits with higher quality. All the fruits were submerged in water using a 500 L reservoir to simulate the same process performed by the coffee washer, which separates the dried fruits or those with granulation problems. Dried fruits or those with grainy problems (float) are less dense than water and float, and these fruits were discarded. Immature fruits were selected manually and discarded due to their astringency. Cherry fruits were considered for fermentation, those with a bronze yellow color throughout the skin's length. The image of the mature Catuaí IAC 62 coffee is shown in Appendix (A2). The selected fruits were placed in plastic containers with cold water and placed in a cool box with ice to keep the temperature low (<4ºC), according to the method used by Carvalho Neto *et al*. (2018). This procedure avoids the fermentation process during coffee transportation from the farm to the laboratory, where the experiments and analyzes were carried out. Selected cherries were highlighted on Appendix (A11).

3.2.3 Incubation

The commercial yeast *Saccharomyces cerevisiae*, of the commercial brand Lallemand ORO® (10^{10} UFC/g), was kindly provided by Lallemand. For the incubation, the manufacturer's ratio was used, with 12 g (0,012 kg) of yeast for each 10 kg of coffee. The yeast was weighed on an analytical balance (Shimadzu, model BL-3200H) and dissolved in distilled water and applied to the coffee mass. in ratio of 10 g of commercial yeast for 500 mL distilled water. For cell count, there were used Neubauer chamber method, with previously prepared solution, diluted 1 ml for 100 ml, according to Equation 3.1, were C_1 and C_2 were quadrant mean counts: After Neubauer chamber count, were found about 2.55 x 10^8 cell/mL of solution.

$$Cell\ count\ \left(\frac{Cell}{ml}\right) = \left(\frac{C_1+C_2}{2}\right) \times 2{,}5 \times 10^5 \times \frac{1}{100} \qquad (3.1)$$

3.2.4 Solid fermentation

The fermentation was carried out in a batch process. Each batch was fermented a volume of 6 L of coffee, divided into open beakers of 2 L, with approximately 2.4 kg of mass. An incubator with temperature control was used for fermentation (Tecnal, model TE-421). The temperature was determined, for each experiment, according to the experimental plan. Samples of 200 g were taken every 4 hours to measure moisture, pH, sugars, and organic acids. Manual turning over the entire grain mass was realized to ensure the aerobic fermentation environment during the sampling.

3.2.5. Drying and final samples treatment

The beans' final drying was carried out in an oven, with forced air circulation (Ethik Technology, Model 400 / 8D) at a controlled temperature and intermittent drying processes, traditionally applied to coffee in conventional processing. After half drying (darkening of the fruit peel), the process occurred in cycles of 8 hours of drying with a temperature of 37.5°C, with rest intervals of 8 hours of the grain mass at room temperature (ISQUIERDO *et al*., 2011). The image of the start of the drying of coffee beans is shown in Appendix (A6).

After drying, the samples were adequately packed in high-strength low density polyethylene bags, identified and packed in a BOD Incubator, at 25 °C (Solab, Model SL-225/364) hermetically sealed to prevent the entry of light and contamination. For 60 days, the coffees were stored and later removed for milling. A mechanical coffee mill carried out the exocarp and dry endocarps' complete removal for samples, located on the farm (Model DRC; Pinhalense). This stage transformers the dry cherries into green raw coffee without any classification (this is a raw coffee, without proper separation by size and removal of defects, just as done on commercial coffees evaluation). After this step, samples were completely placed in plastic packaging, traditionally used to store coffee samples. Thus, the samples were sent directly to the classification, for the separation of defects (impurities and imperfect grains) and separation of only large grains (grains retained in the 16 coffee sieve, without mochas, without clubs, for their sensory analysis (SCA, 2020a).

3.2.6. Samples classification

The samples were classified based on the standards established by IN 08/2003 (Brasil, 2003). In this classification, all intrinsic and extrinsic defects were removed entirely, and the burnt, black grains and skinkers were separated. For the separation of big grains, the classification sieves with the number 11 oblong sieves (separation of mocha grains), classification sieve with round sieves of size 16/64" were used (this Sieve is same as Sieve #16 of coffee classification). Only the grains retained in the 16/64" sieve without straws went through the classification stage. The image of the classified sample is shown in the Appendix (A7).

3.2.7. Physicochemical analysis

3.2.7.1. Moisture and pH during fermentation process

For moisture measurement, part of the whole fruits was placed in previously weighed crucibles and taken to the oven (Nova Ética, model 402-3N) at 105ºC, for 24 hours. After this period, the samples were removed from the oven, placed in desiccators to cool, and then weighed (BRASIL, 1992).

The rest of the coffee fruit sample was crushed with an industrial blender for about a minute (Camargo, 2L 800W / 22000rpm) until a uniform paste was formed and subjected to pH measurement (Instrutherm, model PH-1700), adequately calibrated, according to the methodology described by Velmourogane (2013). All analyzes were performed in triplicate. The image of the ground coffee mass for pH evaluation is presented in the Appendix (A4).

3.2.8. Influence of temperature and fermentation time variables for carbohydrates, residual acids and sensory evaluation

A Central Composite Rotatable Design (CCRD) was proposed to analyze the coffee fermentation process and to find the best process conditions in the studied ranges. The variables studied were temperature (x_1) and fermentation time (x_2) in the process.

For design were used temperature amplitude of Cerrado Mineiro Region and measured on some works (Evangelista *et al*, 2014 a), round up, were defined lower temperature as 15°C and bigger one as 30°C. For fermentation time were defined based on at least 12 hours to 48 hours, as mainly used (Pereira *et al*., 2015, Avallone *et al* 2001).

This experimental design totaling 11 experiments with three central replicates and α equal to 1.4142. The temperature variable (°C) was studied in the range of 11.89 (-α) to 33.10 (α) and the variable fermentation time (hours) was studied in the range of 4.42 (-α) to 55.50 (α). In response to this investigation, the dependent variable was the carbohydrates (sucrose, glucose and fructose), organic acids (acetic, propionic, succinic, and lactic), glycerol concentration and sensory evaluation. Multiple regression was performed to assess the influence of the independent variables studied ($x_1$and x_2) on the dependent variables, and the parameters that showed a significance level greater than 10% were disregarded, that is, in the hypothesis test with *Student's t*-table was considered the maximum error probability of 10%. In the regression analysis of the coffee fermentation results, the independent variables were transformed into the dimensionless form according to Equation (3.2) for temperature (*T*) and Equation (3.3) for fermentation time (*t*). The levels assessed for the CCRD performed with three replicates at the central point is presented in the Appendix (A5).

$$x_1 = \frac{T(°C) - 22.5}{7.5} \tag{3.2}$$

$$x_2 = \frac{t(h) - 30}{18} \tag{3.3}$$

Fermentation was stopped, on fast drying process, just after fermentation time defined on design.

3.2.8.1. Determination of sugars, glycerol and organic acids

For the analyses of sugars and organic acids, the fermented coffee mass (entire fruit: exocarp, mesocarp, endocarp and bean) was placed in aluminum trays and taken to the sun radiation for natural drying until it was dry enough to be ground in an analytical mill (about 12 hours of sun drying, to facilitate mass processing and posterior extraction, with day temperature of 23,2°C. The image of the crushed and dried dough for later grinding is shown in Appendix (A3). The mill used was the Willye knife (STAR FT-50, Fortinox), and the sample was ground, using a 20 mesh sieve to separate the material used for extraction. The crushed samples of the fermented dough were weighed (6 g) and placed in a 125 mL Erlenmeyer with 40 mL of ultrapure water removed from the purifier (Gehaka, Model Master System All), maintaining the same proportion described by Ribeiro *et al*. (2017a). This mixture was taken to a magnetic stirrer for 10 minutes at room temperature to extract the samples. The extract was decanted and centrifuged (10,000 rpm, 4°C for 10 min) in the centrifuge (Heal Force, Model Neofuge 15R) according to the methodology used by Ribeiro *et al*. (2017). The supernatant was filtered through a 0.22 μm cellulose acetate filter for reading in the High Precision Liquid Chromatograph (HPLC) after centrifuging.

The sugars and glycerol concentrations were determined by HPLC (Shimadzu LC-20A) equipped with a refractive index detector, a Hi-plex Ca column (7.0 × 300 mm, Agilent, CA, USA), operated at 85°C and ultra-pure water as the mobile phase at a flow rate of 0.6 mL min^{-1}. The organic acids concentrations were determined by HPLC (Shimadzu LC-20A) equipped with a diode array detector, a Shim-pack VP-ODS C8 phenyl column (150 × 4.6 mm), operated at 30 °C and a flow rate of 0.6 mL min^{-1}. The mobile phase A was a 0.01 M potassium dihydrogen phosphate solution (pH 2.50 with H3PO4), and B was acetonitrile. The elution was programmed as the following: 0.00–3.00 min, 0% B; 3.00–5.00 min, 0–15% B; 5.00–8.00 min, 15 - 0% B; 8.00–10.10 min, 0% B; 10.10–15.00 min, 0-15% B.

3.2.8.2. Sensory analysis

For cupping (sensory analysis of coffee), samples were classified according to Cupping Specialty Coffee protocols from Specialty Coffee Association (SCAA, 2015). Two Q-Graders (Specialty Coffee Professional Grader) (SCAA, 2015) panels were formed for sensory analysis, having total eight certified judges. Samples were judged in sensory attributes from Coffee protocols and evaluated different nuances according to Wheel of Flavors (SCA & WCR, 2016).

Each sample was roasted in two different roast levels: light-medium roast (#65) and medium roast (#55) and tasted at each different roast level for every experiment. The coffee tasting was performed 'at dark'; this is, each sample had a code, and none of the tasters knew what process each code represented. Pictures from different roast levels were shown on Appendix (A8). The roast was performed on a Specialty Coffee Roaster for samples (LABORATTO, Carmomaq, São Paulo, Brasil), before 24 hours of coffee cupping. For sensory analysis, samples were ground in an analytical grinder and performed sensory analysis just as required by the Specialty Coffee Association protocol (SCA, 2020a). The picture from the coffee roasting process is shown in the appendix (A9).

One of the panels was done in Patos de Minas town – at Farroupilha Group's Coffee Laboratory, a place kindly given by the group's Board. This one had the participation of 4 trained judges. The second panel was done in Carmo do Paranaíba town – at Veloso Coffee's Coffee laboratory, a place kindly given by the group's Board, with a participation of 5 trained judges. SCA Sensory Panels assembled on each location are shown in Appendix (A10).

3.3. Results and discussion

3.3.1. Moisture and pH

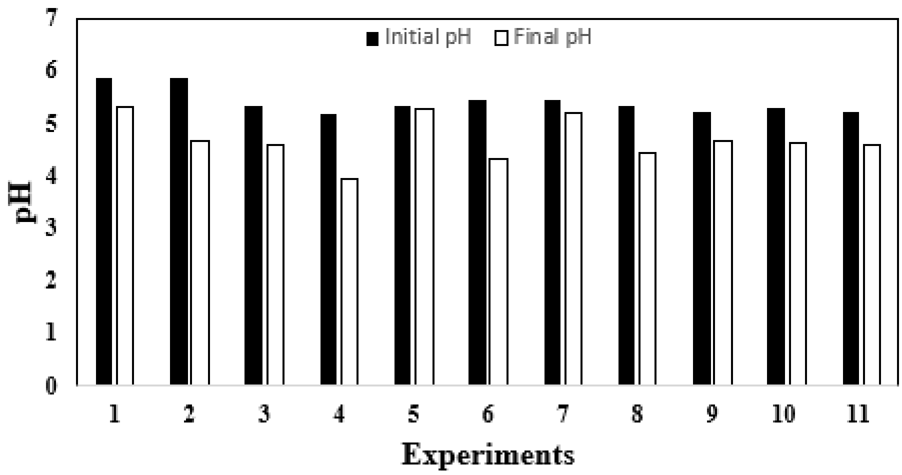
The moisture of the fermentative mass did not vary during the process. The samples' averaged initial moisture was 70.62 ± 1.03, and moisture at the end of the process was 68.67 ± 1.39. Moisture was measured in wet basis.

The fermentative mass's pH had its initial values with an average of 5.4; only two experiments (1 and 2) started with a pH of 5.88. The values were similar to those found by Elhalis *et al*. (2020a) in its spontaneous fermentation (without inoculation), around 5.5. Other studies have shown that the initial pH of coffee beans ranges from 5.0 to 7.0 (KWAK *et al*., 2018; HAILE *et al*., 2019b).

With the advancement of the fermentative process, microorganisms' metabolism uses the sugars available to form soluble organic acids as metabolites (AVALLONE *et* *al*., 2001, KWAK, *et al*., 2019). It thereby promotes the reduction of pH, a fact also observed by Haile *et al*. (2019b). In this work, it was evidenced that the pH reduction varied, with the test temperature, fact also verified by Avallone *et al*. (2001). They found a relationship between the decrease in pH and temperature, describing a lower reduction in pH at night due to low temperature. Figure 3.2 shows a graph comparing the initial pH with the final pH of the fermentation process.

As we can see on Figure 3.2, tests 5 and 6 (same fermentation time), there is possible to see temperature effect on pH, greater temperature provides pH decrease. Beyond this, as shown tests 8 and 9, (same temperature), bigger times, also provides a pH decrease, which show us a relationship of pH with both time and temperature. pH variation can be show at central point without variation.

Figure 3.2- Initial and final pH of the fermentation process.



3.3.2. Influence of temperature and fermentation time variables for carbohydrates, residual acids, and sensory evaluation

The results of the CCRP are shown in Table 3.1. The responses evaluated were sensory analysis of fermented coffee with light-medium roast and medium roast. Other responses evaluated were the concentrations of sugars (sucrose, glucose, and fructose), glycerol, and final organic acids that interfere in the grade of the sensory evaluation of fermented coffee.

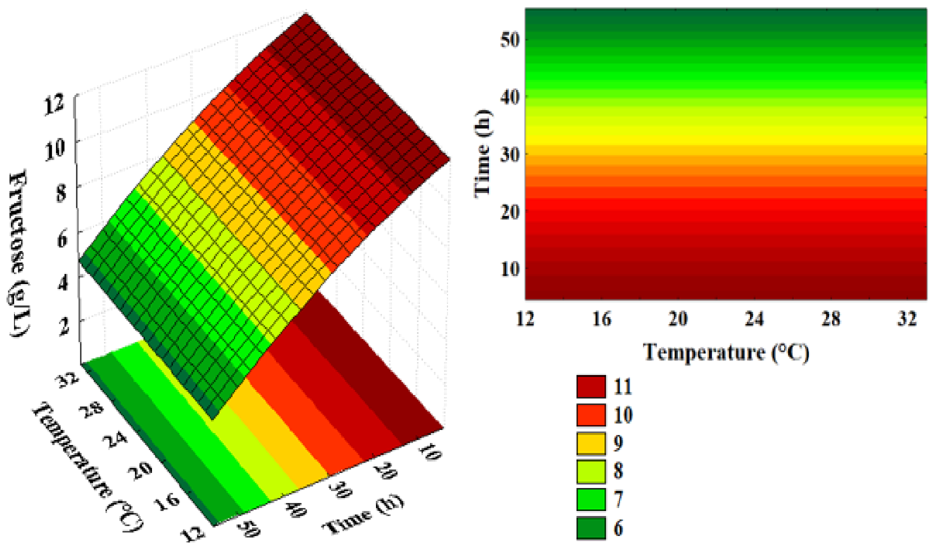
Table 3.1 – Experimental planning with the evaluated responses (sugars, glycerol, organic acids and final grade of sensory analysis) concerning the variables studied.

	Real Value (Coded Value)		**Carbohydrates**			**Alcohols**	**Organic Acids**				**Final Grade**	
	Temperature (x_1) (°C)	Time Fermentation (x_2) (h)	Sucrose (g/L)	Glucose (g/L)	Fructose (g/L)	Glycerol (g/L)	Acetic (mg/g)	Propionic (mg/g)	Succinic (mg/g)	Lactic (mg/g)	Light Medium Roast (SCA)	Medium Roast (SCA)
1	15.00 (-1)	12.00 (-1)	4.5382	6.6934	10.5473	0.1634	2.0000	3.2733	27,667	0.0000	81.30	81.19
2	15.00 (-1)	48.00 (1)	3.0540	7.0421	6.0881	0.5641	9.3333	4.2200	18.000	0.0000	83.55	83.11
3	30.00 (1)	12.00 (-1)	2.6863	7.2341	11.1752	0.3914	13.333	3.3600	0.0000	0.0000	83.53	83.88
4	30.00 (1)	48.00 (1)	0.5159	1.8150	5.4760	0.7682	0.0000	4.6133	0.0000	0.0000	82.06	81.00
5	11.89 (-α)	30.00 (0)	4.0506	8.0709	8.8953	0.1800	48.000	0.0000	4.2133	5.8867	83.54	82.01
6	33.10 (α)	30.00 (0)	1.7347	4.6283	9.5563	0.9095	42.000	0.0000	0.0000	7.5933	84.42	82.98
7	22.50 (0)	4.42 (-α)	4.4759	7.1148	12.2048	0.0000	73.333	0.0000	0.0000	9.4000	82.25	83.44
8	22.50 (0)	55.50 (α)	0.6205	3.0667	5.1005	0.7244	28.000	0.0000	0.0000	3.0667	82.56	82.54
9	22.50 (0)	30.00 (0)	3.8526	5.0698	8.9730	0.7844	22.666	0.0000	0.0000	8.4000	84.47	82.31
10	22.50 (0)	30.00 (0)	3.1957	6.1205	8.7715	0.7071	28.000	0.0000	0.0000	9.1333	84.32	82.41
11	22.50 (0)	30.00 (0)	3.8671	6.7429	9.3328	0.7846	25.333	0.0000	0.0000	8.1333	84.39	82.27

3.3.2.1. Sugars and glycerol

Initial sugars content was very important to determinate consumption and generation of mono and disaccharides. Fructose is sugar with greater initial concentration, showed mean 10,92 g/L (σ=1,29), followed by Glucose mean content 8,09 g/L (σ=0,97) and sucrose with mean content of 4,90 g/L (σ=1,13). In the fermentative process of coffee, a reduction in sugars occurs due to microorganisms' metabolic activity, forming metabolites, such as acids, alcohols, among others (DE BRUYN *et al*., 2016; MARTINEZ *et al*., 2017; ELHALIS *et al*., 2020a). At the end of the fermentation process, the main sugars observed in this work were fructose, glucose, and sucrose, and sucrose was found in lower concentrations. Fructose showed higher initial values, followed by glucose due to sucrose's hydrolysis by the yeasts present, mainly *Saccharomyces cerevisiae* (which the coffee was inoculated), transforming glucose and fructose into the monosaccharides. Yeast consumes glucose first and then fructose due to its metabolism. Figure 3.4 shows the response and contour surface, of the reduced model, of the fructose concentration at the end of the fermentation process concerning the studied variables.

Figure 3.3- Reduced model surfaces for fructose concentration at the end of the fermentation process.



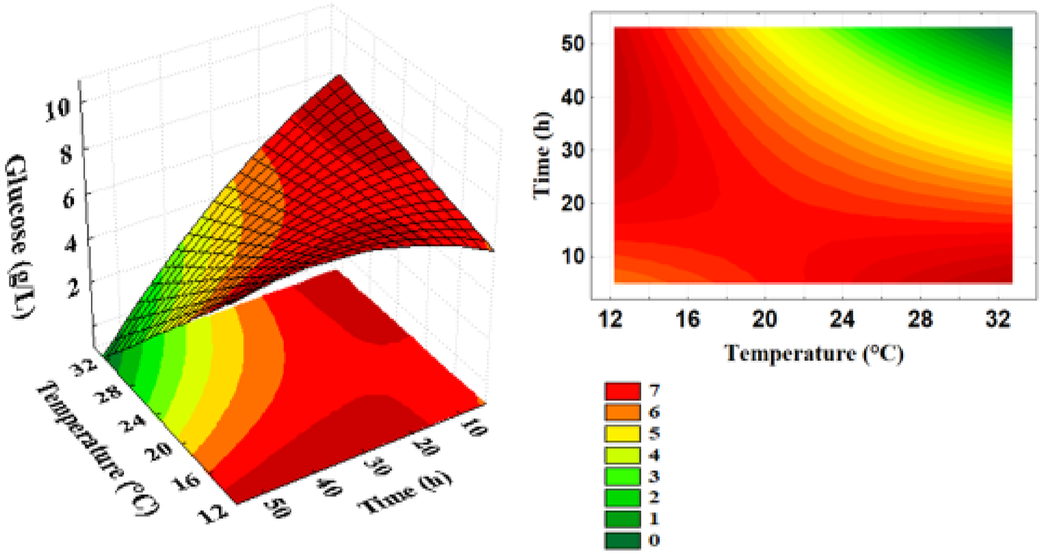
Using the experimental results for fructose concentration ($Y_{Fructose}$), after multiple regression, Equation 3.4 was obtained with the significant parameters ($p \leq 0.10$): x_2 (linear) and x_2^2 (quadratic). The coefficient of determination of this proposed model was

$R^2 = 0.9795$, which means that 97.95% of the experimental data variability was explained by the proposed empirical model equation. An analysis of the regression residues was related to the fructose concentration and it can be noted that these values were randomly around zero and did not show a trend regarding distribution.

$$Y_{Fructose} = 8.97 - 2.53x_2 - 0.33x_2^2 \quad (3.4)$$

Observing Equation 3.4 is possible to know that temperature did not influence the fructose concentration results at the end of the process. As for the fermentation time, it was observed that a reduction in the concentration of fructose is evident in longer times. On the other hand, the glucose concentration-response showed a different profile than fructose concerning the variables studied. As shown in Figure 3.4, the response surface was shaped like a saddle. It is possible to observe that the highest glucose concentration values are in the range of high temperatures and shorter fermentation times and, also, in longer fermentation times with lower temperatures. Figure 3.4 shows the response and contour surface, of the reduced model, of the glucose concentration at the end of the fermentation process concerning the variables studied.

Figure 3.4- Reduced model surfaces for glucose concentration at the end of the fermentation process

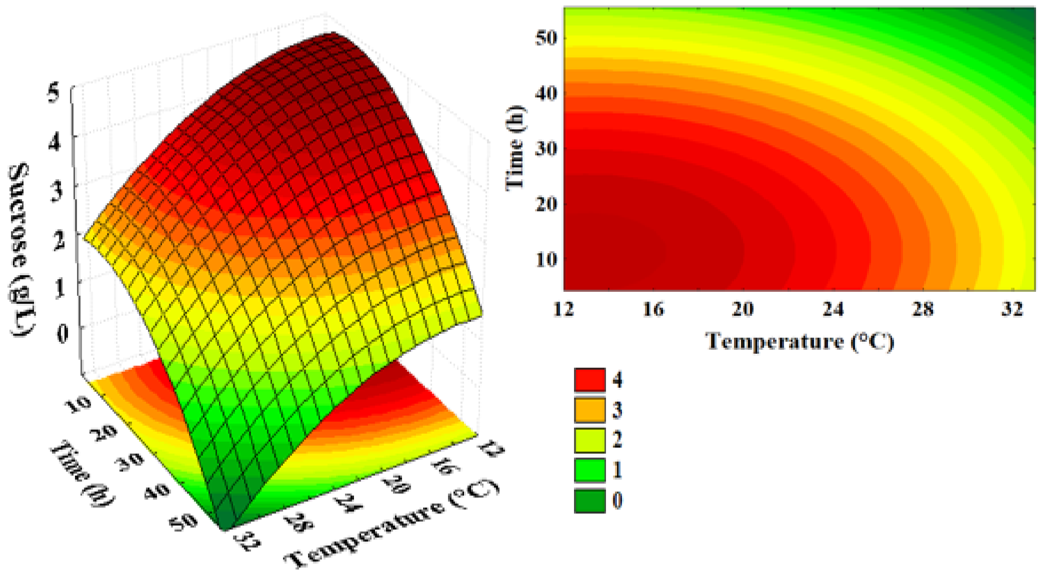


After multiple regression of the fructose concentration experimental results ($Y_{Glucose}$), Equation 3.5 was obtained, with the parameters of the factors that showed a significant effect: x_1, x_2 (linear), x_1x_2 (interaction) and x_2^2 (quadratic). The coefficient of determination of the proposed model was $R^2 = 0.9563$.

$$Y_{Glucose} = 6.14 - 1.20x_1 - 1.35x_2 - 1.44x_1x_2 - 0.50x_2^2 \quad (3.5)$$

As shown in Figure 3.5, the sucrose concentration showed higher values in fermentation time and lower temperatures. Figure 3.5 shows the response and contour surface, of the reduced model, of the sucrose concentration at the end of the fermentation process with the studied variables.

Figure 3.5- Surfaces of the reduced model for sucrose concentration at the end of the fermentation process.



Using the results for sucrose concentration ($Y_{Sucrose}$), after multiple regression, Equation 3.6 was obtained with the significant effects: x_1 and x_2 (linear), x_1^2 and x_2^2 (quadratic). The coefficient of determination of the proposed model was $R^2 = 0.9558$, indicating an appropriate adjustment, which means that the proposed empirical equation explained 95.87% of the experimental data variability. An analysis of the regression residues was related to the sucrose concentration and it can be noted that these values were randomly around zero and did not show a trend regarding distribution.

$$Y_{Sucrose} = 3.64 - 0.96x_1 - 1.14x_2 - 0.38x_1^2 - 0.55x_2^2 \quad (3.6)$$

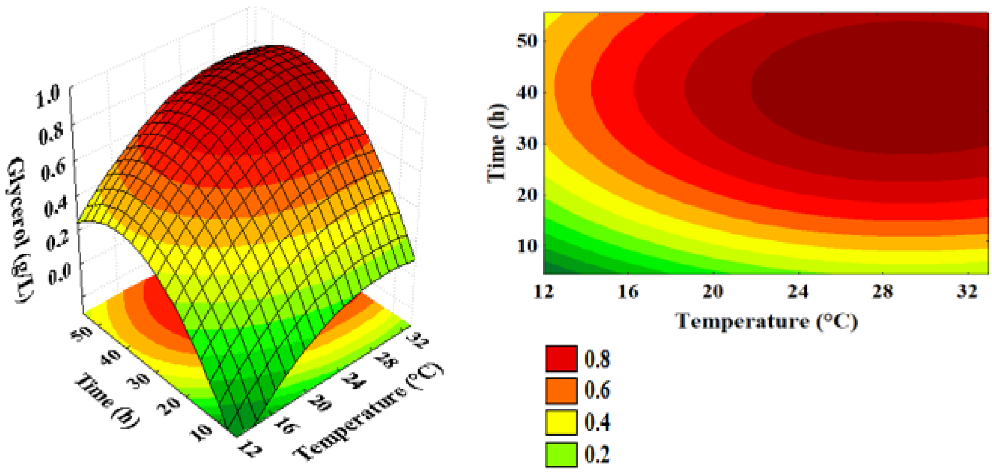
The sugar concentration results showed that the lowest values found were for the experiments with higher fermentation times and temperatures. Elhalis *et al*. (2020b) reported in their works that sucrose and monosaccharides fructose and glucose were practically degraded at the end of fermentation, which was conducted by 24 h at room temperature (between 10 and 30°C), this reduction of sugars content also was observed in other studies, as in De Bruyn *et al*. (2017) and Matinez *et al*. (2017).

In some part of the tests conducted on this work was noticed that sugar's final concentration was still high, evidencing that fermentative mass' sugars were not entirely degraded. Ribeiro *et al*. (2017)a noticed an increase of glucose and fructose at the end of the fermentative process, what may be explained by enzymatic or hydrolytic reactions from fermentative process, that degrade pectinolytic polysaccharides, through polygalacturonase and pectinase enzymes activities, mainly produced by yeasts fermentative process (MASSOUD AND JESPERSEN, 2006). This process which has a great positive impact on coffee quality (LEE *et al*., 2015).

According to Avallone *et al*. (2001), the most easily metabolized sugars are prioritized by microorganisms before the hydrolysis of polysaccharides. This means that the coffee fermentation process is dynamic in the consumption kinetics and sugar generation. De Bruyn *et al*. (2017) found in their study that despite the concentration of sucrose and monosaccharides have decreased, glucose and fructose showed peaks during the fermentation process, also corroborating the hypothesis that these sugars are both substrate and metabolites of biochemical reactions that occurred in the process fermentation. Other authors who also evidenced, in their work, an increase in monosaccharides' levels were Ribeiro *et al*. (2017a). The authors were justifying the same due to the breakdown of polysaccharides. This fact was also discussed by Haile *et al*. (2019a), in which they described that microbial activity and fermentation time determine the final concentration of sugars such as glucose and fructose.

Glycerol concentration present at the end of the process is another interesting response in the study of coffee fermentation. Glycerol is an important metabolite for the formation of quality since it has a sweet taste and a smooth mouth sensation (SWIEGERS *et al*., 2005). In experimental design, the response surface showed an optimum tendency for glycerol concentration in the fermentation time interval between 30 and 50 hours at temperatures above 24°C. Figure 3.6 shows the response and contour surface, of the reduced model, of the glycerol concentration at the end of the fermentation process, about the studied variables.

Figure 3.6- Surfaces of the reduced model for sucrose concentration at the end of the fermentation process



After multiple regression of the glycerol concentration experimental results ($Y_{Glycerol}$), Equation 3.7 was obtained, with the significant effects: x_1 and x_2 (linear), x_1^2 and x_2^2 (quadratic). The coefficient of determination of the proposed model was $R^2 = 0.9615$, indicating an appropriate adjustment, which means that the proposed empirical equation explained 96.15% of the experimental data variability. An analysis of the regression residues was related to the glycerol concentration and it can be noted that these values were randomly around zero and did not show a trend regarding distribution.

$$Y_{Glycerol} = 0.75 - 0.18x_1 + 0.23x_2 - 0.10x_1^2 - 0.19x_2^2 \qquad (3.7)$$

In experiment 7 (see Table 3.1), the glycerol concentration was zero. In this experiment, the fermentation time was the shortest, just 4.42 hours. This fact can be explained because glycerol is a metabolite of sugar degradation by yeasts. It is also not found in mechanically husked grains without fermentation (ELHALIS *et al*., 2020a; ELHALIS *et al*., 2020b). Elhalis *et al*. (2020a) showed the presence of glycerol after 24 hours of fermentation, what may be explained by their process, which removes exocarp and mesocarp partially, reduction polysaccharides content.

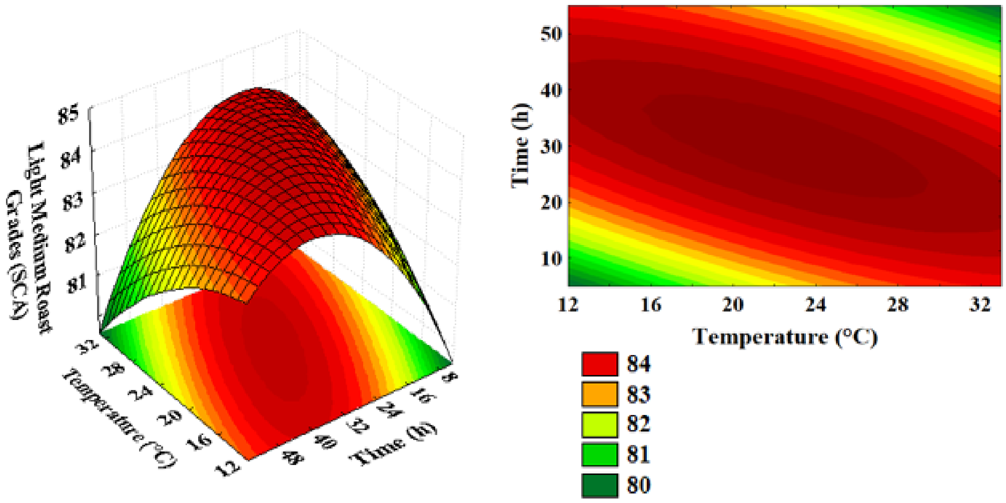
The highest concentrations of glycerol occurred at the points considered central for both variables (22.5 °C and 30 h) and, also, it was observed in experiment 6, where the highest temperature was used in all experimental design (33.1 °C) and at the central point of the fermentation time (30 h). Similar results were observed in work by De Bruyn *et al*. (2017), who found spikes in glycerol concentration during the dry process fermentation and not at the end of it.

3.3.2.2. Sensory analysis

In the sensory analysis, the raw grain's physical evaluation was carried out, as recommended by the Ministério da Agricultura Pecuária e Abastecimento (MAPA) (BRAZIL, 2003). The physical evaluation consisted of the appearance, color, and percentage of burnt grains, representing signs of undesirable fermentation processes. The coffees fermented for 48 hours showed yellow (experiment 4) and yellowish (experiment 2). The other experiments had a green color, characteristic of normal, natural coffees, as defined in the MAPA Normative Instruction, nº 8 of 2003. Based on the same instruction, the same samples' appearance was defined as regular, and the others with a determined aspect were good. In the characteristic of physical defects, the burnt grains, those grains that present brown color in different tones due to fermentative processes, were only identified in experiment 4, at a level of 3% of the total mass. An interesting view is experiment 6, with higher temperatures, but lower fermentation time, what shows important relation time and temperature for degradation of quality.

The second part was the sensory evaluation of the coffees, carried out with the roasted beans in two different roasting levels and tasted according to the SCA classification. The results of the sensory analysis of the fermented coffees, as shown in Table 3.1, showed little variation in their grades, between 81.33 to 84.47 for coffees tasted in the light-medium roast (Agtron # 65 / SCA). For medium roast coffees (Agtron # 55 / SCA), the grades ranged from 81.00 to 83.88. The sensory analysis results of the different roasting types show that regardless of the roasting level used, there is a significant dependence on the coffees' notes' values with the two variables, temperature (x_1) and fermentation time (x_2). Figure 3.7 shows the reduced model's response and contour surface for scoring the average clear roasting according to the SCA concerning the studied variables.

Figure 3.7. Reduced model surface for SCA sensory assessment for light medium roasting (#65, SCA)



Equation 3.8 was obtained using the sensory results in the light-medium roast (YLMR), after multiple regression with significant effects. The coefficient of determination of the proposed model was $R^2 = 0.9397$, indicating a good fit, which means that the proposed empirical adjustment, equation applied 93.97% of the experimental data variability. From the regression residues analysis related to the light-medium roast it can be noted that the residues were randomly around zero and did not show a trend regarding distribution.

$$Y_{LMR} = 84.39 - 0.93x_1x_2 - 0.35x_1^2 - 1.14x_2^2 \quad (3.8)$$

The central levels of both variables showed the best sensory results. The fermentation time ranged from 20 to 40h, and temperatures ranging from 16ºC to 28 °C for the sensory note's optimal range. The response surface shows that shorter fermentation times are required to better sensory evaluation when fermentation occurs at higher temperatures. When the fermentation temperature is lower, it must use a longer fermentation time to reach the sensory evaluation's desired value. The best results were obtained in experiments 9, 10 and 11, from the central point (22.50 °C and 30 h) and experiment 6 (33.10 °C and 30 h), with sensory scores greater than 84 points. A control experiment called a "witness" was used to compare the sensory evaluation grade with experimental design results. In this experiment, from the control, the inoculation with the yeast *Saccharomyces cerevisiae* was not performed. The fermentation control was not performed, and the light-medium roast was performed because it is a commercial standard. The control (without fermentation) had a sensory score of 82.16, according to SCA. This result indicates that the experiments carried out in this work showed sensory results superior to those in the witness. Authors such as Bressani *et al*. (2018), Ribeiro *et al*. (2017), Martinez *et al*. (2017) obtained better results with coffees inoculated with *S. cerevisiae* when compared with the controls.

Similar results were observed by several authors who carried out experiments with the inoculation of microorganisms. Bressani *et al*. (2019), found a maximum score of 84 points, with inoculation of the *S. cerevisiae* strain CMA0543, in the dry process. Pereira *et al*. (2015), Carvalho Neto (2018), Elhalis *et al*. (2020) used 24h, 24h (12 aerobic and 12 anaerobic), and 26 hours of fermentation, respectively, with only Carvalho Neto *et al*. (2018) controlled the temperature at 30 °C, with the other two jobs at room temperature, obtaining exceptional coffees in wet processes. These authors reported similarities in the fermentative processes, where they used inoculation and managed to maintain or improve the coffee quality.

According to Illy and Viani (2005), in the roasting process, the level of soluble carbohydrates, proteins, and chlorogenic acids are degraded in melanoidins through the reactions of Maillard and the sugar caramelization. Moreover, there is the release of other volatile compounds. The best relationship found for aroma precursors in coffee is generally found during the medium-light roasting, a statement that was also evidenced in this work. During the evaluation of the drink potentials after fermentation, the coffee the medium clear roast presented better sensory results than in medium roasting. Table 3.2 presents some works developed with the inoculation of microorganisms in the fermentation of coffee.

Table 3.2 – Inoculation in different cultivars and fermentation conditions

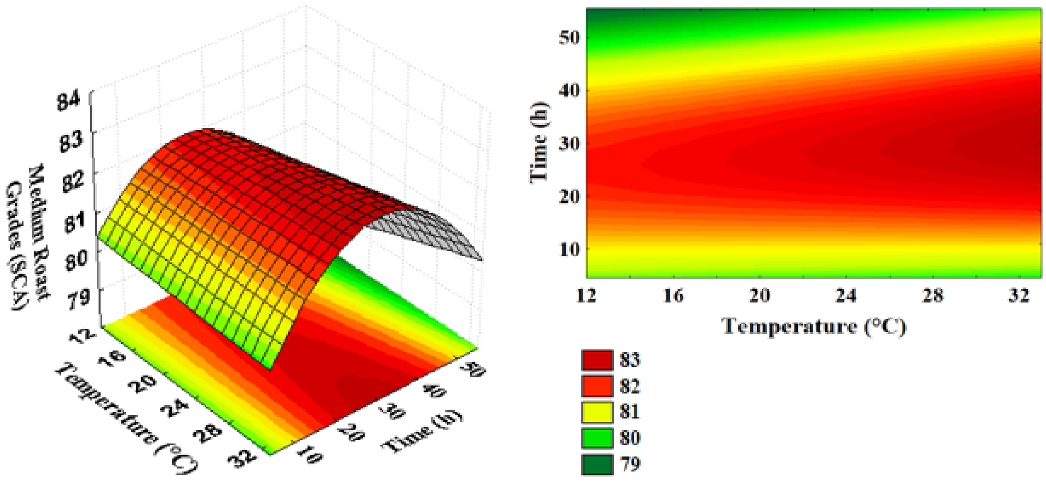
Author	**Cultivar**	**Temperature**	**Fermentation Time**	**Method**	**Inoculation**	**Final Grade**
Elhalis *et al*., 2020a	Bourbon	Air temperature (25-30 °C - day e 10-15 °C night)	36 h	Wet process	Spontaneous	89.50
					Natamycin (anti-Yeast)	84.75
					T. delbrueckii 084	85.50
					Spontaneous	91.50
Bressani *et al*., 2018	Yellow Catuai	Air temperature	16 h	Dry process	*S. Cerevisiae 0543*	84.00
					C. parapsilosis 0544	81.50
	Catuí	30 °C			Spontaneous	80.67

Carvalho Neto *et al*., 2018			12h aerobic and 12h anaerobic	Wet process	*Lactobacilus Plantarum*	80.00
Ribeiro *et al*., 2017	Mundo Novo	Air Temperature	Over drying process 284h	Semi-dry process	*S. Cerevisiae 0200*	80.13
					S. Cerevisiae 0543	82.63
					Spontaneous	-
	Yellow Ouro				*S. Cerevisiae 0200*	83.25
					S. Cerevisiae 0543	82.88
					Spontaneous	81.38
Martinez *et al*., 2017	Yellow Catuaí	Air temperature 14.6 °C – 28.2 °C	352 h	Semi-dry process	*S. Cerevisiae 0543*	81.40
					C. parapsilosis 0544	81.30
					T. delbrueckii 084	81.00
					Spontaneous	81.40
Pereira *et al*., 2015	Catuí	Air temperature (24-32 °C - day and 12-15 °C night)	24 h	Wet process	*P. fermentaris YC.2*	89.00
					P. fermentaris YC.2 Sup.	87.50
					Spontaneous	89.00
Evangelista *et al*., 2014b	Acaiá	Air Temperature	Over Drying process	Semi-dry process	Controle	80.93
					S. cerevisiae YCN 724	79.33
					P. guillermondii YCN 731	74.17
					C. parapsilosis YCN448	80.00
					*S. cerevisiae *YCN 727*	81.08

*YCN 727 = CCMA 0543

Figure 3.8 shows the reduced model's response and contour surface for the average roasting score about the variables studied.

Figure 3.8- Reduced model surface for SCA sensory assessment for medium roasting (#55, SCA)



Using the results for a medium roast (Y_{MR}), after multiple regression, Equation 3.9 was obtained with the significant parameters: $x_1$and x_2 (linear), x_1x_2 (interaction between both factors) and x_2^2 (quadratic). The coefficient of determination of the proposed adjustment was $R^2 = 0.9341$, indicating an appropriate adjustment, which means that the proposed empirical adjustment equation explained 93.41% of the experimental data variability.

$$Y_{MR} = 82.30 + 0.24x_1 - 0.28x_2 + 0.23Xx_1x_2 - 1.20x_2^2 \qquad (3.9)$$

The best sensory results observed were in the fermentation time range between 25 to 35 hours and temperatures above 28°C. The response surface shows that shorter fermentation times can be used to achieve a good sensory evaluation when the fermentation process occurs at higher temperatures. The best result was obtained in experiment 3 (30°C and 12 h) with sensory notes close to 84 points (Table 3.1). The comparison with the control was not performed because the medium roasting is not considered commercial and was only used to analyze the Q-Graders. Based on the results of the two types of toast, it can be seen that the medium-light roast showed a better sensory result when purchased the medium roast in conditions of lower temperature and longer fermentation time.

Lee *et al*. (2016)b used different roasting levels to evaluate the quality of coffee, fermented greens, dark and light roasting in notes of 0-5 for various attributes (sweet, fruity, buttery, caramel, chestnuts, roasted, smoky, spicy, sulfurous). The sensory analysis results showed a higher quality for roasted coffee in dark roasting than in light roasting, different from what was evidenced in this work.

3.3.2.3. Organic acids

As we can observe in Table 3.1, acetic and lactic acid were the main acids found on HPLC, analysis just as found by other authors in fermented coffee with *S. cerevisiae* (BRESSANI *et al*., 2018; PEREIRA *et al*., 2015; CARVALHO NETO *et al*., 2018; EVANGELISTA *et al*., 2014b and EVANGELISTA *et al*., 2015). In lowers concentrations and with less representativity, there was found succinic (except for tests 1 and 2, which succinic is main acid) and propionic acids. None of those acids were on all of the experiments, and none of them presented a correlation with fixed parameters of CCRP (Time and Temperature).

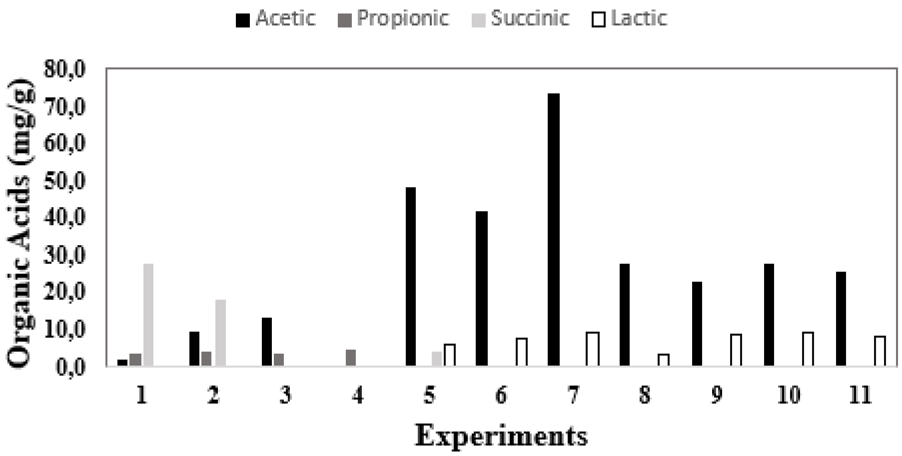
Acetic acid shows concentrations from 0 to 73.33 mg/g, which lower concentration was in E4, which was 30 °C for 48hours and higher concentration on the shorter fermentation time (4,42h), finding the most variated values between 0 and 73.33 mg/g with at least 7 tests with concentrations above 20 mg/g. Otherwise, other authors have not found those great acetic acid concentration at the end of the process, Bressani *et al*. (2018) and Evangelista *et al*. (2014a) found near 10 mg/g of acetic acid, Wang *et al*. (2018) found 2.21 mg/g, the similar value found by Martinez *et al*. (2017).

Succinic acid also showed adverse concentrations among different experiments and did not correlate with time or temperature. Succinic acid greater concentration was found at experiment 1 (27.67mg/g) and this acid was not found in 8 of 11 tests conducted. This acid was found in every other experiment, also in lower concentrations (BRESSANI *et al*., 2018; EVANGELISTA *et al*., 2014b; MARTINEZ *et al*., 2017).

Lactic acid was the second acid with concentration at the end of the process, with absence only on Experiment 1 to 4. Concentrations varying from 3.1 mg/g (longer time) to 9.4 mg/g (shorter time), instead of were found by Carvalho Neto (*et al*. 2018), which lactic acid increased with the advance of fermentation. Other authors found the lactic acid concentration (WANG *et al*., 2018; MARTINEZ *et al*., 2017 and EVANGELISTA *et al*. 2015: near 2 mg/g).

Propionic acid was found only on the four first experiments (1 to 4), with concentrations varying from 3.2 to 4.6 mg/g. It may be responsible for the taste and smell of onions (PIMENTA, 2003). Evangelista *et al*. (2014a) found it concentrations near 2 mg/g and lower values by Martinez *et al*. (2017), and it was not found in many other works (EVANGELISTA *et al*., 2015; RIBEIRO *et al*., 2017a; PEREIRA *et al*., 2015; ELHALIS *et al*., 2020a).

Figure 3.9- Final organic acids concentrations for each experiment



3.4. Conclusion

A small variation in the coffees' sensory quality represents great possibilities for producing a higher quality coffee and can add value to the final product. The use of initiating microorganisms in fermentation can lead to the maintenance of coffee characteristics, showing that these microorganisms can inhibit undesirable metabolites' production. The yeast *Saccharomyces cerevisiae* (Lallemand ORO®) was well suited to the cultivar Catuaí Amarelo, IAC 62, in which it presented good sensory results.

The study of environmental conditions and fermentation time for coffee standardization is extremely important to obtain a quality product. From the point of view of applying the results obtained from the fermentation with the light-medium roast on coffee properties, we realized that the process could be easily employed, as they are consistent with the producing region's temperatures. This fact is already becoming more complex for coffee evaluated in medium roast since the best sensory results do not match the region's natural temperatures, requiring investments that can make the process unfeasible.

On the other hand, these data can be used for regions with different temperatures from the region to which the coffee is being produced. The concentrations of sugars, glycerol, and acids in fermented coffee showed values close to that of the literature and the decrease in pH, which can indicate the completion of fermentation.

Otherwise sugars and Sensory analysis, organic acids do not represented concentrations between literature values and showed no correlations with time/temperature parameters.

3.5. Acknowlegments

The authors would like to thank the managers of the Farroupilha Group - Patos de Minas/MG, who kindly ceded the coffee laboratory for conducting sensory analysis sessions for this study, and the managers of the Veloso Coffee laboratory - Carmo do Paranaíba/MG, who kindly gave up the space to conduct sensory analysis sessions. And Lallemand Brazil who gently supply on yeast. Study with University-Industry-Farms integration.

CAPÍTULO 4 - SENSORY PROFILE OF BEVERAGES PRODUCED FROM FERMENTED COFFEE UNDER DIFFERENT TIME AND TEMPERATURE CONDITIONS

This chapter deals with the elaboration of the sensory profile of Arabica coffee drinks, obtained from beans submitted to fermentation in solid state by inoculation of *Saccharomyces cerevesiae*, under different conditions of time and temperature. The sensory attributes of the drinks were evaluated according to the SCA for two types of roasting, Light Medium Roast (#65) and Medium Roast (#55).

4.1. Introduction

Coffee (*Coffea arábica L.*) is the second most consumed beverage in the world, after water, and its consumption occurs on the 5 continents. The United States is the largest consuming country, with 14% of world demand, followed by Brazil, with 13%. For the 2020 harvest, total consumption of about 167 million bags of coffee is projected. According to the International Coffee Organization (ICO), global production in 2020 is estimated at around 169 million bags, distributed between arabica coffee (56.7%) and robusta coffee (43.3%). Brazil leads to producing countries' ranking, responsible for more than 60 million bags produced, followed by Vietnam and Colombia.

Among all agricultural commodities, few are linked to their quality indicators as coffee, and their price is immensely dependent on their quality and sensory attributes. For some producers and cooperatives, such dependence on quality can be a major inconvenience. At the same time, it can also represent great opportunities since maintenance or increases in quality can represent direct increases in the price received for the grain (DONOVAN *et al.*, 2019). In this context, specialty coffees stand out, those with higher quality than the traditional and reach 80 points according to the Specialty Coffee Association's Sensory Evaluation Methodology (SCA). These coffees represent only 12% of the international market and 15% of the Brazilian market, however sales in Brazil should reach about 1 billion dollars this year. The product is generally priced at 30 to 40% more than the commercial standard (traditional coffee), in some cases exceeding the 100% barrier. In the 2018 Brazilian edition of the Cup of Excellence, the main coffee quality contest in the world, each bag of champion special coffee reached the value of US $ 18,916.00 (BSCA 2020).

The sensory quality of coffee is decisive for its classification as special and is directly linked to its physical characteristics and chemical composition, influenced by several factors such as genetic, environmental, nutritional and operational (PIMENTA, *et al*., 2019). Chemical composition is fundamental for the final development of coffee flavor and, according to Bressani *et al.* (2019), the complex flavors and aromas of coffee result from the combined presence of various chemical compounds, volatile and non-volatile, such as acids, aldehydes, ketones (CHIN *et al*., 2015), sugars, amino acids, fatty acids and phenolic compounds, in addition to the action of enzymes such as proteases and lipases, which contribute to the formation of secondary metabolites (LEE *et al.,* 2015).

One of the factors that strongly interferes in the sensory quality of the drink, especially in the aroma, is the fermentation of coffee (VELMOUROGANE, 2013). The fruits serve as the substrate for the development of bacteria, yeasts and filamentous fungi, supplying them with carbon and nitrogen sources, due to their chemical composition, and the microorganisms promote their fermentation, influencing the final quality of the coffee due to the degradation of some compounds or by the excretion of their metabolic products (ESQUIVEL *et al*., 2012). Lee *et al*. (2017) pointed out that fermented coffee showed floral and fruit notes due to alcohols and ketones formation from aldehydes increasing its quality. In this sense, the microbial composition of the fruit is especially important. However, according to their characteristics, it is also desirable to apply selected microorganisms so that there is a positive impact on the sensory quality of the coffee (HAILE and KANG, 2019). Such microorganisms, also known as starter cultures, can improve the quality of the coffee drink and reduce processing time (SILVA *et al*., 2013) and inhibit the growth of mycotoxin-producing fungi (VAUGHAN *et al*, 2015; SOUZA *et al.,* 2017).

The roasting of the beans at the end of the processing is also a critical step for the composition of the aroma and flavor of the coffee, since during the process reactions such as Maillard, caramelization, Strecker degradation and pyrolysis reactions occur, which together with fermentation, are responsible for forming the unique characteristics of the final product (AKILHOGLU & GÖKMEN, 2014). Many works have been published pointing out the relationship between the degree of roasting and the sensory quality of the drink, often using different parameters such as aroma, flavor, color, bean temperature, pH, and chemical composition (KU MADIHAH *et al*., 2012; TOCI *et al*., 2020; HU *et al*., 2020).

Given the importance of fermentation and the roasting process in the quality of the final product, the objective of this work was to evaluate the influence of different fermentation times and temperatures of Arabica coffee, inoculated with *Saccharomyces cerevesiae* and two different types of roasting in the sensory responses.

This study aimed to analyze the effects of fermentation of Arabica coffee, the Catuaí Amarelo strain, on the physical-chemical characteristics and the product's sensory result and the influence of each coffee quality attribute on coffee final quality

4.2. Materials and methods

4.2.1. Material

The cultivar used in conducting the experiments was Arabica coffee from the Catuaí Amarelo strain, IAC 62, harvested from a rural property located in the city of Carmo do Paranaíba, state of Minas Gerais, Brazil. The area from which the samples were taken is in the geographical coordinates Lat.: *18°57'37"S*/ Long.:*46°36'17"O*, with an altitude of 1,080 meters.

4.2.2. Methods

4.2.2.1. Coffee solid fermentation

The commercial yeast *Saccharomyces cerevisiae*, of the commercial brand Lallemand ORO® (10^{10} UFC/g), was kindly provided by Lallemand. For the incubation, the manufacturer's ratio was used, with 12 g (0,012 kg) of yeast for each 10 kg of coffee. The yeast was weighed on an analytical balance (Shimadzu, model BL-3200H) and dissolved in distilled water and applied to the coffee mass. in ratio of 10 g of commercial yeast for 500 mL distilled water. For cell count, there were used Neubauer chamber method, with previously prepared solution, diluted 1 ml for 100 ml, according to Equation 3.1, were C_1 and C_2 were quadrant mean counts: After Neubauer chamber count, were found about 2.55 x 10^8 cell/mL of solution.

Table 4.1 – Times and temperatures applied in the fermentation of coffee by the yeast *Saccharomyces cerevisiae*

Experiments	Temperature (°C)	Time fermentation (h)
1	15.00	12.00
2	15.00	48.00
3	30.00	12.00
4	30.00	48.00
5	11.89	30.00
6	33.10	30.00
7	22.50	4.42
8	22.50	55.50
9	22.50	30.00
10	22.50	30.00
11	22.50	30.00

4.2.2.2 Drying and processing coffee

After fermentation, the grains were submitted to intermittent drying at 37.5ºC in an oven (Ethik Technology, model 400 / 8D), with rest intervals of the grain mass, in cycles of 8 hours of drying and 8 hours of rest at room temperature, as described by Isquierdo et al. (2011). After drying, the samples were properly packed in high-resistance plastic bags, identified and placed in a BOD Incubator (Solab, model SL-225/364), at 25 °C, for 60 days. After this period, the coffees were benefited from the complete removal of the exocarp and endocarp in a mechanical processing machine (Pinhalense, Model DRC) and then subjected to the separation of defects (impurities and imperfect grains) and separation of only coarse grains (grains retained in the sieve 16/64”, without clubs, which is same of coffee sieve #16). The coffees obtained after these steps were submitted to sensory analysis.

4.2.2.3. Sensory analysis

The cup tests were carried out following the SCA Cupping Specialty Coffee protocols (SCAA, 2015). First, the samples from each experiment were subjected to two types of roasting, Light Medium Roast (#65) and Medium Roast (#55). This procedure was carried out in a roaster for special coffees (Carmomaq, model Laboratto), at an interval of 24 hours before tasting. Immediately before sensory analysis, samples were ground in an analytical mill (G3 model, BUNN, Springfield, Illinois, USA). The ground coffees, including control were then evaluated Sensorily by a panel of 8 Q-graders, who analysed them for attributes flavor, aroma, aftertaste, acidity, body, uniformity, balance, clean cup, sweetness and overall, scoring them between 6 and 10. The final score of each coffee is the sum of attributes scores. According to the SCA- WCR® (2016-2020) flavour wheel, the different nuances of the samples were also evaluated.

4.2.2.4 Statistical analysis

The comparison between the experiments for each roasting was performed by Friedman test. The two types of roasting in each experiment were also compared, for all attributes, by the Mann-Whitney test. To assess the influence of attributes on the samples a Principal Component Analysis (PCA) was performed. These data were analysed using R software, version 3.6.1 (R Core Team, 2015).

4.3 Results and discussion

Table 4.2 presents the evaluations obtained in each attribute for the 11 experiments submitted to Light Medium Roasting and Medium Roasting and compares these two processes for each test. An experiment not subjected to the fermentation process was carried out and taken as a control and received only Light Medium Roasting.

One of the most striking coffee attributes is its aroma, the result of dozens of compounds presents in its composition. Degradation of mucilage in fermentation can promote compounds, especially organic acids, esters, and ketones, contributing to the final aroma (ELHALIS, *et al., 2020*). The application of different fermentation times and temperatures did not promote a significant difference in aroma between coffee samples with light-medium roasting. The variations in these parameters were possibly not sufficient for significant amounts of new compounds to have been produced, to the point of promoting different sensory perceptions. The same occurred for samples with medium roasting. Peñuela-Martínez and co-authors (2018) evaluated the effect of different fermentation process changes, such as delayed pulping time, different fermentation times, and pH and temperature control. The results suggested that it is possible to modulate the drink's acidity and the fragrance/aroma by producing organic acids and alcohols, esters, and ketones in a synergistic combination that can promote different profiles to satisfy the requirements of specialty coffees. The aroma of coffee is established mainly in the roasting process, in which the Maillard reaction, which is responsible for the aroma, and thermally catalyzed reactions occur (LEE *et al.,* 2016a). In this study, there was no significant difference for the aroma between the light medium and medium roasting of each experiment. Likely, the same profile of aromatic compounds developed in the samples since the two towers are similar in the Agtron measurement system. The difference between them did not promote different compounds or quantities that would allow them to be sensorially differentiated. In recent years, several authors have devoted themselves to unraveling the compounds formed in coffee during roasting and their influence on sensory perception (HU *et al.*, 2020; COLZi *et al.,* 2017; BARIE *et al.*, 2015), and more than 1000 volatile organic compounds have been identified. However, the relationship between their presence in the grains and the sensory results is still not well understood.

Table 4.2 - Attribute means for Light Medium Roast and Medium Roast experiments (continues)

	Attributes											
Experiments	**Aroma**		**Uniformity**		**Clean cup**		**Sweetness**		**Flavor**		**Acidity**	
	LMR*	**MR***	**LMR***	**MR***	**LMR***	**MR***	**LMR***	**MR***	**LMR***	**MR***	**LMR***	**MR***
Control	7.56 ^{A}	-	10.00 ^{A}	-	10.00 ^{A}	-	10.00 ^{A}	-	7.64 $^{A, B}$	-	7.03 ^{B}	-
1	7.53 $^{A\ a}$	7.34 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	7.11 $^{A\ a}$	7.28 $^{A\ a}$	7.28 $^{B, C\ a}$	7.38 $^{A\ a}$
2	7.83 $^{A\ a}$	7.75 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	7.39 $^{A, B\ a}$	7.34 $^{A,B\ a}$	7.58 $^{A, B, C\ a}$	7.69 $^{A\ a}$
3	7.89 $^{A\ a}$	7.91 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	7.67 $^{A, B\ a}$	7.88 $^{B\ a}$	7.42 $^{A, B, C\ a}$	7.50 $^{A\ a}$
4	7.58 $^{A\ a}$	7.31 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	7.56 $^{A, B\ a}$	7.31 $^{A,B\ a}$	7.39 $^{A, B, C\ a}$	7.38 $^{A\ a}$
5	7.58 $^{A\ a}$	7.66 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	7.72 $^{A, B\ a}$	7.41 $^{A,B\ b}$	7.25 $^{B, C\ a}$	7.44 $^{A\ a}$
6	7.64 $^{A\ a}$	7.41 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	7.72 $^{A, B\ a}$	7.66 $^{A,B\ a}$	8.00 $^{A\ a}$	7.56 $^{A\ b}$
7	7.58 $^{A\ a}$	7.59 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	7.64 $^{A, B\ a}$	7.53 $^{A,B\ a}$	7.39 $^{A, B, C\ a}$	7.75 $^{A\ b}$
8	7.44 $^{A\ a}$	7.53 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	7.64 $^{A, B\ a}$	7.75 $^{A,B\ a}$	7.58 $^{A, B, C\ a}$	7.44 $^{A\ a}$
9	7.92 $^{A\ a}$	7.38 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	7.58 $^{A, B\ a}$	7.59 $^{A,B\ a}$	7.89 $^{A, C\ a}$	7.50 $^{A\ a}$
10	7.89 $^{A\ a}$	7.66 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	7.92 $^{B\ a}$	7.34 $^{A,B\ b}$	7.44 $^{A, B, C\ a}$	7.47 $^{A\ a}$
11	7.94 $^{A\ a}$	7.72 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	10.00 $^{A\ a}$	7.72 $^{A, B\ a}$	7.50 $^{A,B\ a}$	7.72 $^{A, C\ a}$	7.50 $^{A\ a}$

Table 4.2 - Attribute means for Light Medium Roast and Medium Roast experiments (continuation)

Experiments	**Attributes**									
	Body		**Aftertaste**		**Balance**		**Overall**		**Final Score**	
	LMR*	**MR***	**LMR***	**MR***	**LMR***	**MR***	**LMR***	**MR***	**LMR***	**MR***
Control	7.47 $^{A, C}$	-	7.44 $^{A, B, C}$	-	7.67 $^{A, B}$	-	7.36 $^{A, B}$	-	82.17 $^{A, B}$	-
1	7.39 $^{A, B\ a}$	7.09 $^{A\ a}$	7.50 $^{A, B, C\ a}$	7.38 $^{A\ a}$	7.28 $^{A\ a}$	7.47 $^{A\ a}$	7.25 $^{A, B\ a}$	7.25 $^{A\ a}$	81.33 $^{A\ a}$	81.19 $^{A\ a}$
2	7.64 $^{A, B\ a}$	7.56 $^{A\ a}$	7.78 $^{A, D\ a}$	7.56 $^{A\ b}$	7.69 $^{A, B\ a}$	7.63 $^{A\ a}$	7.64 $^{A, B\ a}$	7.59 $^{A,B\ a}$	83.56 $^{A, B\ a}$	83.13 $^{B\ a}$
3	7.61 $^{A, B\ a}$	7.59 $^{A\ a}$	7.58 $^{A, B, C\ a}$	7.53 $^{A\ a}$	7.72 $^{A, B\ a}$	7.47 $^{A\ a}$	7.47 $^{A, B\ a}$	7.75 $^{A,B\ a}$	83.53 $^{A, B\ a}$	83.88 $^{B\ a}$
4	7.36 $^{A, B\ a}$	7.34 $^{A\ a}$	7.11 $^{B, C\ a}$	7.34 $^{A\ a}$	7.53 $^{A, B\ a}$	7.16 $^{A\ b}$	7.53 $^{A, B\ a}$	7.16 $^{A,B\ a}$	82.06 $^{A, B\ a}$	81.00 $^{A,B\ a}$
5	7.56 $^{A, B\ a}$	7.28 $^{A\ a}$	7.31 $^{B, C, D\ a}$	7.34 $^{A\ a}$	7.47 $^{A, B\ a}$	7.38 $^{A\ a}$	7.06 $^{A\ a}$	7.50 $^{A,B\ b}$	81.81 $^{A\ a}$	82.00 $^{A,B\ a}$
6	7.75 $^{A, B\ a}$	7.50 $^{A\ a}$	7.75 $^{A\ a}$	7.53 $^{A\ a}$	7.94 $^{B\ a}$	7.53 $^{A\ b}$	7.61 $^{A, B\ a}$	7.78 $^{A,B\ a}$	84.42 $^{A, B\ a}$	82.97 $^{A,B\ a}$
7	7.31 $^{B\ a}$	7.59 $^{A\ a}$	7.69 $^{A, B, C\ a}$	7.41 $^{A\ a}$	7.58 $^{A, B\ a}$	7.72 $^{A\ a}$	7.39 $^{A, B\ a}$	7.84 $^{A,B\ b}$	82.25 $^{A, B\ a}$	83.44 $^{A,B\ a}$
8	7.58 $^{A, B\ a}$	7.59 $^{A\ a}$	7.39 $^{A, B, C\ a}$	7.47 $^{A\ a}$	7.39 $^{A, B\ a}$	7.34 $^{A\ a}$	7.53 $^{A, B\ a}$	7.41 $^{A,B\ a}$	82.56 $^{A, B\ a}$	82.53 $^{A,B\ a}$
9	7.78 $^{A, B\ a}$	7.56 $^{A\ a}$	7.86 $^{A\ a}$	7.47 $^{A\ a}$	7.75 $^{A, B\ a}$	7.47 $^{A\ a}$	7.69 $^{A, B\ a}$	7.41 $^{B\ a}$	84.47 $^{B\ a}$	82.31 $^{A,B\ b}$
10	7.83 $^{A\ a}$	7.59 $^{A\ a}$	7.61 $^{A, B, C\ a}$	7.38 $^{A\ a}$	7.89 $^{B\ a}$	7.38 $^{A\ b}$	7.75 $^{B\ a}$	7.59 $^{A,B\ a}$	84.31 $^{B\ a}$	82.41 $^{A,B\ a}$
11	7.47 $^{A, B\ a}$	7.38 $^{A\ a}$	7.67 $^{A, B, C\ a}$	7.34 $^{A\ b}$	7.89 $^{B\ a}$	7.53 $^{A\ b}$	7.81 $^{B\ a}$	7.31 $^{A,B\ b}$	84.33 $^{B\ a}$	82.28 $^{A,B\ b}$

LMR = Light Medium Roast; MR = Medium Roast.

* Averages obtained from the evaluations of 8 Q-graders;

Different capital letters in the same column indicate a significant difference ($p < 0.05$) between the experiments, according to the Friedman test. Different lowercase letters on the same line, for each attribute, indicate significant difference ($p < 0.05$) between the two types of roasting, according Mann-Whitney test.

All fermentation experiments showed excellent uniformity for both types of roasting. This attribute refers to the consistency of different cups for each sample. If cups from the same sample have different flavors, that aspect's value should not be high (SCAA, 2015). Thus, it was expected that no significant differences would be found between samples for this attribute.

The experiments that suffered light-medium roasting showed no significant difference for the clean cup, and the same occurred for the medium roasting. All analyzed samples received a maximum score. In coffee, defects are negative interference that impairs their quality, such as a strange taste or aroma of mold (SCAA, 2015), and their presence lowers the note of coffee. For this reason, the result obtained was desirable.

An essential attribute in the quality of coffee, especially in mature beans, is its sweetness, accentuated by the caramelization reaction during the roasting process. Good quality coffees usually have more pronounced sweetness, with hints of caramel, honey, or chocolate, and allow them to be drunk without the need for added sugar. Coffee with little sweetness is considered astringent (green coffee) or bitter (SCAA, 2015). Since fermentation consumes sugars to form acids (LEE et al., 2017), a reduction in the samples' sugar concentrations could have occurred. However, there was no significant difference between experiments with different fermentation times and temperatures, both in light medium and medium roasting, which indicates that the consumption of sugars by yeast did not affect the sensory perception of sweetness in any of the samples. Similar results were obtained by Evangelista et al. (2014a) and can be explained by the degradation of pulp, exocarp, and mucilage polysaccharides into smaller sugars fructose and glucose. The roasting operation were also not able to promote a significant difference for each sample, indicating that the reactions triggered the same sensory profile of sweetness.

Changes in the sugar composition of coffee beans, promoted by fermentation, can also affect the taste. Among the trials that suffered light-medium roasting, there was a significant difference only between experiments 1 and 10. In the latter, the grains fermented for a longer time and at a higher temperature than to the former, and the change in the chemical composition of the grains could be noticed sensorially. A significant difference was also found for the tests submitted to medium roasting between experiments 1 and 3. In this case, the higher fermentation temperature of test 3 was sufficient to promote sensorially noticeable changes. Hundreds of compounds already identified are responsible for the taste and aroma of the coffee. The microbial activity and the extent of fermentation determine the concentrations of secondary metabolites, influencing the taste of free sugars, such as glucose and fructose, and free amino acids, which contribute to the formation of volatile and non-volatile compounds in the Maillard reaction during roasting (HAILE and KANG*,* 2019). Subfermented coffee beans contain residual mucilage and sugar, which prevent drying and create an environment conducive to developing spoilage bacteria and fungi. Overfermentation stimulates the production of undesirable compounds, namely propionic and butyric acids, which impart strange flavors, such as onion flavor (HAILE and KANG*,* 2019). The type of roasting influenced experiments 5 and 10 since they showed a significant difference between them in terms of flavor, which indicates that the formation of compounds in Maillard and caramelization's reactions were more impactful for these two experiments.

Another attribute of great impact on the sensory quality of coffees is the acidity, which can be pleasant or not, depending on the nature of the drink's predominant acid. Pleasant acidity increases the perception of sweetness and gives the coffee fresh fruit characteristics (SCAA, 2015). The fermentative process converts sugars to organic acids, and the greater the fermentation, the greater the conversion is expected. The results obtained for the experiments submitted to light-medium roasting confirm this, since significant differences were observed between those experiments with greater differences in time or temperature, and consequently in the extent of fermentation. Concerning the experiments submitted to medium roasting, there was no significant difference between any of the tests, which may have occurred due to the cancellation of acids' sensory effect due to the higher degree of roasting. This process can promote changes in coffee compounds. The effect of light medium and medium roastings could be seen in experiments 6 and 7. According to Evangelista et al. (2014b), the presence of small amounts of acetic acid at the end of fermentation contributes to the acidity of the drink, as well as the presence of succinic acid (BUFFO *&* CARDELLI-FREIRE *et al.*, 2004). Citric acid, an intermediate compound in plant’s metabolic cycle, can contribute to the acidity and fruity or slightly fruity flavors of drinks if present at the end of fermentation (IGAMBERDIEV *&* EPRINTSEV *et al.* 2016). Mota and et al. (2020) evaluated the influence of fermentation conditions on the sensory quality of coffee inoculated with the yeasts *S. cerevisiae* and *T. delbrueckii*, at 18 °C, 22 °C and 30 °C, for 30 and 70 h. In all samples, acetic, citric, succinic, malic and lactic acids were found at the end of fermentation.

For good quality coffees, a light-medium, medium or full-bodied drink is expected. The more “slimy” and “heavy”, the bigger the body has the drink. Already a coffee with less body has characteristics in the mouth as "light-medium" and "delicate". Among the light medium roasting tests, a significant difference was noted only between experiments 7 and 10, whose fermentation times were 4.42h and 30h, respectively. The difference in fermentation times culminated in total amounts of different solids in the extraction, which was noticed in the sensory evaluation of the drink. Among the experiments submitted to medium roasting, no significant differences were found. The lighter the grain roast, the lighter the body. Likewise, the darker the roast, the fuller the sensation will be. In the comparison between the logs of each experiment, there was no significant difference between them for any of the trials, which indicates that the difference in this process did not change the body of the drinks.

The aftertaste or residual flavor attribute is defined as the persistence of the flavor, the characteristics perceived on the palate and which remain after the coffee is expelled from the mouth. Very short or unpleasant residual flavors are not desirable, which can lower the coffee score (SCAA, 2015). Generally, for higher quality coffees, fuller, this flavor can refer to dark chocolate, for softer and acidic coffees such completion can be quick and allude to citrus fruits. For the tests submitted to light medium roasting, significant differences were found between tests 5 and 6, 4 and 6, 5 and 9, 2 and 4, and 4 and 9 and it was noted that, for these pairs of experiments, the longest time or fermentation temperature promoted the difference of the attribute in the sensory evaluation. The fermentative process promotes the formation of volatile and non-volatile compounds (Evangelista et al., 2014a), and the greater intensity or extension of the process may have allowed the formation of different sensory profiles in terms of aftertaste. There was no significant difference between the medium roasting experiments, and possibly the most intense roasting may have promoted the reduction or suppression of compounds related to the residual flavor. When comparing the towers in each test, Experiments 2 and 11 showed a significant difference and, for both, the increase in the degree of roasting reduced the grade for the attribute in question.

Flavor, finish, acidity and body of the drink end up forming a set, contrasting or complementing each other. This effect is called balance (SCAA, 2015) and a balanced coffee has these attributes in perfect harmony. Among the tests submitted to light-medium roasting, there was a significant difference between experiments 1 and 6, 1 and 10 and 1 and 11, indicating that longer times and higher temperatures seem to favor the drink's balance. Medium roasting did not promote significant differences between the tests. When the light-medium and medium roastings were compared in each experiment, a significant difference was observed for experiments 4, 6, 10 and 11. For them, the higher degree of roasting reduced the balance of the drink.

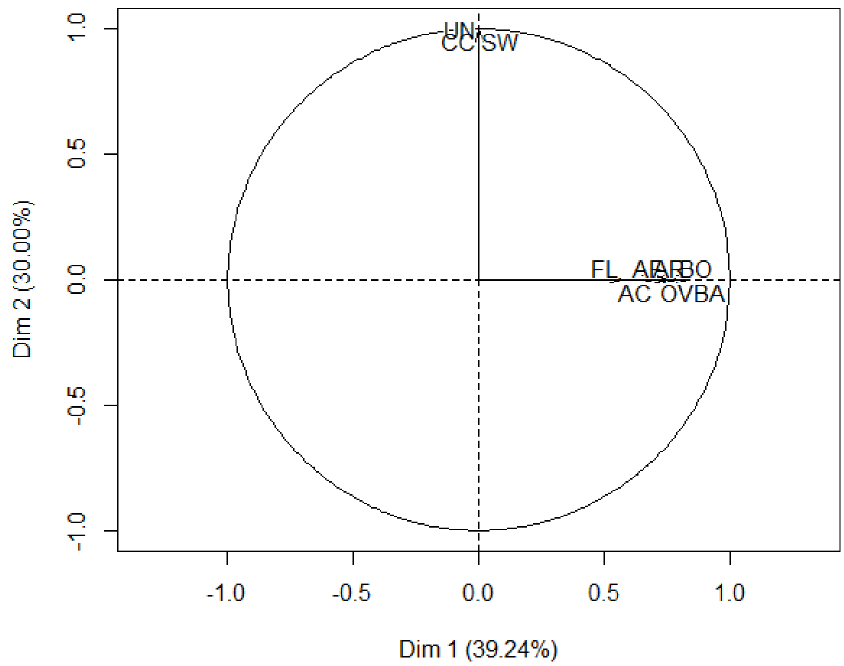
The overall attribute reflects total coherence concerning the sensory assessment of each of the attributes. It refers to the taster's impression of the complexity and the stimulus aroused during and after tasting. For light-medium roasting, pairs of experiments 5 and 10 and 5 and 11 showed a significant difference, which indicates that higher fermentation temperatures may promote better indexes for this attribute during the same period. As for medium roasting, a significant difference was noted only between tests 1 and 7. In this case, the higher temperature allowed a better index in this attribute even for a shorter time. When comparing the light medium and medium towers for the experiments, tests 5, 7, and 11 showed a significant difference. However, it is not possible to state that the more intense roasting can improve or worsen this attribute's evaluation.

The final score of the coffee is attributed by adding the scores of each attribute, individually, and must be equal to or greater than 80 for the coffee to be considered as special. All experiments evaluated sensorially, both submitted to light-medium roasting and medium roasting received scores above 80 and can be classified as special coffees. In general, higher fermentation temperatures during the 30 hours period led to higher punctuation of the drinks, when subjected to light-medium roasting, indicating that the volatile and non-volatile compounds formed during the process may have contributed beneficially to the sensory profile of the drink. This same relationship cannot be established for samples submitted to medium roasting. The degree of roasting also seems to influence drink's final quality since the higher notes in the light-medium roasting were reduced with the medium roasting. Regardless of the fermentation condition and the type of roasting applied, all samples evaluated in this study can also be classified as soft drinks, which corresponds to drinks with a pleasant taste, sweet, without astringency or harshness (SCAA, 2015).

Many works have been carried out to ferment the coffee to improve its final quality. Velmourougane (2013) studied the natural fermentation of Arabica and Robusta coffees and noticed that for both the pH during the process decreased, the temperature of the fermented mass increased and the cup quality was slightly higher when compared to enzyme-processed coffees, for example. Ribeiro et al. (2018) evaluated the bacterial diversity during wet coffee fermentation of the three coffee varieties—Mundo Novo (MN), Ouro Amarelo (OA), and Catuaí Vermelho (CV) and thirty-six mesophilic bacteria and six lactic acid bacteria were identified. The Sensory analysis showed sensations of acidity (OA and CV), bitterness, chocolate, nuts (MN), and sweetness (CV). The authors considered that their findings are relevant to future select starter bacteria for coffee processing to improve the quality and standardization of quality. Peñuela-Martínez *et al*. (2018) assessed different fermentation wet processes. They evaluated their effect on coffee quality (C. arabica) and organic acid concentrations, and volatile organic compounds content in the green coffee beans. They found that the best quality was obtained from the treatments that used short process times and low temperatures. The assessed processes lead to the conclusion that it is possible to improve coffee quality by introducing changes in the fermentation process and modulating the acidity and fragrance of the final product. Mota *et al*. (2020) evaluated the effect of *Saccharomyces cerevisiae* (CCMA 0543) and *Torulaspora delbrueckii* (CCMA 0684) inoculation on the quality of natural and pulped natural processed coffee in different producing regions. They reported that yeast inoculation modified the Sensory profile and increased the coffee beverage scores by up to 5 points. *S. cerevisiae* inoculation was most suitable for pulped natural coffee, and *T. delbrueckii* inoculation showed the best performance in natural coffee.

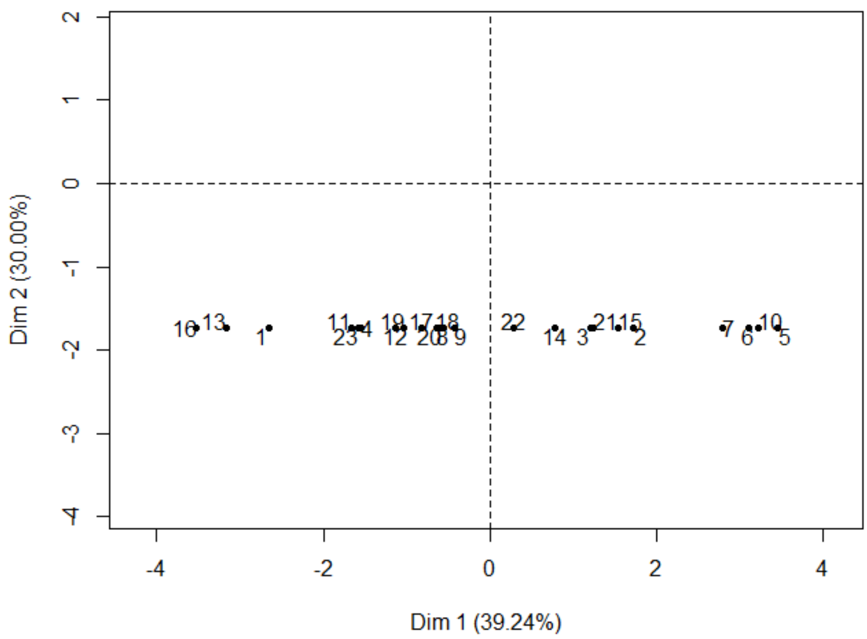
Figure 4.1 illustrates the Principal Component Analysis (PCA) of the experiments carried out under different conditions of time and temperature and subjected to light medium and medium roastings. With the first two main components it is possible to explain 69.24% of the total variability of coffee score, and the first component explains 39.24%, being that great majority of coffee attributes classified on first component. The graphic representation of the main components allows the characterization of the attributes described in the different coffee samples. The first main component (x-axis) is a global coffee index and the higher its value, the better the quality of the drink. This component is positively correlated to the attribute's aroma, flavor, acidity, body, aftertaste, balance and overall, which had the greatest influence and high contribution to the sensory profile. The second component is positively correlated to the attribute's uniformity, clean cup and sweetness. The spatial dispersion of the samples in the PCA is illustrated in Figure 4.2.

Figure 4.1 – Variables factor map (PCA)



AR = aroma; UN = uniformity; CC = clean cup; SW = sweetness; FL = flavor; AC = acidity; BO = body; AF = aftertaste; BA = balance; OV = overall.

Figure 4.2 – Individuals factor map (PCA)



Samples 1 to 11 – Experiments 1 to 11 with Light Medium Roast; sample 12 – control with Light Medium Roast; Samples 13 to 23 – experiments 1 to 11 with Medium Roast. (R Software)

Experiments 2 and 6 with light medium roasting were more correlated to the flavor attribute. Experiments 2 and 3 with light medium roasting, and 3 and 7 with medium roasting, were more correlated to body, balance, overall, aroma and aftertaste attributes. Experiments 6, 9, 10 and 11, with light medium roasting, correlated especially with the body and balance attributes. For the other experiments, it was not possible to establish a correlation with the evaluated attributes, through the PCA, and the attributes uniformity, clean cup and sweetness could not be correlated to any of the evaluated tests.

4.4. Conclusion

Aroma, uniformity, clean cup and sweetness were not affected by different fermentation conditions, either by the type of roasting. Acidity was influenced by fermentation in experiments with light-medium roasting, and greater extensions of the process seem to contribute positively to the attribute. The higher degree of roasting seems to cause a reduction in the acidity of the drink. The body attribute was influenced by fermentation, but not by roasting, while the aftertaste was influenced by both. The balance of the drink was also affected by fermentation, and higher degrees of roasting seems to influence it in a negatively. The overall, higher fermentation temperatures increased the notes of this attribute, but the roasting effect could not be well established. Through the PCA it was possible to correlate aroma, flavor, acidity, body, aftertaste, balance and overall, as the attributes that had the greatest influence and high contribution to the sensory profile. This study expands the horizons for sensory analysis as a tool to increase coffee quality through processing such as fermentation.

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CAPÍTULO 5 - CONCLUSÕES FINAIS

O uso de microrganismos iniciadores dos processos de fermentação é de extrema importância não apenas para a modulação de novos sabores para as bebidas do café mas também na manutenção da qualidade, independente do quão extremas fossem as condições da fermentação.

Também foi possível estabelecer ajustes matemáticos para determinar as taxas de degradação de açúcares básicos, como frutose, glicose e sacarose, e ajustes, que com base nas variáveis tempo e temperatura de fermentação determinar ajustes para as notas SCA, com base em diferentes níveis de torra.

A integração entre a Academia (UFU), a indústria (Lallmand) e as fazendas produtoras foi de grande importância para que este trabalho pudesse ser desenvolvido, e desta forma, abrindo as portas para que a pesquisa no ramo cafeeiro possa avançar ainda mais, com este elo recém criado.

CAPÍTULO 6 - SUGESTÕES PARA TRABALHOS FUTUROS

- Avaliar diferentes cepas de leveduras para a cultivar do café arábica, da linhagem Catuaí Amarelo, IAC 62;
- Estudar o efeito da fermentação em diversas regiões produtoras de café, principalmente aquelas com pouca tradição em cafés especiais;
- Realizar a combinação de diferentes microrganismos, tais como leveduras e lactobactérias para a condução da fermentação do café.

Utilizar as equações do planejamento para modelar condições de campo, integrando-se a temperatura observada para encontrar o tempo ótimo de fermentação

CAPÍTULO 7 – REFERÊNCIAS BIBLIOGRÁFICAS

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CAPÍTULO 8 – APÊNDICE

Apêndice (A1): Imagem de satélite da fazenda.

FONTE: GOOGLE EARTH PRO, 2019

Apêndice (A2): Imagem do café da variedade Catuaí IAC 62 maduro.

FONTE: Acervo pessoal do Autor

Apêndice (A3): Amostras de café triturada e seca.

FONTE: Acervo pessoal

Apêndice (A4): Massa de café triturada para avaliação do pH, por aproximadamente 1 minuto no liquidificador Industrial (Camargo, 2L 800W / 22000rpm).

FONTE: Acervo pessoal

Apêndice (A5): Levels of independent variables studied in the central composite rotatable design (CCRD).

Factor	Level				
	-1.4142 (-α)	-1	0	+1	+1.4142 (+α)
Temperature (ºC)	11.89	15.00	22.5	30.00	33.10
Fermentation time (h)	4.42	12.00	30	48.00	55.50

Apêndice (A6): Início da secagem do café na estufa.

FONTE: Acervo pessoal

Apêndice (A7): Amostra de café classificada.

FONTE: Acervo pessoal

Apêndice (A8): Diferentes níveis de torra.

a) torra média clara (#65) b) média escura (#55).

FONTE: Acervo pessoal

Apêndice (A9): Imagem do modelo de torrador utilizado na preparação das amostras.

FONTE: Acervo pessoal

Apêndice (A10): A imagem dos Painéis Sensoriais SCA montados nas duas diferentes localidades: a) laboratório do Grupo Farroupilha, Patos de Minas – MG e b) laboratório do Grupo Veloso Coffee, Carmo do Paranaíba – MG.

(a)

(b)

FONTE: Acervo pessoal

Apêndice (A11): Evolução da maturação do café Catuaí Amarelo.

FONTE: Gentilmente Cedido pelo colega Leandro Reis Silva