UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ZOOTECNIA E ENGENHARIA DE ALIMENTOS

CAROLINA MEDEIROS VICENTINI-POLETTE

Extraction of sunflower seed oil (*Helianthus annuus*) by supercritical fluid and pressurized ethanol for enrichment of highly digestible dairy product

"Extração de óleo de semente de girassol (*Helianthus annuus*) por fluido supercrítico e por etanol pressurizado para enriquecimento de produto lácteo de alta digestibilidade"

Pirassununga
2022

CAROLINA MEDEIROS VICENTINI-POLETTE

Extraction of sunflower seed oil (*Helianthus annuus*) by supercritical fluid and pressurized ethanol for enrichment of highly digestible dairy product

"Extração de óleo de semente de girassol (*Helianthus annuus*) por fluido supercrítico e por etanol pressurizado para enriquecimento de produto lácteo de alta digestibilidade"

Versão Corrigida

Tese apresentada à Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo, como parte dos requisitos para a obtenção do titulo de Doutora em Ciências do programa de Engenharia de Alimentos.

Área de Concentração: Ciências da Engenharia de Alimentos

Orientadora: Profª. Drª. Alessandra Lopes de Oliveira

Pirassununga

2022

Ficha catalográfica elaborada pelo
Serviço de Biblioteca e Informação, FZEA/USP,
com os dados fornecidos pelo(a) autor(a)

M633e

Medeiros Vicentini-Polette, Carolina
Extraction of sunflower seed oil (Helianthus annuus) by supercritical fluid and pressurized ethanol for enrichment of highly digestible dairy product / Carolina Medeiros Vicentini-Polette ; orientadora Alessandra Lopes de Oliveira. -- Pirassununga, 2022.
155 f.

Tese (Doutorado - Programa de Pós-Graduação em Engenharia de Alimentos) -- Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo.

1. supercritical carbon dioxide. 2. pressurized liquid extraction. 3. goat milk. 4. sunflower oil. 5. tocopherol. I. Lopes de Oliveira, Alessandra, orient. II. Titulo.

HONORING

In 2008 I vowed not to give up. I honor each one who helped me to get here – incluing my own younger versions - we did it!

ACKNOWLEDGMENTS

A **Deus**, representativo do Universo, que me trouxe até aqui;
To God, representative of the Universe, who brought me here;

Ao meu marido, **Leandro Polette**, por acreditar em mim e me incentivar a ser uma pessoa melhor, todos os dias. Obrigada por aflorar a melhor versão que há em mim. Eu te amo!
To my husband, Leandro Polette, for believing in me and encouraging me to be a better person every day. Thank you for bringing out the best version of me. I love you!

À minha família, especialmente aos meus pais, **Kátia** e **José Luis**, meus irmãos, **Pedro** e **Guilherme**, e aos meus filhos, **Gabriel** e **Gabrielli**, por me tornarem quem eu sou;
To my family, especially my parents, Katia and Jose Luis, my brothers, Pedro and Guilherme, and my children, Gabriel and Gabrielli, for making me who I am;

À minha sogra, **Ana Paula**, por toda a ajuda para que eu pudesse finalizar este estudo em tempos de pandemia;
To my mother-in-law, Ana Paula, for helping me finish this study in times of pandemic;

Aos meus amigos, especialmente a **Amanda Vale**, que por quase duas décadas tem sido um porto seguro, e um exemplo de vida; e à **Elaine Silva**, por tornar meus dias mais alegres;
To my friends, especially Amanda Vale, who for almost two decades has been a safe place, and an example of life; and Elaine Silva, for making my days happier;

À **Marta Verruma**, por ser minha luz no fim do túnel há uma década. Obrigada pela amizade, pela sabedoria, pela paciência, e por nunca desistir de mim;
To Marta Verruma, for being my light at the end of the tunnel for a decade. Thank you for your friendship, your wisdom, your patience, and for never giving up on me;

À Profª Drª **Alessandra Lopes de Oliveira**, minha orientadora, pelos infinitos ensinamentos, e pela imensa paciência apesar de todos os empecilhos nesta jornada. Obrigada por me tornar uma pessoa mais forte;
To Prof. Dr. Alessandra Lopes de Oliveira, my advisor, for the endless teachings, and for the immense patience despite all the obstacles on this journey. Thank you for making me a stronger person;

Aos professores e pesquisadores que fizeram parte desta jornada, especialmente a Profª Drª **Eliana Setsuko Kamimura** e à Profª Drª **Cintia Bernardo Gonçalves** (*in memoriam*), por toda a competência e leveza. Vocês tornam o mundo um lugar melhor;
To the professors and researchers who were part of this journey, especially Prof. Dr. Eliana Setsuko Kamimura and Prof. Dr. Cintia Bernardo Gonçalves (in memoriam), for all their competence and lightness. You make the world a better place;

À toda a equipe do LTAPPN, especialmente aos amigos **Gabriela Bittencourt** e **Paulo Ramos**, que deram cor à esta jornada, e ao **Nilson Ferreira**, por todo o auxílio a paciência;
To the whole LTAPPN team, especially to friends Gabriela Bittencourt and Paulo Ramos, who gave color to this journey, and Nilson Ferreira, for all the help and patience;

A todos os alunos de iniciação científica que passaram por meu projeto, especialmente **Isabella Mothe**, **Beatriz Yamada** e **Joyce Sponchiado**. Vocês têm um futuro brilhante! Obrigada por tudo;

To all the scientific initiation students that have been through my project, especially Isabella Mothe, Beatriz Yamada, and Joyce Sponchiado. You have a bright future! Thank you for everything;

À Caramuru Sementes, que gentilmente doou as sementes de girasol utilizadas neste estudo;

To Caramuru Sementes, who kindly donated the sunflower seeds used in this study;

À todos que colaboraram, direta ou indiretamente, neste estudo;

To everyone who collaborated, directly or indirectly, in this study;

Por fim, à **Elza**, **Lana**, **Mia**, **Michael**, **Tina**, **Zeca** e **Nelson**, meus amados animais, por serem meu poço de amor, carinho e diversão todos os dias. Obrigada por surgirem em minha vida.

Finally, to Elza, Lana, Mia, Michael, Tina, Zeca, and Nelson, my beloved animals, for being my well of love, care, and fun every day. Thank you for coming into my life.

Funding: This work was supported by the Brazilian agency Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - Process nº 2018/18024-7); the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – Process nº 304573/2019-1); and the Brazilian agency Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship (Process nº 001).

ABSTRACT

We identified the best conditions for extraction of sunflower seed oil (*Helianthus annuus*) enriched with tocopherols, using the technologies of supercritical extraction with supercritical carbon dioxide (SFE-scCO$_2$) and pressurized liquid extraction (PLE) with ethanol as solvent. *Altis 99* sunflower seed was chosen because of its oil and α-tocopherol content, which is considered of high nutritional quality. The raw material was characterized by classical bromatological analyses. Experimental planning was applied to evaluate the influence of process variables on total yield and tocopherol content (α, β, γ, and δ) for obtaining oil by both SFE and PLE. Central Compound Rotated Design [CCRD] was used for both SFE and PLE. SFE design was performed two independent variables (temperature, ºC; pressure, MPa), and two axial points (-α, + α). PLE design considered the static purging time of the solvent (min), the temperature (ºC) and the rinse volume of solvent (ethanol, %), number of cycles = 4, and P = 10.34 MPa. For yield, the variables that influenced SFE and PLE were pressure and temperature, respectively. Among the material obtained by SFE and PLE, oil and extract, respectively, we analyzed, in addition to yield and tocopherol content, the profile of total and free fatty acids (FAs), estimated composition in triacylglycerols acidity and oxidative stability. In the application study, the tocopherol-rich sunflower oil was incorporated into goat milk yogurt sweetened with xylitol, a viable and inclusive option for low-calorie and highly digestible dairy consumption. After conducting an interest survey with 204 consumers, the enriched yogurt was sensorially described by the free profile technique, with 9 evaluators. A product acceptance test was also applied to 36 evaluators. The mean results obtained in the characterization of dehulled sunflower seeds were 98.76 ± 0.21 % dry matter; 2.70 ± 0.05 % mineral matter; 23.50 ± 0.26 % crude protein; and 12.88 ± 0.27 % crude fiber. As for the ethereal extract, the average obtained was 55.07 ± 0.30 %. The highest oil recovery obtained by SFE was 87.6 % of the total oil in the seed (55 %), where pressure proved significant ($p \leq 0.05$) in the process. In PLE, the statistical analysis of the experimental planning indicated that only the temperature had an effect on the responses, adjusted to a linear model that proved significant ($p \leq 0.05$) and predictive. In this process the highest oil recovery was 93.9%. When comparing the products from the different processes, the highest concentration of tocopherols was observed in the oil obtained via SFE-scCO$_2$ at 60 ºC and 18 MPa, where 91.17 mg of tocopherols / 100 g of oil were obtained. Of these, 88 mg are related to α-tocopherol. In the analysis of the process variables in this response, a significant (and negative) influence of temperature was observed. Although smaller, the extract obtained via PLE also showed a high amount of tocopherols, up to 83.16 mg / 100 g (77 % of which were α-tocopherols) under the

conditions of 56 ºC and 110 % of the extraction cell volume (34 mL) of solvent volume used in the rinse. However, in the statistical analysis of the influence of the process variables on this response, none proved significant at the 5% level. As for the profile of total AGs, there was no difference between the oils obtained by PLE, the one optimized by SFE-scCO_2 and the commercial one, suggesting that both processes do not interfere in the quality of the product obtained. Under the optimized extraction conditions, the oils obtained by SFE-scCO_2 and PLE presented total titratable acidity of 3.59 and 3.17 g oleic acid/100 g of oil, respectively. The acidity of the commercial oil and the oil obtained via soxhlet using hexane or petroleum ether in the same raw material showed an acidity of 0.36 and 2.52 g oleic acid / 100 g oil, respectively, indicating that the pressurized extractions resulted in more acidic oils than the non-pressurized (soxhlet). The methodology used for free fatty acids analysis proved to be feasible for its qualitative determination, and suggests similarity between the main total and free fatty acids (such as linoleic, oleic, and palmitic) observed regardless of the process, although minority fatty acids may vary. There was interest from the consumer public in yogurt made with sunflower oil. Sensory analysis showed that the main attributes described were: fatty appearance, characteristic aroma, milky flavor, homogeneity and consistency. There was no statistical difference for acceptance between the natural or oiled yogurts ($p \geq 0.05$), both scored on the positive part of the scale: using a 7-point scale, the natural yogurt showed an average of 5.03 ± 1.6, while the enriched yogurt was scored as 4.61 ± 1.2.

Keywords: supercritical carbon dioxide; pressurized liquid extraction; goat milk; sunflower oil; tocopherol.

RESUMO

Identificou-se as melhores condições para extração de óleo de semente de girassol (*Helianthus annuus*) enriquecido com tocoferóis, utilizando-se as tecnologias de extração supercrítica com dióxido de carbono supercrítico (SFE-scCO_2) e extração por líquido pressurizado (PLE) tendo o etanol como solvente. Optou-se pela semente de girassol *Altis 99* dado seu teor de óleo e de α-tocoferol, que é considerado de alta qualidade nutricional. A matéria-prima foi caracterizada por análises bromatológicas clássicas. Foram aplicados planejamentos experimentais para avaliar a influência das variáveis de processo no rendimento total e no teor de tocoferóis (α, β, γ e δ) para a obtenção de óleo tanto por SFE quanto por PLE. Design Central Composto Rotativo [DCCR] tanto para SFE como para PLE. A SFE foi realizada com duas variáveis independentes (temperatura, ºC; pressão, MPa) e dois pontos axiais (-α, + α). O design aplicado à PLE considerou o tempo de purga estática do solvente (min), a temperatura (ºC) e o volume de lavagem do solvente (etanol, %), número de ciclos = 4, e P = 10,34 MPa. O óleo e o extrato, respectivamente obtidos pela SFE e PLE, foram analisados através do rendimento e teor de tocoferóis, o perfil de ácidos graxos (AGs) totais e livres, composição estimada em triacilgliceróis acidez e estabilidade oxidativa. No estudo da aplicação, o óleo de girassol rico em tocoferóis foi incorporado em iogurte de leite de cabra adoçado com xilitol, uma opção viável e inclusiva no consumo de laticínios com baixa caloria e alta digestibilidade. Após realização de pesquisa de interesse com 204 consumidores, o iogurte enriquecido foi sensorialmente descrito pela técnica de perfil livre, com 9 avaliadores. Também se aplicou teste de aceitação do produto, com 36 avaliadores. As médias dos resultados obtidos na caracterização das sementes de girassol descascadas foram 98,76 ± 0,21 % matéria seca; 2,70 ± 0,05 % matéria mineral; 23,50 ± 0,26 % proteína bruta; e 12,88±0,27 % fibra bruta. Quanto ao extrato etéreo, a média obtida foi de 55,07 ± 0,30 %. A maior recuperação de óleo obtida por SFE foi de 87,6 % do total de óleo na semente (55%), onde a pressão se mostrou significativa ($p \leq 0,05$) no processo. Na PLE, a análise estatística do planejamento experimental indicou que apenas a temperatura apresentou efeito nas respostas, ajustadas à um modelo linear que se mostrou significativo ($p \leq 0,05$) e preditivo. Neste processo, a maior recuperação de óleo foi de 93,9%. Na comparação dos produtos oriundos dos diferentes processos, a maior concentração de tocoferóis foi observada no óleo obtido via SFE-scCO_2 na condição de 60 ºC e 18 MPa, onde obteve-se 91,17 mg de tocoferóis/100 g de óleo. Destes, 88 mg são referentes ao α-tocoferol. Na análise das variáveis de processo nesta resposta, observou-se influência significativa (e negativa) da temperatura. Embora menor, o extrato obtido via PLE também apresentou alta quantidade de tocoferóis, até 83,16 mg/100 g (sendo 77% do tipo α) nas

condições de 56 ºC e 110 % do volume da célula de extração (34 mL) de volume de solvente usado no enxague. Porém, na análise estatística da influência das variáveis do processo nesta resposta, nenhuma se mostrou significativa ao nível de 5%. Quanto ao perfil de AGs totais, não houve diferença de perfil entre os óleos obtidos por PLE, o otimizado por SFE-scCO_2 e o comercial, sugerindo que ambos os processos não interferem na qualidade do produto obtido. Nas condições otimizadas de extração, os óleos obtidos por SFE-scCO_2 e PLE apresentaram acidez titulável total de 3,59 e 3,17 g ácido oleico/100 g de óleo, respectivamente. A acidez do óleo comercial e do óleo obtido via soxhlet usando hexano ou éter de petróleo na mesma matéria-prima mostrou uma acidez de 0,36 e 2,52 g ácido oleico/100 g de óleo, respectivamente, indicando que as extrações pressurizadas resultaramem óleos mais ácidos que a não pressurizada (soxhlet). A metodologia utilizada para análise de AGs livres se mostrou viável para sua determinação qualitativa, e sugere similaridade entre os principais AGs totais (como linoleico, oleico e palmítico) e livres observados independentemente do processo, embora AGs minoritários possam variar. Houve interesse do público consumidor no iogurte elaborado com óleo de girassol. A análise sensorial mostrou que os principais atributos descritos foram: aparência gordurosa, aroma característico, sabor lácteo, homogeneidade e consistência. Não houve diferença estatística para aceitação entre os iogurtes natural ou com óleo ($p \geq 0,05$), ambos pontuados na parte positiva da escala: utilizando uma escala de 7 pontos, o iogurte natural apresentou uma média de 5,03 ± 1,6, enquanto o iogurte enriquecido foi pontuado como 4,61 ± 1,2.

Palavras Chave: dióxido de carbono supercrítico; extração com líquido pressurizado; leite de cabra; óleo de girassol; tocoferol.

LIST OF FIGURES Page

LIST OF TABLES

Page

SUMMARY

CHAPTER I

I.1. INTRODUCTION

Hexane has been used for decades in the conventional extraction of vegetable oils and fats. The extraction systems depend on the objective of the process and the physical properties of the material to be extracted as well as the product obtained. In the conventional hexane extraction method, the raw material must undergo pre-treatment, heating and rolling, which can take up to 20 min at 90-95 °C. After extraction, the solvent needs to be recovered from the product and the residue, both for the achievement of a quality product and for environmental issues (EGGERS; JAEGER, 2003).

In a conventional batch extraction, the extractors are filled with the material to be extracted and then liquid hexane is added. Then there is a waiting time, and the miscella, a mixture consisting of solvent plus oil, is drained from the system, leaving for further separation. This system demands a long waiting time and a large amount of solvent (EGGERS; JAEGER, 2003).

To increase the efficiency of the system, several extractors can be aligned, so as to generate a semi-continuous extraction, where the solvent passes from one extractor to another. Thus, the solvent increases in concentration at each step, approaching its full extraction capacity before being drained off. This process can also be performed in counter-current, i.e., starting with the extractor with the most depleted material, and ending with the most recently inserted (EGGERS; JAEGER, 2003).

However, there is a global movement for toxic, non-renewable solvents to be replaced by alternatives that are both economically and ecologically viable.

This change becomes even more challenging when we consider the competitiveness of the market and the need to protect the environment, showing that the new alternatives must not only complement the current ones, but cause a rupture with the harmful ones (CHEMAT; VIAN; CRAVOTTO, 2012).

In this thesis, two technologies were discussed: supercritical fluid extraction (SFE), with carbon dioxide (CO_2) as the solvent, and pressurized liquid extraction (PLE), with ethanol as the solvent.

According to Dunford et al. (2003), the use of supercritical extraction in food engineering has historical precedent, from its application to decaffeinate coffee, to the present day, especially with raw materials and natural products, since it is considered a "green" extraction process. This technology has been implemented in several countries, such as Germany, the United States, France, and Japan.

The SFE is based on the contact of the solid matrix packed in a fixed bed extractor with the solvent in a supercritical state, usually CO_2, which flows under pre-established conditions of pressure (P) and temperature (T) so that the solvent in a supercritical state has physical properties similar to liquids and gases. In addition to the fluid properties, altered by P and T, this extraction technique is also influenced by extrinsic factors, such as sample characteristics, interaction with target compounds with the solvent and between them, and environmental factors (SHARIF et al., 2014; SILVA; ROCHA-SANTOS; DUARTE, 2016). Therefore, it is important that the application be evaluated for each type of matrix and under specific conditions.

Adouni et al. (2022) observed the antioxidant activity of *Asparagus stipularis* Forssk extracts obtained by SFE; Dos Santos et al. (2021) compared different extraction methods of Brazilian "pacová" oilseeds for the determination of specific compounds by SFE; Santos et al. (2020) determined functional compounds in blue shark liver oil obtained by SFE; Asl et al. (2020) studied the SFE of phytosterols and tocopherols from rapeseed oil waste; Rai et al. (2016a; 2016b), Fiori et al. (2009), and Salgin et al. (2006) studied the variables for sunflower oil SFE; Przygoda and Wejnerowska (2015) observed the extraction of tocopherol-enriched oils from Quinoa seeds by SFE; Boutin et al. (2011) modelled SFE from rapeseeds and sunflower seeds; Nimet et al. (2011) observed the SFE of sunflower oil using subcritical propane as a co-solvent; Davarnejad et al. (2010) determined the solubility SFE of tocopherols from crude palm oil; Bravi et al. (2007) improved the SFE of α-tocopherol-enriched oil from grape seeds; Leo et al. (2005) studied the SFE of oil and α-tocopherol from almond seeds; Uquiche et al. (2004) observed the SFE of red pepper oleoresin; Lameira et al. (1997) extracted lipids from cashew kernel by SFE; Cocero and Calvo (1996) studied the SFE of sunflower seed oil using ethanol mixtures as co-solvent.

PLE, on the other hand, which is an Accelerated Solvent Extraction (ASE), is a technique that involves high P and T during the process. In this way, the solvent remains liquid at temperature conditions above its boiling point. Specifically, in this research, the extraction with pressurized liquid with intermittent purging of the extract was studied, which is different from an extraction process with a constant flow of pressurized solvent. In this process, the plant matrix is conditioned in the fixed bed extractor, and the solvent is pumped into the extractor to fill the voids. Once the process P and T are reached, the first cycle begins. In each cycle (C), the solvent is in contact with the matrix for a certain time called static time (St). After that, part of the extract is collected while a new volume

of solvent (rinse volume) is pumped into the extractor. During the collection of the purged extract, the pressure and temperature in the system remain constant. At the end of this period, after collecting the extract, a new cycle begins. The total rinse volume of the solvent to be used in the extraction is divided into different proportions that depend on the number of cycles adopted; eg 300 mL in 3 cycles, each cycle will receive 100 mL. At the end of the last cycle, all remaining extract is removed from the extractor with a Nitrogen (N_2) purge (Cornelio-Santiago et al., 2022). By manipulating the temperature, time and number of cycles, it is possible to obtain high yields in a considerably reduced manufacturing time when compared to conventional extraction techniques (RICHTER et al., 1996).

The use of ethanol as solvent has been studied in the last decades. Cornélio-Santiago et al. (2022) studied the oil extraction from pequi and sacha inchi almonds by PLE; Adouni et al. (2022) observed the antioxidant activity of *Asparagus stipularis Forssk* extracts obtained by PLE; Alves et al. (2022) detailed the extraction of bioactive compounds from Monteverdia aquifolia leaves by PLE; Toda et al. (2021) determined the conventional and pressurized ethanolic extraction of oil from spent coffee grounds; Baümler et al. (2017) observed the diffusion of tocopherols, phospholipids and sugars during oil extraction from sunflower collets (not pressurized); Colivet et al. (2016) discussed the influence of the bed height on the kinetics of watermelon seed oil extraction (PLE); Sineiro et al. (1998) studied the sunflower seeds oil extraction by ethanol from partially defatted prepressed in pulsed and nonpulsed extractors.

Nevertheless, new technologies are promising only when they are advantageous. In the case of vegetable oil extractions, it is imperative that the process be of high yield, and that the oil obtained is of equivalent or superior quality to conventional oils. Process optimization is used to achieve this.

Optimization is the process of maximizing the amount of something desired, or minimizing something undesired, and the parameters that provide such optimization are called "optimal conditions", and are part of the "optimal design". This requires the determination of independent variables that result in one or more dependent variables. Optimization often aims at minimizing the process costs, within the possibility of the system and the available material. It is the application of scientific methods and techniques whose decisions are based on mathematical models to maximize or minimize the criterion or function (TZIA, 2003).

Sunflower seed combines both a high oil content and biocompounds of interest. Sunflower seed (*Helianthus annuus*) is rich in vitamin E, and is notable for its content of

α-tocopherol, a high biological value tocopherol. In common sunflower oil, 91 to 97 percent of the total tocopherol is of the α-tocopherol type. Seeds with high oil content can reach 1,120 mg/kg of vitamin E (GUNSTONE, 2011).

However, consumption of pure oil is not attractive in human diet. Therefore, this study suggests using the enriched oil as part of an already functional food: goat's milk yogurt. According to Haenlein (2004), goat milk products, such as yoghurt, has been seeked in many developed countries. In addition, it is a great consumption alternative for people with cow milk allergies and other gastro-intestinal ailments.

Although goat's and cow's milks have similar compositions, the goat's milk present some important characteristics, such as a high percentage of short and medium chain fatty acids when compared to cow's milk (CEBALLOS et al., 2009). Average goat milk fat is much higher in butyric (C4:0), caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), and linoleic (C18:2) fatty acids, but lower in stearic (C18:0), and oleic acid (C18:1) (CEBALLOS et al., 2009; HAENLEIN, 2004; JENNESS, 1980).

In addition, goat's milk is less allergenic when compared to cow's milk, due to the α-S1 casein presented in higher quantities in cow's milk than in goat's milk, besides having different molecular and antigenic structures. This protein is related to the allergenic characteristic in milk (Ceballos et al., 2009; Ribeiro, 1998).

The enrichment of goat milk yogurt with compounds of nutritional interest has been widely studied in recent years (ALQAHTANI et al., 2021; BEZERRIL, 2021; MAZZAGLIA et al., 2020), as well as its sensory characteristics (ALQAHTANI et al., 2021; ÁM et al., 2020), but no studies using vegetable oils as an enrichment factor were found.

This study stands out for presenting in a comparative and simplified way the extraction of sunflower oil by green technologies, comparing it with its commercial version, contributing with data that will facilitate the use of this system in larger scales in the near future, resulting in a product of lower environmental impact and better nutritional quality. We also sought to exemplify the potential use of the oils obtained through application in a goat milk yogurt, increasing its nutritional quality.

I.1.1. Structure

This PhD. Thesis had as main hypothesis that the pressurized extraction using green solvents provides high yield in the extraction of sunflower seed oil, and also that

this oil is rich in tocopherols of high biological value that can be applied, in its raw form, in the formulation of a functional dairy product.

It is divided into 7 chapters. In the first, a succinct and general introduction to the topic of study is presented, including its structure (Figure 1) and the objectives of this research.

Chapter II consists of a review of relevant literature. It addresses green extraction techniques applicable to vegetable oils, the importance of vitamin E and its presence in sunflower seeds. Factors relating to the consumption of goat's milk (and yogurt) are also noted.

Chapter III brings the characterization of the raw material used in this study, and an in-depth detail on the optimization processes applied for the optimization of sunflower oil extraction using both SFE and PLE. The focus was applied on the yield and tocopherol content of the obtained oil. After optimization, the oils were characterized as to their fatty acid and triacylglycerol composition. In this chapter, the kinetics of the SFE extraction can also be observed. Chapter III was written in partnership with the authors: Beatriz Satie Yamada, who collaborated in the optimization process using both SFE and PLE systems; MSc. Paulo Silva Ramos, who assisted in the statistical analysis and data interpretation; MSc. Marta Gomes Silva, responsible for tocopherol analysis; Dr. Cintia Bernardo Gonçalves (*in memoriam*) assisted in the calculation and interpretation of the lipid composition of the obtained oils; and Dr. Alessandra Lopes de Oliveira, who assisted since the conceptualization to the final writing.

In Chapter IV, PLE extraction kinetics are observed for both ground and rolled sunflower seeds in the continuous and intermittent processes. This chapter briefly describes the behavior of the extraction kinetics of ground and rolled sunflower seeds extracted by PLE. This material was presented by the scientific initiation student Beatriz Satie Yamada at the International Symposium for Scientific and Technological Initiation at USP (SIICUSP) in 2021, except for the topic "Introduction", being indicated to the international stage of the event.

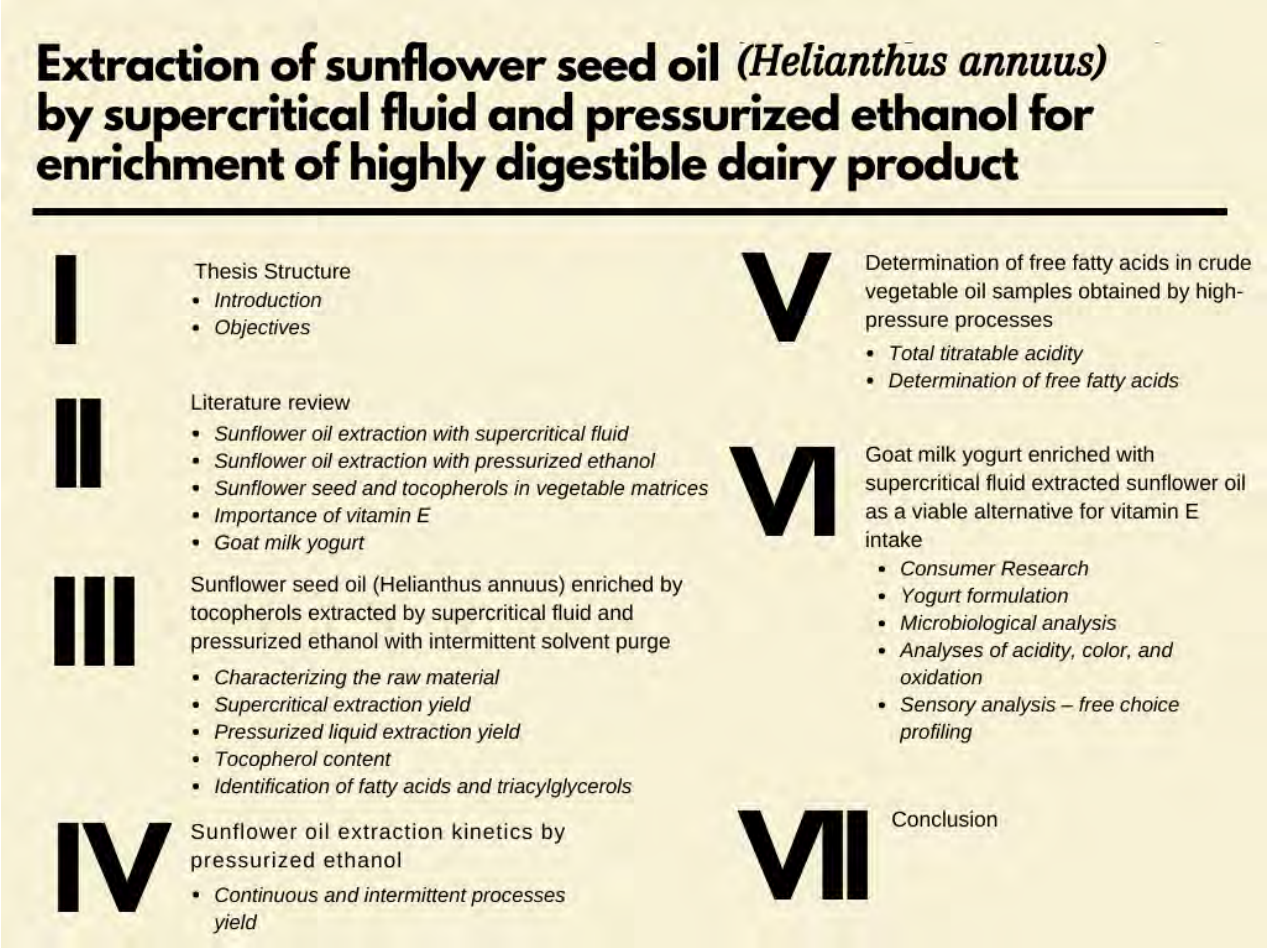
Chapter V represents an article already published in Food Chemistry: X Journal, in 2021, and brings an innovative methodology for the qualitative analysis of free fatty acids in vegetable oils (sunflower and soybean). Chapter V was elaborated with the collaboration of the following authors: MSc. Paulo Silva Ramos, who assisted in every step of the investigation and method determination; Dr. Cintia Bernardo Gonçalves (*in memoriam*) who provided the knowledge and tools for fatty acid and triacilgrlycerol

interpretation; and Dr. Alessandra Lopes de Oliveira, who once more assisted since the conceptualization to the final writing.

Then, in Chapter VI, the application of sunflower oil obtained by SFE, optimized, in a goat milk yogurt enriched and sweetened with xylitol is presented. Pre-consultation with potential consumers, the formulation of the enriched yogurt, its physical, chemical and microbiological characterization, and finally its sensory characterization is available. Chapter VI was written together with the authors: Joyce da Silva Sponchiato, who helped in the designing of the study; MSc. Handray Fernandes de Souza and MSc. Marina Vieira de Carvalho, who provided great aid in the microbiological analysis; Dr. Anneliese de Souza Traldi, responsible for the goat's milk obtaining; Dr. Marta Regina Verruma-Bernardi, responsible for he sensory evaluation design and interpretation; and Dr. Alessandra Lopes de Oliveira, who managed the study.

Finally, Chapter VII concludes, critically, the totality of the studies stated in this thesis.

Figure 1 – Thesis structure



Source: own authorship

I.2. OBJECTIVES

I.2.1. General objectives

Optimize the extraction of sunflower oil enriched in α-tocopherol by the green extraction processes: by pressurized ethanol, and by supercritical carbon dioxide, evaluating its parameters and extraction behavior. Subsequently, formulate a goat milk yogurt using enriched sunflower oil with tocopherol as a source of vitamin E.

I.2.2. Specific objectives

a) Characterize the composition of the sunflower seeds (*Helianthus annuus*);
b) Evaluate the relevant parameters of process for optimization of sunflower oil extraction by supercritical fluid, favoring high yield and high tocopherol concentration;
c) Evaluate the relevant parameters for optimization of sunflower oil extraction by pressurized ethanol, favoring high yield and high tocopherol concentration;
d) Characterize the extracted oils regarding physical and chemical parameters;
e) Formulate a goat milk yogurt enriched with α-tocopherol.

REFERENCES

ADOUNI, K.; BENDIF, H.; ZOUAOUI, O.; KRAUJALIS, P.; FLAMINI, G.; VENSKUTONIS, P. R.; ACHOUR, L. Antioxidant activity of extracts obtained by high-pressure extraction procedures from *Asparagus stipularis Forssk*. **South African Journal of Botany**, v. 146, p. 789-793, 2022.

ALQAHTANI, N. K.; DARWISH, A. A.; EL-MENAWY, R. K.; ALNEMR, T. M.; ALY, E. Textural and organoleptic attributes and antioxidant activity of goat milk yoghurt with added oat flour. **International Journal of Food Properties**, v. 24, n. 1, p. 433-445, 2021.

ALVES, T. P.; TRIQUES, C. C.; PALSIKOWSKI, P. A.; DA SILVA, C.; FIORESE, M. L.; DA SILVA, E. A.; FAGUNDES-KLEN, M. R. Improved extraction of bioactive compounds from Monteverdia aquifolia leaves by pressurized-liquid and ultrasound-assisted extraction: Yield and chemical composition. **The Journal of Supercritical Fluids**, v. 181, p. 105468, 2022.

ÁM, C. G.; GARCÍA, R.; GÓMEZ-PEREA, A.; OLIVA, L.; MI, L. B.; OLVEIRA, G. Satisfaction with the hospital menu and incorporation of drinkable goat milk yogurt as a dessert. **Nutricion hospitalaria**, 2020.

ASL, P. J.; NIAZMAND, R.; YAHYAVI, F. Extraction of phytosterols and tocopherols from rapeseed oil waste by supercritical CO_2 plus co-solvent: A comparison with conventional solvent extraction. **Heliyon**, v. 6, n. 3, p. e03592, 2020. DOI: 10.1016/j.heliyon.2020.e03592

BAÜMLER, E. R.; CARRÍN, M. E.; CARELLI, A. A. Diffusion of tocopherols, phospholipids and sugars during oil extraction from sunflower collets using ethanol as solvent. **Journal of food engineering**, v. 194, p. 1-8, 2017.

BEZERRIL, F. F.; MAGNANI, M.; PACHECO, M. T. B.; DE SOUZA, M. D. F. V.; FIGUEIREDO, R. M. F.; DOS SANTOS LIMA, M.; DO EGYPTO, R. D. C. R. *Pilosocereus gounellei* (xique-xique) jam is source of fibers and mineral and improves the nutritional value and the technological properties of goat milk yogurt. **LWT**, v. 139, p. 110512, 2021.

BOUTIN, O.; DE NADAÏ, A.; PEREZ, A. G.; FERRASSE, J. H.; BELTRAN, M.; BADENS, E. Experimental and modelling of supercritical oil extraction from rapeseeds and sunflower seeds. **Chemical Engineering Research and Design**, v. 89, n. 11, p. 2477-2484, 2011.

BRAVI, M.; SPINOGLIO, F.; VERDONE, N.; ADAMI, M.; ALIBONI, A.; D'ANDREA, A.; DE SANTIS, A.; FERRI, D. Improving the extraction of α-tocopherol-enriched oil from grape seeds by supercritical CO_2. Optimisation of the extraction conditions. **Journal of Food Engineering**, v. 78, n. 2, p. 488-493, 2007. DOI: 10.1016/j.jfoodeng.2005.10.017.

CEBALLOS, L. S.; MORALES, E. R.; DE LA TORRE ADARVE, G.; CASTRO, J. D.; MARTÍNEZ, L. P.; SAMPELAYO, M. R. S. Composition of goat and cow milk produced under similar conditions and analyzed by identical methodology. **Journal of food Composition and Analysis**, v. 22, n. 4, p. 322-329, 2009.

CHEMAT, F.; VIAN, M. A.; CRAVOTTO, G. Green extraction of natural products: concept and principles. **International journal of molecular sciences**, v. 13, n. 7, p. 8615-8627, 2012.

COCERO, M. J.; CALVO, L. Supercritical fluid extraction of sunflower seed oil with CO_2 - ethanol mixtures. **Journal of the American Oil Chemists' Society**, v. 73, n. 11, p. 1573-1578, 1996.

COLIVET, J.; OLIVEIRA, A. L. de; CARVALHO, R. A. de. Influence of the bed height on the kinetics of watermelon seed oil extraction with pressurized ethanol. **Separation and Purification Technology**, v. 169, p. 187-195, 2016.

CORNELIO-SANTIAGO, H. P.; BODINI, R. B.; MAZALLI, M. R.; GONÇALVES, C. B.; RODRIGUES, C. E.; DE OLIVEIRA, A. L. Oil extraction from pequi (*Caryocar brasiliensis Camb.*) and sacha inchi (*Plukenetia huayllabambana sp. Nov.*) almonds by pressurized liquid with intermittent purge: The effects of variables on oil yield and composition. **The Journal of Supercritical Fluids**, p. 105527, 2022.

DA SILVA, R. P. F. F.; ROCHA-SANTOS, T. A. P.; DUARTE, A. C. Supercritical fluid extraction of bioactive compounds. **TrAC Trends in Analytical Chemistry**, v. 76, p. 40-51, 2016.

DAVARNEJAD, R.; AHMAD, Z.; SATA, S. A.; MORAVEJI, M. K.; AHMADLOO, F. Mutual solubility study in supercritical fluid extraction of tocopherols from crude palm oil using CO_2 solvent. **International Journal of Molecular Sciences**, v. 11, n. 10, p. 3649-3659, 2010. DOI: 10.3390/ijms11103649.

DOS SANTOS, L. C.; ÁLVAREZ-RIVERA, G.; SÁNCHEZ-MARTÍNEZ, J. D.; JOHNER, J. C. F.; BARRALES, F. M.; DE OLIVEIRA, A. L.; CIFUENTES, A.; IBAÑÉZ, E.; MARTÍNEZ, J. Comparison of different extraction methods of Brazilian "pacová" (*Renealmia petasites Gagnep*.) oilseeds for the determination of lipid and terpene composition, antioxidant capacity, and inhibitory effect on neurodegenerative enzymes. **Food Chemistry: X**, v. 12, p. 100140, 2021.

DUNFORD, N. T.; KING, J. W.; LIST, G. R. Supercritical Fluid Extraction in Food Engineering. In: TZIA, C.; LIADAKIS, G. Extraction optimization in Food Engineering. USA: Marcel Dekker, 2003.

EGGERS, R.; JAEGER, P. T. Extraction Systems. In: TZIA, C.; LIADAKIS, G. Extraction optimization in Food Engineering. USA: Marcel Dekker, 2003.

FIORI, L. Supercritical extraction of sunflower seed oil: experimental data and model validation. **The Journal of Supercritical Fluids**, v. 50, n. 3, p. 218-224, 2009. https://doi-org.ez67.periodicos.capes.gov.br/10.1016/j.supflu.2009.06.011

GUNSTONE, F. D. **Vegetable Oils in Food Technology: Composition, Properties and Uses**. Reino Unido: Wiley-Blackwell, 2011.

HAENLEIN, G. F. W. Goat milk in human nutrition. **Small ruminant research**, v. 51, n. 2, p. 155-163, 2004.

JENNESS, R. Composition and characteristics of goat milk: review 1968−1979. **Journal of Dairy Science**, v. 63, n. 10, p. 1605-1630, 1980.

LAMEIRA, C. P.; COELHO, G. L. V.; MOTHE, C. G. Extração de lipídeos da amêndoa de castanha de caju com CO_2 supercrítico. **Ciência e Tecnologia de Alimentos,** Campinas, v. 17, n. 4, p. 405-407, 1997.

LEO, L.; RESCIO, L.; CIURLIA, L.; ZACHEO, G. Supercritical carbon dioxide extraction of oil and α-tocopherol from almond seeds. **Journal of the Science of Food and Agriculture**, v. 85, n. 13, p. 2167-2174, 2005. DOI: 10.1002/jsfa.2244.

MAZZAGLIA, A.; LEGAROVÁ, V.; GIAQUINTA, R.; LANZA, C. M.; RESTUCCIA, C. The influence of almond flour, inulin and whey protein on the sensory and microbiological quality of goat milk yogurt. **LWT**, v. 124, p. 109138, 2020.

NIMET, G.; DA SILVA, E. A.; PALÚ, F.; DARIVA, C.; DOS SANTOS FREITAS, L.; NETO, A. M.; CARDOZO FILHO, L. Extraction of sunflower (*Heliantus annuus* L.) oil with supercritical CO_2 and subcritical propane: Experimental and modeling. **Chemical Engineering Journal**, v. 168, p. 262-268, 2011. DOI: 10.1016/j.cej.2010.12.088

PRZYGODA, K.; WEJNEROWSKA, G. Extraction of tocopherol-enriched oils from Quinoa seeds by supercritical fluid extraction. **Industrial Crops and Products**, v. 63, p. 41-47, 2015. DOI: 10.1016/j.indcrop.2014.09.038.

RAI, A.; MOHANTY, B.; BHARGAVA, R. Fitting of broken and intact cell model to supercritical fluid extraction (SFE) of sunflower oil. **Innovative Food Science & Emerging Technologies**, v. 38, p. 32-40, 2016b. DOI: 10.1016/j.ifset.2016.08.019.

RAI, A.; MOHANTY, B.; BHARGAVA, R. Supercritical extraction of sunflower oil: A central composite design for extraction variables. **Food chemistry**, v. 192, p. 647-659, 2016a. DOI: 10.1016/j.foodchem.2015.07.070.

RIBEIRO, S. D. A. Caprinocultura: criação racional de caprinos. São Paulo: Livraria Nobel. 1998. 320 p.

RICHTER, B. E.; JONES, B. A.; EZZELL, J. L.; PORTER, N. L.; AVDALOVIC, N.; POHL, C. Accelerated solvent extraction: a technique for sample preparation. **Analytical chemistry**, v. 68, n. 6, p. 1033-1039, 1996.

SALGIN, U.; DÖKER, O.; ÇALIMLI, A. Extraction of sunflower oil with supercritical CO_2: Experiments and modeling. **The Journal of Supercritical Fluids**, v. 38, n. 3, p. 326-331, 2006. DOI: 10.1016/j.supflu.2005.11.015).

SANTOS, D. N.; SILVA, F. S.; VERDE, A. B.; BITTENCOURT, G. M.; DE OLIVEIRA, A. L. Determination of functional compounds in blue shark (*Prionace glauca*) liver oil obtained by green technology. **Grasas Y Aceites**, v. 71, n. 2, p. e354-e354, 2020.

SHARIF, K. M.; RAHMAN, M. M.; AZMIR, J.; MOHAMED, A.; JAHURUL, M. H. A.; SAHENA, F.; ZAIDUL, I. S. M. Experimental design of supercritical fluid extraction–A review. **Journal of Food Engineering**, v. 124, p. 105-116, 2014.

SINEIRO, J.; DOMÍNGUEZ, H.; NÚÑEZ, M. J.; LEMA, J. M. Ethanolic extraction of sunflower oil in a pulsing extractor. **Journal of the American Oil Chemists' Society**, v. 75, n. 6, p. 753-754, 1998.

TODA, T. A.; VISIOLI, P. D. C. F.; DE OLIVEIRA, A. L.; DA COSTA RODRIGUES, C. E. Conventional and pressurized ethanolic extraction of oil from spent coffee grounds: Kinetics study and evaluation of lipid and defatted solid fractions. **The Journal of Supercritical Fluids**, v. 177, p. 105332, 2021.

TZIA, C. Optimization. In: TZIA, C.; LIADAKIS, G. Extraction optimization in Food Engineering. USA: Marcel Dekker, 2003.

UQUICHE, E.; DEL VALLE, J. M.; ORTIZ, J. Supercritical carbon dioxide extraction of red pepper (*Capsicum annuum L.*) oleoresin. **Journal of Food Engineering,** v. 65, n. 1, p. 55-66, 2004.

CHAPTER II: LITERATURE REVIEW

When discussing extraction processes, it is necessary to understand mass transfer. In both SFE and PLE, and regardless of the solvent, it is considered that the pure solvent pass through a fixed bed extractor packed with solid particles, uniformly, so that it solubilizes the components present in the matrix.

The solvent flow through the matrix characterizes the solute mass transfer from the solid phase to the liquid or supercritical fluid phase (solvent), continuously, until equilibrium is reached (MOURA, 2004).

The solubility difference is responsible for such compound migration, since the solvent has a greater ability to solubilize and separate the compound of interest than other components present in the system. According to Berk (2018), this process happens by a sequence of steps, starting with the moistening of the solid surface with solvent. Then, there is diffusion of the solvent within the solid matrix, with solubilization of extractables; Subsequently, diffusion of solutes from the interior of the solid particles to their surface is promoted. Finally, dispersion of the solutes in the solvent surrounding the solid particles occurs.

Solid-liquid extraction processes, such as SFE and PLE, discussed in this thesis, are applied in many different areas, and are very present in the study of foods. However, each raw material has particular characteristics, and therefore must be evaluated individually (KHODDAMI; WILKES; ROBERTS, 2013), thus determining the best extraction conditions for the compounds of interest.

II.1. Sunflower oil extraction by supercritical fluid

For extraction of plant components, both traditional methods with organic solvents and supercritical extraction methods can be used (ANDREO; JORGE, 2006).

In gas-liquid interaction, the critical point of a pure substance is usually defined as the temperature and pressure at which the gaseous and liquid phases become indistinguishable; then, when a substance is heated and compressed to its critical point, the formation of the "supercritical phase" is observed - in the case of pure carbon dioxide (CO_2), the critical point is found at 31.1 ºC and 7.4 MPa (DUNFORD et al., 2003), being demoninated as supercritical carbon dioxide ($scCO_2$).

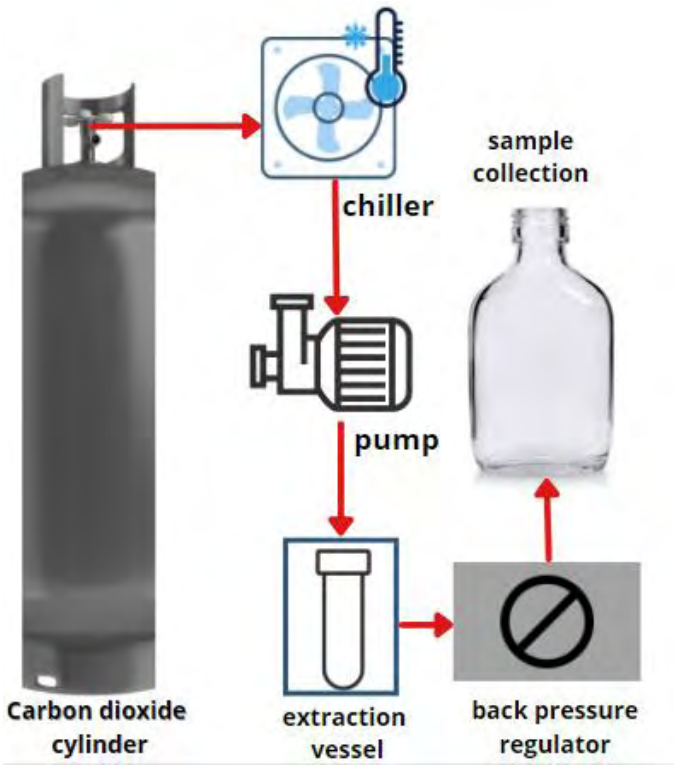
One of the particularities of supercritical fluid extraction (SFE) is the malleability of density in the supercritical region, since an increase in temperature leads to a decrease

in density. Thus, under supercritical conditions, the solvent density approaches the values of the "liquid" densities, and the SFE starts to act as a liquid solvent. At the same time, as the temperature is increased, the solvent density decreases and approaches the "gas" density (DUNFORD et al., 2003). Thus, the solvent used in SFE has both liquid and gaseous solvent characteristics.

The supercritical extraction process facilitates the contact of the plant substrate and supercritical CO_2 in the extractor under constant pressure (P) and temperature (T) conditions. P is controlled by a high pressure pump and a micrometric back pressure control. The gas-separated extract was collected continuously in a smaller collection chamber on ice. The volume of CO_2 is measured by a pump flow meter. The extraction system was automated, with operations and variables (i.e., T, P, and CO_2 flow) controlled by software (Process Suit by SFE, Thar, Waters, Milford, USA).

The summarized process of SFE without the use of co-solvents can be seen in Figure 1. It is important to note that, for the extraction to occur properly, the operating conditions (pressure and temperature) must be previously defined and applied, in a static period, before the beginning of the extraction. This ensures that all the extraction cell is under the same conditions. Then, to start the extraction, the CO_2 flow rate is adjusted. It is pumped into the extraction cell and must be constant, maintaining the defined pressure and temperature. The compound is continuously extracted from the matrix, and collected at the end of the extraction line. It is important that this collection is performed at low pressure and often in cooled flasks, to avoid the loss of volatile compounds. The CO_2 can be recovered in its gaseous state and the sample can be collected after the end of the extraction period.

Figure 1 – Simplified flow diagram of supercritical fluid extraction system



Source: own authorship.

Upon contact with the supercritical solvent, in SFE, the solid substrate (matrix) absorbs it, expanding both its cellular structure and its intracellular channels. Thus, there is a facilitation of mass transfer, coupled with the solubilization of compounds, which are transferred from inside the matrix to its surface (diffusion). Then, the solubilized compound is transferred from the surface to the supercritical solvent, being sent to the sample collection vessel (BRUNNER, 1994).

Since the fluid has a high density, with apolar interactions, it therefore has an intense extractive power (MAUL et al., 1996).

For obtaining vegetable oils, extraction with $scCO_2$ allows good separation of phytosterols and vitamin E, in a more efficient and pure way and with lower waste when compared to the conventional process (ASL et al., 2020). Lameira et al. (1997) demonstrated that increasing the pressure resulted in an increase in the efficiency of lipid extraction from cashew nuts with $scCO_2$.

According to Maul et al. (1996), extraction with $scCO_2$ is indicated for lipophilic compounds and apolar substances. Since tocopherol (vitamin E) is fat-soluble, the technique shows promise for the extraction process. Producing clean products and waste, CO_2 has already been used for decades for the supercritical extraction of natural products.

Moreover, $scCO_2$ presents advantageous characteristics to the extraction, when compared to conventional methods (REVERCHON; OSSEO, 1994; UQUICHE et al., 2004; MONROY, 2018).

To increase the efficiency and better use of the solvent, in the extraction using SFE, the CO_2 flow can be maintained with a pump, using a gas recovery system, such as water or activated carbon (EGGERS; JAEGER, 2003).

Supercritical extraction of sunflower oils is not an unknown process, already in 1996, the group of Prof. Maria Cocero in Spain started studies on the yield of sunflower oil extraction using $scCO_2$ with ethanol (COCERO; CALVO, 1996). Nimet et al. (2011) extracted sunflower oil using $scCO_2$ and propane in a subcritical state, and more recently Rai et al. (2016a; 2016b) also researched and modeled the sunflower oil extraction process with supercritical fluid. These researches sought extraction with high yield, and directed this project. This project stands out by associating high yield and vitamin E enrichment of the oil simultaneously.

The use of $scCO_2$ for product enrichment has been studied in recent years. Uquiche, Millao and Del Valle (2021) evaluated the effect of extrusion as a densification process on the yield and quality of $scCO_2$–extracted red pepper oleoresin, and maximized the extraction yield and carotenoid yield using SFE. Menezes et al. (2021) developed a process that was efficient to obtain Tucumã-of-Pará oil rich in carotenoids by $scCO_2$ extraction. Vladic et al. (2020) stated that plum kernel seeds possess high potential as an alternative oil source due a high amount of oleic acid and tocopherols when extracted using $scCO_2$.

II.2. Sunflower oil extraction with pressurized ethanol

The use of alcohol in sub or supercritical conditions dispenses the use of catalysts, enabling an efficient extraction in reduced time and with some advantages, such as increased solubility of various compounds, improved mass transfer and, mainly, ease in separating and purifying the products after extraction (SILVA; OLIVEIRA, 2014; RATHORE; MADRAS, 2007).

The extraction with pressurized liquid (PLE), with ethanol as solvent, has currently been characterized as an efficient technique since it promotes extractions with reduced time. High temperatures (T) reduce the viscosity of the solvent, and facilitate its penetration and diffusion without degradation of solutes, and high pressure (P) maintains the solvent in the liquid state under conditions of T, in which it would be in the vapor

state at atmospheric pressure (KAUFMANN; CHRISTEN, 2002; WANG; WELLER, 2006).

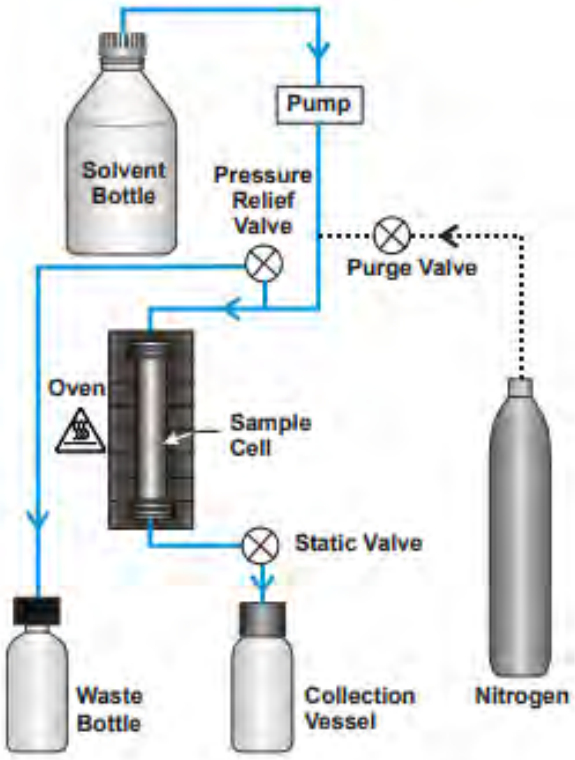
The operation of a PLE extractor can be seen in Figure 2. The Dionex ASE 150 Accelerated Solvent Extractor (Thermo Scientific) performs an intermittent process with extract purge system. The equipment is maily composed by a solvent bottle and its pump; a purge valve responsible for adding the nitrogen to the system; a pressure relief vale; a stainless steel extraction cell (34, 66, or 100 mL), inserted into an closed oven; an static valve; and two bottles: a waste bottle, connected to the pressure relief vale, and a collection bottle, where the extract is stored during the process. All the processes are automatized, and samples are extracted one at a time, and the extraction process is typically completed in 15 to 25 minutes. The posible variables are: temperatura, static time, rinse volumen, purge time, static cycle, and cell type.

In this study, the rinse volume of the solvent, chosen according to the capacity (%) of the extraction cell, and divided by the number of cycles comes into contact with the matrix in each cycle at a set time. After establishing the equilibrium for P (fixed at 10.34 MPa) which remains constant throughout the extraction, a fraction of the extract is purged and collected for each cycle. During the collect, a new fraction of rinse solvent enters the cell simultaneously. At the last stage, the extract remaining in the extractor is purged with N_2 to ensure the entire material is collected. Although ethanol was used in this study, this extractor is suitable for using various solvents such as isopropanol, hexane, and water. Since the extractor cell is kept in an oven (on laboratory scale), its temperature is constant and uniform. The high temperature reduces the viscosity and increases the diffusivity of the solvent in the matrix, improving the interaction between the compounds and the solvent so that solubility is increased while increasing the desorption of the compound from the matrix.

Extraction with pressurized liquid solvent can be done with continuous or intermittent solvent flow. In both operations, the extraction cell is filled with the matrix (raw material). The set is taken to the equipment's oven, which is then programmed according to the desired variables. In PLE with intermittent purge of the extract, the studied process variables are T, solvent rinse volume (VS), number of cycles (C), P, and static time (St) and purge time. The pump is responsible for sending the solvent to the cell that fills the void volume in the fixed bed extractor, which is then pressurized. At this point, with high temperature, the solvent is expanded, increasing the pressure to the set point. Then, the extraction enters a static period (St), after this period, the extract is purged

and a new volume (rinse volume) enters the extractor, concomitantly with the collection, keeping the pressure and temperature constants. From then on, a new cycle (C) begins, in which the solvent will remain in contact with the matrix for the same static time (St). The cycles will be repeated according to the setting or experimental design and the total rinse volume (VS), expressed as a percentage of the extraction cell volume, will be divided by the number of cycles adopted in each extraction. At the end of the cycles, there is the final purge of the extract remaining in the extractor, using nitrogen (N_2) at 150 psi. At this point, the collection cell, containing solvent + extracted compounds, can be obtained, and treated according to the proper procedure.

Figure 2 - Flowchart of the pressurized extraction process



Source: THERMO SCIENTIFIC. Dionex ASE 150 Accelerated Solvent Extractor Operator's Manual. 2011. Available at: https://tools.thermofisher.com/content/sfs/manuals/75688-Man-ASE-ASE-150-Operators-Dec2011-065207-03.pdf. Access: nov. 2021.

The efficiency of ethanol under atmospheric pressure conditions in solubilizing tocopherols was demonstrated by Baümler et al. (2016). In this experiment it was demonstrated that almost all of the oil was extracted from sunflower seed pellets within the first thousand seconds (approximately 17 minutes) for both T = 50 and 60 ºC. In

addition, the authors found that, compared to extraction with hexane, ethanol extracted 70% less crystallizable waxes and at least 38% more tocopherols and phospholipids.

Oliveira et al. (2014) studied the conditions for extraction of compounds from pitanga seeds using pressurized liquid ethanol. Aiming at this, they examined the effect of four process variablesT, St, C, and the VS at each cycle. According to the authors, T, St and their interactions showed significant effects ($P<0.05$) and had a positive effect on yield. The effect of T is higher than that of St. However, the C and VF used in each cycle had no effect, suggesting that the use of large amounts of solvent is not required to obtain higher yields. Interestingly, T, St and St×T are significant variables for both linear and quadratic models. However, for quadratic models, C is also significant. The authors believe that the longer the contact time between the solvent and the matrix, the better the performance.

Colivet et al. (2016) evaluated extractor cell size on the efficiency of sunflower seed oil extraction yield, and concluded that the only significant difference in the oil extraction yield is related to the T, since temperature of extraction had a significant effect on the extraction efficiency of oils and compounds with antioxidant activity, with higher yields of oil obtained at 80 ºC and better antioxidant activity at temperatures of 60 ºC. Thus, although the raw material is different from the seed used in this study, their results help us to define a single cell size (extractor) for the process.

PLE has been used, in recent years, for the extraction of vegetable oils, such as soybean oil (RODRIGUES; CARDOZO-FILHO; DA SILVA, 2017), and also for the extraction of specific compounds, such as the enrichment of galician algae extracts with high quality fatty acids and antimicrobial and antioxidant properties (OTERO et al., 2018).

However, no studies have been found using PLE as a way to enrich sunflower oil with tocopherols.

Based on these previous studies, the design for sunflower seed oil extraction occurred with fewer cycles, volume and cell size, in order to use the least amount of solvent possible, and application of milder temperatures, seeking a green and efficient extraction.

Thus, it can be seen that the use of ethanol by the industry for sunflower oil extraction presents very promising characteristics, using a green, safe, economical and sustainable solvent.

II.3. Sunflower seed and tocopherols in plant matrices

In nature, eight substances have been found to have vitamin E activity: α-, β-, γ- and δ-tocopherol; and α-, β-, γ- and δ-tocotrienol (SEN; KANNA; ROY, 2006). In this study, we will focus on tocopherols.

According to the Institute of Medicine (U.S.A.), from the age of 14 years, it is recommended to consume at least 15 mg/day of vitamin E, with 1,000 mg/day being the maximum tolerable. Its deficiency can lead to peripheral neuropathy, skeletal myopathy, and retinopathy pigmented, among others, and can come from both malnutrition and genetic deficiencies (NATIONAL RESEARCH COUNCIL, 2006).

After evaluating data from 4,322 individuals aged 60 years or older, Fisberg et al. (2013) report that the Brazilian elderly have a high inadequacy of intake of nutrients, recognized as protectors against chronic diseases. Inadequacies were identified for vitamins E, D, A, calcium, magnesium and pyridoxine in both genders. The most worrisome cases were for vitamins D and E, with almost 100% inadequacy in most regions of the country.

Nimet et al. (2011) found an average of 0.79 mg of vitamin E per gram of sunflower oil when extracted with CO_2. Considering that the minimum daily requirement is 15 mg of vitamin E (NATIONAL RESEARCH COUNCIL, 2006), approximately 19 g of oil would be required to supply 100 % of the recommended intake, considering full absorption (100 % bioavailability).

In recent decades, obtaining tocopherols by SFE has also been evaluated using other plant matrices. Davarnejad et al. (2010) studied the solubility of tocopherols in crude palm oil when extracted with $scCO_2$ (80, 100 and 120 ºC). The authors reported that pressure and temperature affected the solubility of tocopherol in CO_2, and the maximum solubility obtained (approximately 2.27%) was obtained at conditions of 5.44 and 7.68 MPa, and at 120 and 100 ºC, respectively.

Puah et al. (2007) evaluated the solubility of tocopherols from palm oil extracted by supercritical fluid (14 to 30 MPa, 40 to 80 ºC). According to the results obtained in the study, the solubility of tocopherols ranged from 0.94×10^{-3} to 0.018 mg / g CO_2, and the highest concentration was obtained at 22 MPa and 40 ºC. According to the authors, the solubility of tocopherols in palm oil may have been decreased by the extraction of other more soluble compounds, such as TAGs.

Asl et al. (2020) evaluated rapeseed oil extraction using $scCO_2$. The ideal parameters found were 40 ºC, 40 MPa and 5% ethanol as co-solvent, in addition to pre-

treatment with saponification. According to the authors, this condition allowed the recovery of α-tocopherol, and its content can be up to 3 times higher in the oil obtained by SFE than in that obtained conventionally by Soxhlet. The use of ethanol may have increased the solubility of the compounds (ASL et al., 2020).

Bravi et al. (2007) extracted oil from crushed grapes seeds by $scCO_2$. Aiming to obtain α-tocopherol, the optimal conditions were 25 MPa and 80 °C, resulting in 265 ppm of α-tocopherol. The authors reported that, compared to conventional extraction with hexane, higher α-tocopherol concentration was obtained (265 and 44 ppm for $scCO_2$ and hexane, respectively), but lower extraction yield.

In quinoa seeds, Przygoda and Wejnerowska (2015) evaluated the extraction of tocopherol-rich oil by SFE (30 to 130 ºC, 12.5 to 28.5 MPa, 15 - 180 min). The authors found a condition at 130 ºC and 180 min, in which it was possible to obtain tocopherol content of 336.0 mg/100 g of oil. This is a significant result because it is more than four times higher than that obtained with classical hexane extraction. In addition, the extraction time was directly proportional to the quinoa oil yield and tocopherol content, regardless of the P and T conditions.

Finally, the chemical composition of sunflower seed has been studied for various purposes, such as for broiler feed (MANTOVANI et al., 2000) and for increasing the stability of the extracted oil (OLMEDO et al., 2018; PRADHANANGA; MANANDHAR, 2018). However, no records of its use for enrichment of human food products, specifically, have been found. This application shows great promise due to the high presence of vitamin E in this oil, in addition to the ease of access to the seed and the extraction technology using $scCO_2$, combined with the nutritional needs of the population, especially the elderly.

II.4. Goat milk yogurt

Yogurt is a fermented dairy food recognized for its health benefits, with the presence of the lactic acid bacteria *Streptococcus salivarius ssp. termophilus* and *Lactobacillus delbrueckii ssp. Bulgaricus*, which have therapeutic properties (INOUE et al., 1998).

The choice for goat milk comes from its high digestibility, due to the smaller fatty acid chains, combined with a low allergenic potential when compared to cow's milk. Thus, it is more suitable for consumption by the elderly and intolerant people, facilitating digestion and absorption of the product (HAENLEIN, 2004). In addition, goat milk can

be obtained from the institution where the experiment will be conducted, making the process feasible.

Besides the digestibility, goat's milk is known as hypoallergenic (CARVALHO et al., 2022). This characteristic is associated with α-S1 casein, which is considered a major cause of cow's milk allergies and is found in lesser amounts in goat's milk. Its molecular and antigenic structure differs in the milk of both species (CEBALLOS et al., 2009; RIBEIRO, 1998).

According to Cruz et al. (2016), the fats present in goat milk are formed mostly by medium and short chain fatty acids. In contrast to cow's milk, its fat globules are smaller and therefore more rapidly absorbed. This results in better digestibility and lower residues in the intestine.

Still, it has similar properties to cow's milk - while cow's milk has, on average, 87.7 % moisture, 4.5 % lactose, 3.4 % protein, 3.7 % fat, and 0.7 % minerals, goat's milk has, for the same attributes, 87.5, 4.5 3.1, 3.9, and 0.8 %, respectively (CRUZ et al., 2016).

In recent years, goat's milk yogurt has been a vehicle for several nutritional compounds, and is the basis for enriched and nutritious foods. Silva et al. (2022) incorporated phenolic-rich ingredients from integral Isabel grape to improve the nutritional, functional and sensory characteristics of probiotic goat milk yogurt. Mazzaglia et al. (2020) evaluated the influence of almond flour, inulin and whey protein on the sensory and microbiological quality of goat milk yogurt, reducing the goaty aroma and flavor and improving the flavor of the goat milk products. Feng et al. (2019) evaluated the quality characteristics and antioxidant activities of goat milk yogurt with added jujube pulp, suggesting that the pulp can effectively improve the flavor and the health benefits of the final product.

Although goat milk can have a marked characteristic flavor, some technological procedures (such as homogenization and cold storage) are capable of diminishing such an attribute (MORGAN; GABORIT, 2001).

Uysal-pala et al. (2006) evaluated the sensory differences between goat milk yogurts produced with milks from different goat species. The authors reported that lactation periods affected the sensory characteristics of the yogurts and that in describing attributes, even yogurt produced with cow's milk (control) was described as "goaty".

Thus, the concern with the sensory characteristics of the product is also a constant. Current studies seek alternatives to increase the acceptance of goat milk yogurt, enriched

or not (BEZERRIL et al., 2022; SILVA et al., 2022; BULUT; TUNÇTÜRK; ALWAZEER, 2021; JIA; LIU; SHIU, 2021; DE SANTIS et al., 2019).

REFERENCES

ANDREO, D.; JORGE, N. Antioxidantes naturais: técnicas de extração. **CEPPA,** v. 24, n. 2, p. 319-336, 2006.

ASL, P. J.; NIAZMAND, R.; YAHYAVI, F. Extraction of phytosterols and tocopherols from rapeseed oil waste by supercritical CO_2 plus co-solvent: A comparison with conventional solvent extraction. **Heliyon**, v. 6, n. 3, p. e03592, 2020.

BAÜMLER, E. R.; CARRÍN, M. E.; CARELLI, A. A. Extraction of sunflower oil using ethanol as solvent. **Journal of Food Engineering**, v. 178, p. 190-197, 2016.

BERK, Z. Extraction. In: BERK. Z. (Ed.). **Food process engineering and technology**. 3 ed. Oxford: Academic Press, 2018. 289-310 p.

BEZERRIL, F. F.; PIMENTEL, T. C.; DA SILVA SANT'ANA, A. M.; DE SOUZA, M. D. F. V.; DE MEDEIROS, L. L.; GALVÃO, M.; MADRUGA, S.M.; QUEIROGA, S. C. M.; MAGNANI, M. Lacticaseibacillus casei 01 improves the sensory characteristics in goat milk yogurt added with xique-xique (*Pilosocereus gounellei*) jam through changes in volatiles concentration. **LWT**, v. 154, p. 112598, 2022.

BRUNNER, G. Gas extraction: an Introduction to Fundamentals of Supercritical Fluids and the Application to Separation Processes. 1. ed. *Darmstadl*, Alemanha: Steinkopff, 1994. 387p.

BULUT, M.; TUNÇTÜRK, Y.; ALWAZEER, D. Effect of fortification of set-type yoghurt with different plant extracts on its physicochemical, rheological, textural and sensory properties during storage. **International Journal of Dairy Technology**, v. 74, n. 4, p. 723-736, 2021.

CARVALHO, C. C. de; BODINI, R. B.; SOBRAL, P. J. A.; OLIVEIRA, A. L. Ice creams made from cow's and goat's milks with different fat concentrations: physical-chemical and sensory properties. **Food Science and Technology**, v. 42, p. e79721, 2022.

CEBALLOS, L. S.; MORALES, E. R.; DE LA TORRE ADARVE, G.; CASTRO, J. D.; MARTÍNEZ, L. P.; SAMPELAYO, M. R. S. Composition of goat and cow milk produced under similar conditions and analyzed by identical methodology. **Journal of food Composition and Analysis**, v. 22, n. 4, p. 322-329, 2009.

COCERO, M. J.; CALVO, L. Supercritical fluid extraction of sunflower seed oil with CO_2 - ethanol mixtures. **Journal of the American Oil Chemists' Society**, v. 73, n. 11, p. 1573-1578, 1996.

COLIVET, J.; OLIVEIRA, A. L. de; CARVALHO, R. A. de. Influence of the bed height on the kinetics of watermelon seed oil extraction with pressurized ethanol. **Separation and Purification Technology**, v. 169, p. 187-195, 2016.

CRUZ, A. G.; ZACARCHENCO, P. B.; OLIVEIRA, C. A. F.; CORASSIN, C. H. **Química, bioquímica, análise sensorial e nutrição no processamento de leite e derivados.** 1.ed. Rio de Janeiro: Elsevier, 2016.

DE SANTIS, D.; GIACINTI, G.; CHEMELLO, G.; FRANGIPANE, M. T. Improvement of the sensory characteristics of goat milk yogurt. **Journal of food science**, v. 84, n. 8, p. 2289-2296, 2019.

DUNFORD, N. T.; KING, J. W.; LIST, G. R. Supercritical Fluid Extraction in Food Engineering. In: TZIA, C.; LIADAKIS, G. Extraction optimization in Food Engineering. USA: Marcel Dekker, 2003.

EGGERS, R.; JAEGER, P. T. Extraction Systems. In: TZIA, C.; LIADAKIS, G. Extraction optimization in Food Engineering. USA: Marcel Dekker, 2003.

FENG, C.; WANG, B.; ZHAO, A.; WEI, L.; SHAO, Y.; WANG, Y.; CAO, B.; ZHANG, F. Quality characteristics and antioxidant activities of goat milk yogurt with added jujube pulp. **Food chemistry**, v. 277, p. 238-245, 2019.

FISBERG, R. M.; MARCHIONI, D. M. L.; CASTRO, M. A. D.; VERLY JUNIOR, E.; ARAÚJO, M. C.; BEZERRA, I. N.; PEREIRA, R. A.; SICHIERI, R. Ingestão inadequada de nutrientes na população de idosos do Brasil: Inquérito Nacional de Alimentação 2008-2009. **Revista de Saúde Pública**, v. 47, p. 222s-230s, 2013.

HAENLEIN, G.F.W. Goat milk in human nutrition. **Small Ruminant Research**, v.51, n.2, p.155-163, 2004.

INOUE, K.; SHIOTA, K.; ITO, T. Preparation and properties of ice cream type frozen yogurt. **International Journal of Dairy Technology**, v.51, n.2, p.44-50, 1998.

JIA, W.; LIU, Y.; SHI, L. Integrated metabolomics and lipidomics profiling reveals beneficial changes in sensory quality of brown fermented goat milk. **Food Chemistry**, p. 130378, 2021.

KAUFMANN, B.; CHRISTEN, P. Recent extraction techniques for natural products: microwave-assisted extraction and pressurized solvent extraction. **Phytochemical Analysis**, v. 13, p. 105–113, 2002.

KHODDAMI, A.; WILKES, M.; ROBERTS, T. Techniques for analysis of plant phenolic compounds. **Molecules**, v. 18, n. 3, p. 2328–2375, 2013.

LAMEIRA, C. P.; COELHO, G. L. V.; MOTHE, C. G. Extração de lipídeos da amêndoa de castanha de caju com CO_2 supercrítico. **Ciência e Tecnologia de Alimentos,** Campinas, v. 17, n. 4, p. 405-407, 1997.

MAUL, A. A.; WASICKY, R.; BACCHI, E. M. Extração por fluido supercrítico. **Revista Brasileira de Farmacognosia**, v. 5, n. 2, p. 185-200, 1996.

MAZZAGLIA, A.; LEGAROVÁ, V.; GIAQUINTA, R.; LANZA, C. M.; RESTUCCIA, C.The influence of almond flour, inulin and whey protein on the sensory and microbiological quality of goat milk yogurt. **LWT**, v. 124, p. 109138, 2020.

MENEZES, E. G. O.; BARBOSA, J. R.; PIRES, F. C. S.; FERREIRA, M. C. R.; SILVA, A. P. D. S.; SIQUEIRA, L. M. M.; DE CARVALHO JUNIOR, R. N. Development of a new scale-up equation to obtain Tucumã-of-Pará (*Astrocaryum vulgare Mart.*) oil rich in carotenoids using supercritical CO_2 as solvent. **The Journal of Supercritical Fluids**, p. 105481, 2021.

MONROY, Y. M. **Extração e fracionamento de compostos fenólicos de milho roxo, de própolis verde e de própolis vermelha usando tecnologia supercrítica.** 2018. 291 f. Tese (Doutorado) – Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Campinas, 2018.

MORGAN, F.; GABORIT, P. The typical flavour of goat milk products: technological aspects. **International Journal of Dairy Technology**, v.54, n.1, p.38-40, 2001.

MOURA, L. S. Obtenção por extração supercrítica do extrato de funcho (*Foeniculum vulgare*): determinação das isotermas de rendimento global, de parâmetros cinéticos e do equilíbrio de fases. Campinas, SP: [s.n.], 2004.

NATIONAL RESEARCH COUNCIL. Institute of Medicine. Food and Nutrition Board. **Dietary Reference Intakes**: The Essential Guide to Nutrient Requirements Washington: The National Academies Press; 2006.

NIMET, G.; DA SILVA, E. A.; PALÚ, F.; DARIVA, C.; DOS SANTOS FREITAS, L.; NETO, A. M.; CARDOZO FILHO, L. Extraction of sunflower (*Heliantus annuus* L.) oil with supercritical CO_2 and subcritical propane: Experimental and modeling. **Chemical Engineering Journal**, v. 168, p. 262-268, 2011. DOI: 10.1016/j.cej.2010.12.088

OLIVEIRA, A. L.; DESTANDAU, E.; FOUGÈRE, L.; LAFOSSE, M. Isolation by pressurised fluid extraction (PFE) and identification using CPC and HPLC/ESI/MS of phenolic compounds from Brazilian cherry seeds (*Eugenia uniflora L.*). **Food chemistry**, v. 145, p. 522-529, 2014.

OTERO, P.; QUINTANA, S. E.; REGLERO, G.; FORNARI, T.; GARCÍA-RISCO, M. R. Pressurized liquid extraction (PLE) as an innovative green technology for the effective enrichment of Galician algae extracts with high quality fatty acids and antimicrobial and antioxidant properties. **Marine drugs**, v. 16, n. 5, p. 156, 2018.

RAI, A.; MOHANTY, B.; BHARGAVA, R. Fitting of broken and intact cell model to supercritical fluid extraction (SFE) of sunflower oil. **Innovative Food Science & Emerging Technologies**, v. 38, p. 32-40, 2016b.

RAI, A.; MOHANTY, B.; BHARGAVA, R. Supercritical extraction of sunflower oil: A central composite design for extraction variables. **Food chemistry**, v. 192, p. 647-659, 2016a.

RATHORE, V.; MADRAS, G. Synthesis of biodiesel from edible and non-edible oils in supercritical alcohols and enzymatic synthesis in supercritical carbon dioxide, **Fuel**, v. 86, p. 2650–2659, 2007.

REVERCHON, E.; OSSEO, L. S. Comparison of processes for the supercritical carbon-dioxide extraction of oil from soybean seeds. **Journal of the American Oil Chemists Society,** v. 71, n. 9, p. 1007-1012, 1994.

RIBEIRO, S. D. A. Caprinocultura: criação racional de caprinos. São Paulo: Livraria Nobel. 1998. 320 p.

RODRIGUES, G. de M.; CARDOZO-FILHO, L.; DA SILVA, C. Pressurized liquid extraction of oil from soybean seeds. **The Canadian Journal of Chemical Engineering**, v. 95, n. 12, p. 2383-2389, 2017.

SEN, C. K.; KHANNA, S.; ROY, S. Tocotrienols: Vitamin E beyond tocopherols. **Life sciences**, v. 78, n. 18, p. 2088-2098, 2006.

SILVA, C.; OLIVEIRA, J. V. Biodiesel production through non-catalytic supercritical transesterification: current state and perspectives, **Brazilian Journal of Chemical Engineering**, v. 31, p. 271-285, 2014.

SILVA, F. A.; DO EGYPTO, R. D. C. R.; DE SOUZA, E. L.; VOSS, G. B.; BORGES, G. D. S. C.; DOS SANTOS LIMA, M.; PINTADO, M. M. E.; DA SILVA VASCONCELOS, M. A. Incorporation of phenolic-rich ingredients from integral valorization of Isabel grape improves the nutritional, functional and sensory characteristics of probiotic goat milk yogurt. **Food Chemistry**, v. 369, p. 130957, 2022.

UQUICHE, E.; DEL VALLE, J. M.; ORTIZ, J. Supercritical carbon dioxide extraction of red pepper (*Capsicum annuum L.*) oleoresin. **Journal of Food Engineering,** v. 65, n. 1, p. 55-66, 2004.

UQUICHE, E.; MILLAO, S.; DEL VALLE, José M. Extrusion affects supercritical CO_2 extraction of red pepper (*Capsicum annuum L.)* oleoresin. **Journal of Food Engineering**, v. 316, p. 110829, 2022.

UYSAL-PALA, C.; KARAGUL-YUCEER, Y.; PALA, A.; SAVAS, T. Sensory properties of drinkable yogurt made from milk of different goat breeds. **Journal of sensory studies**, v. 21, n. 5, p. 520-533, 2006.

VLADIC, J.; GAVARIC, A.; JOKIC, S.; PAVLOVIC, N.; MOSLAVAC, T.; POPOVIC, L.; MATIAS, A.; AGOSTINHO, A.; MARIJA, B.; VIDOVIC, S. Alternative to Conventional Edible Oil Sources: Cold Pressing and Supercritical CO_2 Extraction of Plum (*Prunus Domestica L.)* Kernel Seed. **Acta Chimica Slovenica**, v. 67, n. 3, p. 778-784, 2020.

WANG, L.; WELLER, C. L. Recent advances in extraction of nutraceuticals from plants. **Trends in Food Science & Technology**, v. 17, p. 300–312, 2006.

CHAPTER III: SUNFLOWER SEED OIL (*HELIANTHUS ANNUUS*) ENRICHED BY TOCOPHEROLS EXTRACTED BY SUPERCRITICAL FLUID AND PRESSURIZED ETHANOL WITH INTERMITTENT SOLVENT PURGE

ABSTRACT

This research sought to identify the optimal conditions for obtaining tocopherol enriched sunflower seed oil by supercritical fluid extraction (SFE, carbon dioxide) and by pressurized liquid extraction (PLE, ethanol). Extractions were evaluated for total yield, tocopherol content (α, β, γ, and δ), fatty acid profile, and triacylglycerols in the products obtained both by PLE, the optimized condition for yield in SFE, and in commercial oil. To identify the optimal conditions for the extraction of sunflower seed oil enriched with tocopherols, Central Compound Rotated Design [CCRD] was used for both SFE and PLE. SFE design was performed two independent variables (temperature, ºC; pressure, MPa), and two axial points (-α, + α). PLE design considered the static purging time of the solvent (min), the temperature (ºC) and the rinse volume of solvent (ethanol, %). Resembling the number of cycles (C = 4), P was set at 1,500 psi (10.34 MPa). For yield, the variables that influenced SFE and PLE were pressure and temperature, respectively. However, related to tocopherol contents, there was a variation: in the oil obtained by SFE, temperature affected the α-tocopherol content, while pressure influenced γ-tocopherol and δ-tocopherol. Thus, the total tocopherol content was influenced by both variables. In the oil obtained by PLE, no variable influenced the tocopherol contents. The greatest recovery of crude oil obtained by SFE was 87.6%, and 93.9% by PLE. The greatest content of tocopherols in SFE oil was observed at 60 ºC and 18 MPa (91.17 mg of tocopherols/100 g). The oil obtained via PLE presented up to 83.16 mg/100 g of tocopherols. There was no difference in fatty acid profile among the products. The two fatty acids in greatest content in sunflower oil are linoleic and oleic, and the three triacylglycerols estimated most frequently are groups C54:5 (linoleic-linoleic-oleic) and C54:6 (linoleic-linoleic-linoleic). The study of quality products obtained by innovative technology suggest the possibility of using ethanol, a green solvent, safe for health and renewable to obtain sunflower oil, instead of hexane.

Keywords: green extraction; PLE; SFE.

III.1. INTRODUCTION

Hexane has been used for decades in the conventional extraction of vegetable oils and fats. After extraction, the crude oil goes through a series of steps, being filtered, degummed (to remove phospholipids), deacidified, bleached, and deodorized to then be considered a refined oil suitable for culinary use (WAKELYN; WAN, 2003).

The extraction processes depend on the desired purpose and the physical properties of the material to be extracted, as well as the product obtained. In the conventional hexane extraction method, the raw material must undergo pretreatments, such as heating and lamination, which can reach 20 min at 90-95 ºC. After extraction, the solvent needs to be recovered from the product and the residue, both to reach a high-quality product and by environmental issues (EGGERS; JAEGER, 2003).

In a conventional batch extraction, the extractors are filled with the material to be extracted and then hexane is added. Then there is a waiting time, and the miscella, a mixture consisting of solvent plus oil, is drained from the system, for later separation. Lately, the solvent batch system has been less used due to the long waiting time and a large amount of solvent used (EGGERS; JAEGER, 2003).

For the extraction of vegetable compounds, both traditional methods with organic solvents and supercritical extraction methods can be used (ANDREO; JORGE, 2006). In gas-liquid interaction, the critical point of a pure substance is usually defined as the temperature and pressure at which the gas and liquid phases become indistinguishable. Then, when a substance is heated and compressed to its critical point, the formation of the “supercritical phase” is observed - in the case of pure carbon dioxide (CO_2), the critical point is found at 31.1 ºC and 7.4 MPa (DUNFORD et al., 2003).

The extraction of vegetable oils and fats by green technologies has been studied by several authors, as discussed in Chapter I.

The use of supercritical extraction in food engineering has a historical precedent from its application to decaffeinate coffee, to the present day, especially with raw materials and natural products, since it is considered a “green” extraction process (DUNFORD et al., 2003). This technology has been implemented in several countries, such as Germany, the United States, France, and Japan.

One of the particularities of the supercritical fluid extraction (SFE) is the malleability of density in the supercritical region since an increase in temperature leads to a decrease in density. Thus, in supercritical conditions, the density of the solvent approaches the values found in "liquids", and the SFE begins to act as a liquid solvent. At

the same time, once the temperature is increased, the density of the solvent decreases and approaches the “gas” (DUNFORD et al., 2003). Thus, the solvent used in SFE has both liquid and gaseous solvent characteristics.

Optimization is the process of maximizing the amount of something desired or minimizing something undesired, and the parameters that provide such optimization, called "optimal conditions", are part of the "optimal design". For this, there is the determination of independent variables that result in one or more dependent variables (TZIA, 2003).

Pressurized liquid extraction (PLE), with ethanol as a solvent, has currently been characterized as an efficient technique since it promotes extractions with reduced time. High temperatures (T) decrease the viscosity of the solvent and facilitate its penetration and diffusion without degradation of the solutes, and the high pressure (P) keeps the solvent in the liquid state under T conditions, in which it would be at the vapor state in atmospheric pressure (KAUFMANN; CHRISTEN, 2002; WANG; WELLER, 2006).

Cornelio-Santiago et al. (2022) described the oil extraction from pequi and sacha inchi almonds by PLE using isopropanol as the solvent. The authors evaluated the effects of the static time and temperature for oil extraction yield, free fatty acids (FFAs), total phenolic content (TPC) and β-sitosterol content, and concluded that static time and temperature influenced positively the yield and TPC, but not β-sitosterol content and FFAs.

Thus, it is observed that the use of ethanol in the industry for the extraction of sunflower oil has very promising characteristics - in addition to the extraction itself, there is the possibility of aligning processes using a green, safe, and more accessible solvent.

The sunflower seed (*Helianthus annuus*) is rich in vitamin E, standing out for the content of α-tocopherol of high biological value. In common sunflower oil, 91 to 97% of the total tocopherol is type α. Seeds with great oil content can reach 1,120 mg/kg of vitamin E (GUNSTONE, 2011).

For obtaining vegetable oils, extraction with supercritical carbon dioxide [$scCO_2$] allows good separation of phytosterols and vitamin E in a more efficient and pure method and with less waste when compared to the conventional process (ASL et al., 2020). Besides, Baümler et al. (2016) demonstrated the efficiency of ethanol in solubilizing tocopherols under atmospheric pressure conditions. The experiment demonstrated that almost all oil was extracted from the sunflower seed pellets within the first thousand seconds (approximately 17 min), both for T = 50 and 60 ^{o}C. In addition, the authors found

that, compared to hexane extraction, ethanol extracted 70 % less crystallizable waxes and at least 38 % more tocopherols and phospholipids.

This study stands out for presenting in a comparative and simplified way the extraction of sunflower oil enriched by tocopherols using green technologies, supercritical carbon dioxide, and pressurized ethanol, comparing them with its commercial version, resulting in a product with less environmental impact and better nutritional quality.

III.2. MATERIALS AND METHODS

III.2.1. Reagents

CO_2 99 % [SFE-scCO_2] (Linde, Sertãozinho, Brazil); absolute ethanol 99.5 % [PLE] (Dinâmica, Indaiatuba, Brazil); n-hexane [tocopherol analysis; fatty acid profile] (VWR, Fontenay-sous-Bois, France); n-hexane ACS [determination of ethereal extract] (Êxodo Científica, Hortolândia, Brazil); ethyl acetate (Tedia, Fairfield, USA), glacial acetic acid analytical grade (Lab Synth, Diadema, Brazil), tocopherol set (Calbiochem, Darmstadt, Germany) [tocopherol analysis].

The reagents used in the esterification process to determine the fatty acid profile are: sodium hydroxide 99 % (Êxodo Científica, Hortolândia, Brazil); sodium chloride 99 % (Êxodo Científica, Hortolândia, Brazil); sodium sulfate 99 % (Synth, Diadema, Brazil); methyl alcohol 99.8 % (Dinâmica, Indaiatuba, Brazil); hexane 98.5 % (Êxodo Científica, Sumaré, Brazil); and boron trifluoride 14 % in methanol (Aldrich, St. Louis, USA).

III.2.2. Obtaining and characterizing the raw material

The seeds originate from sunflower plants (*Helianthus annuus*), variety Altis 99 (Atlântica Sementes). For characterization, the sunflower seeds were husked, and dried in a forced circulation oven, until they reached a constant mass. The seeds were crushed, sieved, and stored at -22 °C.

The husked sunflower seed was characterized by the quantification of dry matter (DM, method 925.40), mineral matter (MM, method 950.49), crude protein (CP, method 950.48), crude fiber (CF, method 935.53), ethereal extract (EE, method 948.22), non-nitrogen extract (NNE), acid detergent fiber (ADF), ash-free neutral detergent fiber (NDF), nitrogen in the ADF (N-ADF), and nitrogen in the NDF (N-NDF). Such analyzes were performed at the Nutrition Laboratory of the Department of Zootechnics, Faculty of Zootechnics and Food Engineering, University of São Paulo (ZAZ / FZEA / USP), in

triplicate, according to the Association of Official Analytical Collaboration [AOAC] (2005). The determination of ethereal extract occurred by Soxhlet with hexane (ZENEBON et al., 2008), in triplicate.

III.2.3. Preparation of raw material for extraction

To evaluate the extraction processes, rolled seeds with particle size under 2 mm (<10 mesh) were used, without separation of the husks, aiming at simplifying the process.

The diameter of the particles was determined using a set of sieves from the Tyler series and stirrer (BRASIL, 2009), from 10 to 48 mesh (2 to 0.3 mm). The mass retained in the respective sieves was weighed on a semi-analytical scale. The average diameter was determined by the retained masses per sieve.

$$d_m = \frac{\sum_{i=1}^{n}(W_i\, d_i)}{\sum_{i=1}^{n} W_i} \quad (1)$$

where: d_i is the nominal opening of the i-th sieve (mm); d_{i+1} is the nominal sieve opening greater than the i-th sieve (mm); W_i is the mass of the material retained in the i-th sieve (g).

To calculate yield, regardless of the process used, the total mass of oil obtained was considered as total yield in relation to the mass of raw seeds, while recovery was considered as the percentage of oil obtained in relation to the oil content observed in conventional extraction via Soxhlet (55.07 %).

III.2.4. Supercritical extraction

SFE-scCO_2 was performed on Thar-SFC equipment provided by Thar Technologies Inc. (Pittsburgh, USA). The system included high pressure and cosolvent pumps, heat exchangers, two separators, an extractor, and a collector. The sample (≅10 g) was placed in a cylindrical stainless steel extractor basket (290 cm^3, with glass beads to fill the empty space) which was then inserted into the extraction system.

Anticipating dynamic extraction, CO_2 remains in contact with the matrix for 20 min so that equilibrium conditions are achieved and established. From this moment on, CO_2 flows under constant flow conditions (10 g CO_2/min) during the extraction time, also preestablished by the study of kinetics. All equipment is automated, operated by the

Process Suit for SFE software (Thar, Waters, Milford, USA). The collection was performed continuously during extraction in a flask immersed in an ice bath.

III.2.4.1. Kinects

The kinetics of supercritical extraction is normally studied for each different raw material, to establish the fixed extraction time that will be used in the dynamic extraction process. In this test, 10 g of rolled and sieved seeds were placed in a cylindrical extractor basket. The condition chosen to profile the extraction kinetics (25 MPa and 60 ºC) was chosen based on the discussions by Rai et al. (2016a), Nimet et al. (2011), and Cocero and Calvo (1996) (Table 1), and the condition that provided good performance (preferably with mild T) was chosen, in order to preserve the tocopherols.

Table 1 – Sunflower oil extraction research under supercritical conditions

Pressure (MPa)	Temperature (°C)	References
34.5	80	Rai et al. (2016a)
40.0	80	Rai et al. (2016a)
25.0	40	Nimet et al. (2011)
20.0	60	Cocero and Calvo (1996)

Source: own authorship

The extraction was performed in triplicate and the extraction time was determined according to the behavior of the material (rolled sunflower seeds) during the SFE - $scCO_2$ with a flow rate of 10 g CO_2/min (FIORI, 2009). This flow rate and the mass of seeds stored in the fixed bed extractor were chosen due to the limitations of the equipment (collection). The collections of the extracted sunflower oil were performed periodically every 20 min for 460 min.

III.2.4.2. Experimental design for supercritical fluid extraction of sunflower oil

To identify the optimal conditions for the extraction of sunflower seed oil enriched with tocopherols, a Central Compound Rotated Design [CCRD] was used, with two independent variables, and two axial points ($-\alpha$, $+\alpha$) (Table 2).

Table 2 – Levels of independent real variables and rotational central composite design for supercritical extraction of sunflower oil

Variables	Levels				
	$-\alpha$	-1	0	+1	$+\alpha$
Temperature (ºC)	46	50	60	70	74
Pressure (MPa)	18	20	25	30	32

Source: own authorship

The extraction yield and the content of tocopherols were evaluated. Literature and preliminary tests were used to determine the levels for the independent variables, T (ºC) and P (MPa) (Tables 1 and 2). The central points of the design applied for extraction were selected for kinetics studies. The extraction time was 4 h.

III.2.5. Pressurized liquid extraction (PLE)

The oil was also extracted by PLE in a Dionex ASE 150 extractor (Thermo Scientific), using 34 mL extraction cells filled with 10 g rolled and sieved sunflower seeds.

The PLE used in this experiment performs an intermittent process with extract purge system. The rinse volume of the solvent, chosen according to the capacity (%) of the extraction cell, and divided by the number of cycles comes into contact with the matrix in each cycle at a set time. After establishing the equilibrium for P (fixed at 10.34 MPa) which remains constant throughout the extraction, a fraction of the extract is purged and collected for each cycle. During the collect, a new fraction of rinse solvent enters the cell simultaneously. At the last stage, the extract remaining in the extractor is purged with N_2 to ensure the entire material is collected. The extraction time was ≅ 30 min.

III.2.5.1. Experimental design for obtaining sunflower oil with pressurized ethanol

In conventional extraction of vegetable oils, the ratio of solute to solvent (usually hexane) remains at 1:1 (m:m). In this countercurrent solvent extraction process, the minimum number of cycles adopted is four (KEMPER, 2005), and this number was assumed in this study.

The initial conditions (preliminary tests) were stipulated according to the studies of other seeds and similar methods found in the literature (ROSTAGNO et al., 2004), and from preliminary tests.

The variables studied in the optimization of the extraction process with pressurized ethanol (without dilution) of sunflower oil enriched with tocopherols were the contact time (St, min), the temperature (T, ºC) and the rinse volume of solvent (ethanol, VS, %). Resembling the number of cycles (C = 4), P was set at 1,500 psi (10.34 MPa).

For the selection of variables, linear experimental design (Box, Hunter, and Hunter) was used, with three variables, and the extraction yield as a response (Table 3). Significant variables were selected at 90 % ($p \leq 0.1$).

After selecting variables, a CCRD was applied to two independent variables, and two axial points ($-\alpha$, $+\alpha$), to identify the optimal conditions for obtaining sunflower seed oil enriched with tocopherols, having VS and T as independent variables (Table 3), and extraction yield and tocopherol content as dependent variables. After extraction, the ethanol was recovered in a rotary evaporator (MARCONI, MA-120, Piracicaba, BR) at 40 ºC, in a vacuum condition, for approximately 30 min (until constant mass).

Table 3 – Levels of the three factors of the experimental designs with two and three independent variables for extraction with pressurized ethanol

Design	**Independent variables**	-α	**-1**	**0**	**+1**	+α
Selection of variables (2^3)	Contact time (min)	-	5	7	9	-
	Temperature (ºC)	-	40	50	60	-
	Rinse Solvent Volume (%)	-	80	100	120	-
Linear and quadratic	Temperature (ºC)	56	60	70	80	84
	Volume of solvent (%)	82	90	110	130	148

Rinse Solvent volume is expressed in % of extraction cell volume
Source: own authorship;

III.2.6. Tocopherol content

Tocopherol contents were evaluated in all crude sunflower oils and extracts obtained via SFE and PLE and in commercial (refined) oil.

The sample (1 ± 0.01 g) was diluted to 10 mL with n-hexane, vortexed for about 1 minute, and filtered through a regenerated cellulose membrane with a 0.45 µm pore (Pall, Diadema, Brazil). For the quantification of the β, γ, and δ-tocopherol types, 10 mL were used. For the α-tocopherol analysis, 0.25 mL of sample was used, which was then diluted to 10 mL with n-hexane.

In the analysis, a chromatographic system composed of Rad Pump III (Lab Alliance, Massachusetts, USA) and fluorescence detector LC 305 (Lab Alliance, Massachusetts, USA), version 3.11, was used. The injector was a Rheodyne type with a 250 µL sampling loop.

The analysis was performed in an isocratic system, using a mobile phase composed of n-hexane: ethyl acetate: glacial acetic acid (98.8: 0.7: 0.5, v/v/v) and a flow rate of 1.5 mL / min. The separation occurred on a 60 Lichrospher Si 60 silica column (Merk, Germany) (5 µm of film, 125 mm in length, and 4 mm internal diameter).

In monitoring the types of tocopherols, the wavelengths of 294 nm were used for excitation and 326 nm for emission. Quantification was performed by external standardization, using a tocopherol standard set (α, β, γ, and δ-tocopherols). This analysis was performed in duplicate.

III.2.7. Identification of fatty acids (FA) and probable composition of triacylglycerols (TAGs)

For the analysis of the fatty acid profile, the crude oil samples were saponified and esterified according to method 969.33 (AOAC, 2005), using hexane as a solvent. After esterification, the samples were diluted to 10 % in hexane and injected into the chromatograph.

The fatty acid profile of sunflower oils was analysed by Gas Chromatography coupled to the Mass Spectrometer (GC-MS), equipped with a Split injector (1:40) (Shimadzu, GCMS-2010), with an automatic injector (model AOC-5000) at 250 ºC and P = 300 kPa. The capillary column used was the SP 2560 (100 m × 0.25 mm × 0.2 μm), with helium as the carrier gas, at a flow rate of 1.59 $mL.min^{-1}$.

The oven started at 100 ºC, remaining for 1 min, and was gradually increased (5 ºC) to 195 ºC, and then, with a minor increase (2 ºC), the temperature was established at 250 ºC. The reading occurred between minutes 11 and 60.5, and a mass range between 40 and 350 *m/z*. Peaks with an area ≥ 0.5% were considered in the integration.

The probable content of triacylglycerols (TAGs) in sunflower oils was determined using the statistical algorithm suggested by Antoniosi Filho et al. (1995). TAGs with a frequency ≥ 0.6% were selected.

III.2.8. Statistic

The CCRD was planned and analyzed by the Statistica software 13.5.0.17 (Tibco software, Palo Alto, California) to determine the optimal conditions for extraction. The physical and chemical analyzes received treatment by analysis of variance (ANOVA), 95%, using the same software. The probable TAGs were determined using the MATLAB R2013a Inc. software.

III.3. RESULTS

III.3.1. Characterization of raw material

The averages of the results obtained in the characterization of the hulled sunflower seeds were: 98.76±0.21 % DM; 2.70±0.05 % MM; 23.50±0.26 % CP; and 12.88±0.27 % CF. No significant amounts of NNE, ADF, NDF, N-ADF, and N-NDF were found. As for the ethereal extract, the average obtained was 55.07±0.30 %.

III.3.2. Granulometry

The average diameter of the particles used for extraction was 0.84 mm (Table 4). This diameter corresponds to the rolled seeds, according to industrial use, and selected at < 30 mm, for partial separation of the husks. Several authors report that the particle size is inversely proportional to the extraction yield (RAI et al., 2016a, 2016b; NIMET et al., 2011; FIORI, 2009; SALGIN et al., 2006; LEO et al., 2005) due to the density of the sample in the cell extraction. The breakdown and, consequently, the smaller size of the sunflower seed particles increases the accessibility to the oil, since it facilitates the penetration of supercritical carbon dioxide, resulting in greater mass transfer and extraction yield (RAI et al., 2016a).

Table 4 – Granulometry of sunflower seeds used for oil extraction

Sieves		Retained mass	
(*mesh*)	(mm)	(g)	(%)
10	2	1.14	0.57
14	1.41	47.82	23.93
20	0.84	66.68	33.37
28	0.59	38.86	19.45
35	0.42	35.73	17.88
48	0.3	8.35	4.18
> 48	> 0.30	0.44	0.22
Average diameter (< 2. > 0.3 mm)	0.84		

Source: own authorship

For sunflower seeds, specifically, Salgin et al. (2006) demonstrated that the extraction yield is inversely proportional to the particle size used since the highest oil yield was obtained with 0.23 mm particles (91.7%), while for 0.55 particles mm, 1.09 mm and 2.18 mm the yields were, respectively, 57.2 %, 31.9 %, and 26.9 %.

In larger particles, there is the effect of intraparticle diffusion, considerably decreasing the extraction yield. High yields in shorter times are achieved by decreasing the size of the particles used since the diffusion time can be very long in large particles (SALGIN et al., 2006).

The following maximum yields and granulometry used were also found in the literature, respectively: 54 %, 75 mm (RAI et al., 2016b); and 39 %, 0.20 mm (FIORI et al., 2009). In this study, where particles with an average diameter of 0.84 mm were used, up to 93 % of the oil in the seeds was recovered. This result is quite satisfactory, considering the ease of sample preparation (lamination and simple separation by sieve).

III.3.3. Supercritical Fluid Extraction (SFE)

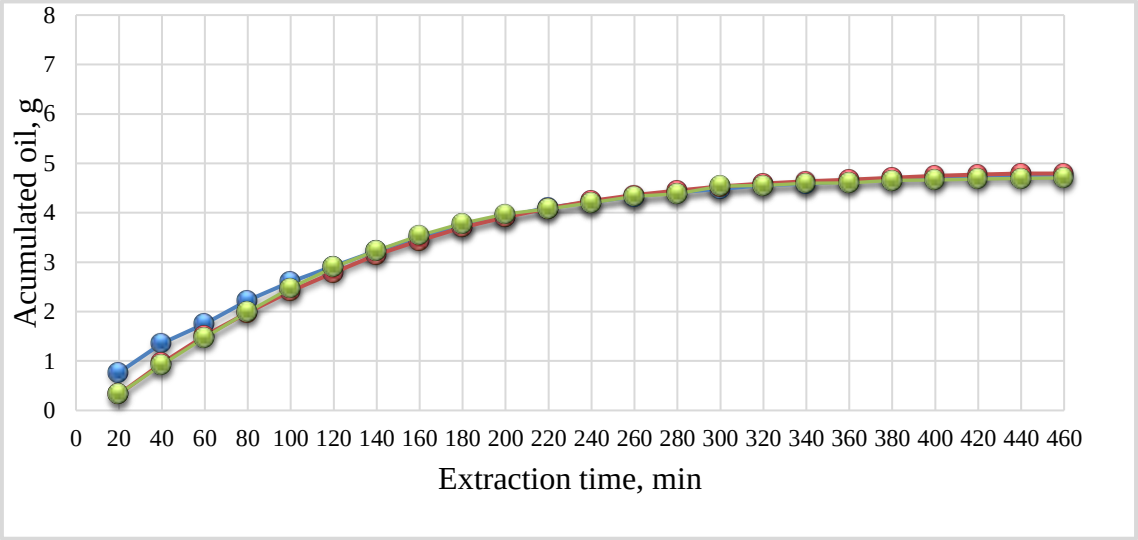
Anticipating the SFE and for comparison, the total oil content in the sunflower seed used in this study was obtained by classical hexane extraction (ethereal extract), resulting in 55.07±0.30 % w/w. This value was very close to that obtained by Rai et al. (2016b), Hartman et al. (1999), and by Salgin et al. (2006), who obtained 54.37 %, 54.80 %, and up to 53.41 %, respectively. However, lower values are found in the literature, such as 41; 42.2; 45.1; 47.83; and 52 %, as reported by Nimet et al. (2011), Correia et al. (2010), Boutin et al. (2011), Hartman et al. (1999), and Salgin et al. (2006), respectively.

According to Rai et al. (2016b) and Kemper (2005), the sunflower oil content in the seeds can vary for several reasons, among them the cultivation conditions, such as ambient temperature, soil properties and nutrition, and availability of water.

III.3.3.1. SFE kinetic

To choose the extraction time to be performed in the study of the optimization of the SFE process, the extraction kinetics was evaluated. According to the extraction curve (Figure 1), the 240 min (four hours) period was determined as viable, indicating the end of the decreasing rate phase, where the sample obtained, on average, 42.2 % of its mass extracted at 25 MPa, 60 ºC and 10 g CO_2/min conditions. Considering that the oil content in the sample is 55.07 g of oil /100 g of seed, the oil recovery in this condition was 76 %.

Figure 1 – Kinetics of supercritical extraction of sunflower oil (25 MPa, 60 ºC and 10 g CO_2 / min)



Source: own authorship

In 240 min, the increasing extraction rate begins to stabilize (Figure 1), reaching the plateau that indicates the start of extraction controlled by the process of oil diffusion through the particles until it reaches the surface. The longer the extraction time, the greater the total yield, however, the processing time must be optimized to be economically viable. Usually, the extraction time used in the processes is related to the region of the curve where a constant extraction rate is noted (0-80 min, Figure 1). In this phase, the external surface of the particles is covered with the solute, which is easily accessible, and convection is the dominant mass transfer mechanism.

The total yield of the extraction promotes data to analyze the effects of T and P in the process and is closely related to the solubility of the solute in the supercritical condition (PEREIRA; MEIRELES, 2010). However, in this study, there was a progressive decrease in the rate of extraction after 120 minutes of extraction, although the sunflower seed had a high content of extractable material and was in a rolled format, as used in the industry. Such behavior may demonstrate some resistance to mass transfer or material shape (BRUNNER, 2005) since the rolled seed has a smaller contact surface than ground materials. Even so, in just 240 minutes, 76 % recovery was achieved under the applied conditions.

According to Moura et al. (2012), due to the operational cost, the process is usually limited to the region of the curve that concentrates 50 to 70% of extraction. It must be

considered that the relatively short extraction time is one of the characteristics of the best operating conditions (PEREIRA; MEIRELES, 2010).

The total mass of CO_2 used in this kinetics was 2,484 g at the end of 240 min. In conventional extraction of vegetable oils using hexane, the ratio of solvent to solute (S/F) is 1/1 (w/w) (KEMPER, 2005), or S/F = 1.

In this study, the extraction time equal to four hours was chosen, based on the curve obtained when evaluating the accumulated extraction over time (Figure 1), and on the literature. Besides, a fixed CO_2 flow was maintained, since according to Rai et al. (2016a) the extraction rate is constant and is not influenced by the flow at the beginning of the extraction, and therefore the output concentration of sunflower oil is independent of the flow, suggesting equilibrium.

Leo et al. (2005) evaluated the extraction, by $scCO_2$, of almond seed oil enriched with tocopherols. The evaluated parameters were P (35 to 55 MPa), T (35 to 50 ºC), and CO_2 flow (10 to 30 kg h^{-1}). The authors concluded that the optimal co-extraction condition for oil and tocopherols, including their maximum recovery, was obtained in the first two and three hours of extraction.

III.3.3.2. Yield of sunflower oil extraction by SFE

The yield of sunflower oil extracted with $scCO_2$ varied considerably in the temperature and pressure ranges studied, from 10.73 to 48.23%, which implies an oil recovery of 19.48 and 87.58%, respectively (Table 5). Selection of variables for PLE will be discussed in topic III.3.4.1.

In the statistical analysis of the effects of the process variables (P and T) in this response, it was found that P was the only one that had a significant and positive effect (Figure 2A) at the level of 5% significance.

Table 5 – Yield of sunflower oil by supercritical carbon dioxide (SFE) and by pressurized ethanol (PLE) in function of the process variables, T and P for SFE and T and VS for PLE.

Run			SFE				PLE			
	Coded variables		Variables		Yield (%)		Variables		Yield (%)	
			T	P	Total	Recovery[1]	T	VS	Total	Recovery[1]
1	-1	-1	50	20	30.27	54.97	60	90	42.97	78.03
2	-1	1	50	30	47.89	86.96	60	130	43.03	78.13
3	1	-1	70	20	10.73	19.48	80	90	51.23	93.02
4	1	1	70	30	47.32	85.93	80	130	51.03	92.67

5	-1,41	0	46	25	36.92	67.04	56	110	41.51	75.37
6	1,41	0	74	25	24.99	45.38	84	110	51.73	93.93
7	0	-1,41	60	18	12.95	23.52	70	82	46.65	84.71
8	0	1,41	60	32	48.23	87.58	70	148	48.13	87.39
9	0	0	60	25	41.67	75.67	70	110	46.39	84.24
10	0	0	60	25	39.84	72.34	70	110	47.33	85.94
11	0	0	60	25	42.90	77.90	70	110	47.47	86.20

T = Temperature, ºC; P = Pressure, MPa; SV = Volume of Solvent, % ; [1]= in relation to the ethereal extract (55.07 %). Source: own authorship

In the extraction of sunflower oil by $scCO_2$, the extraction yield is proportional to the density of the solvent, and increases with the applied pressure (NIMET et al., 2011). According to Salgin et al. (2006), the extraction rate enhances with increasing CO_2 flow. However, the extraction is almost linear at the beginning of the extraction, resulting in a single curve, which suggests that the influence is provoked by the total amount of CO_2 used in a given time per fixed amount of seeds, and not necessarily its flow. In our study, 10 g of CO_2 per min of extraction were used for a fixed amount of seeds (10 g), resulting in 2.4 kg of CO_2 / 4 h.

According to Boutin et al. (2011), the yield of sunflower oil extraction by $scCO_2$ can be influenced by small variations in parameters P and T, especially at the beginning of the extraction, which is of great importance in optimization for industrial purposes.

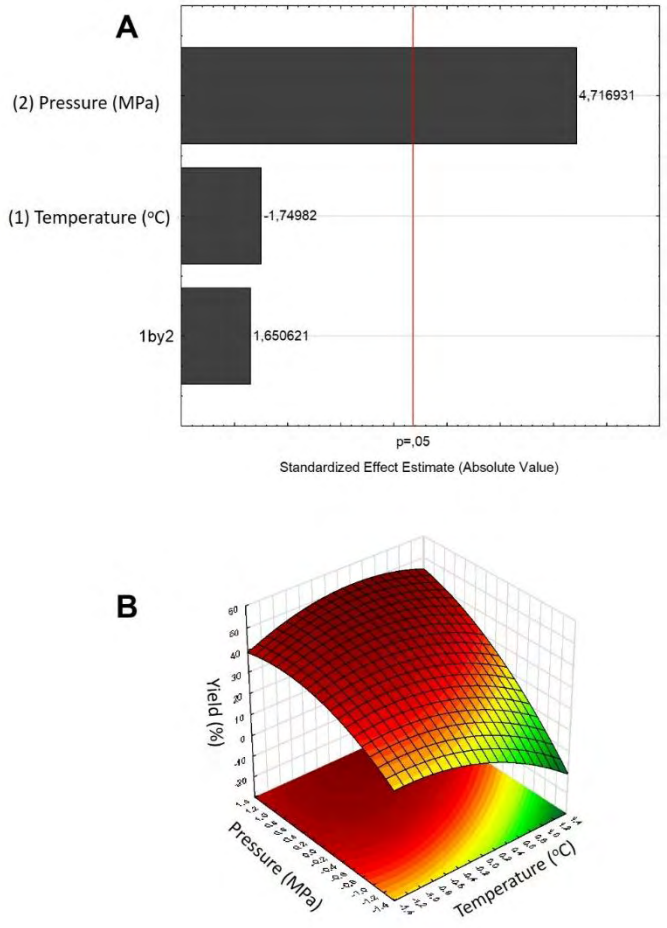
Using $scCO_2$, the maximum yield of sunflower oil obtained by Rai et al. (2016b) was about 54.37 % when the extraction was performed at 80 °C, 40 MPa, particle size of 0.75 mm, and solvent flow rate of 10 g / min with a cosolvent of 5 %.

Fiori (2009) obtained up to 39% of sunflower oil (28 MPa and 40 ºC), using husked seeds with an average diameter equal to 0.195 mm. For seeds with husks, the maximum obtained was 23 %, with an average diameter of 0.312 mm, under the same conditions. In this study, the experimental design used made it possible to identify the optimal point of extraction of sunflower oil with $scCO_2$, reaching a yield of 48 % or an oil recovery of 87.58 % (Table 5).

As for the effect of T on the extraction yield, in the study by Rai et al. (2016a) the accumulated oil yield was proportional to the increase in T, probably due to the increased solubility of fatty acids present in the oil. The solubility of sunflower oil in $scCO_2$, with or without ethanol as a cosolvent, was directly proportional to the extraction rate.

Using the Yield and coded variables (Table 5), in the statistical analysis of a model to predict the behavior of P and T in sunflower oil yield (Y), at least one of the variables was significant (*), P (Equation 2), as already found in the Pareto diagram (Figure 2A).

Figure 2 – Pareto chart for estimated effects of the linear model (A) and adjusted response surface in the quadratic model (B) for yield in obtaining sunflower oil by supercritical carbon dioxide



Source: own authorship

When analyzing the analysis of variance (ANOVA) and the estimated regression coefficients (R^2) (Appendix I) which compose the quadratic model, it is observed that all parameters showed significance (*) at the level of 5% (Equation 3), in addition to an excellent fit.

$$Y_{SFE} = 37.23^{*} - 5.03T + 13.55^{*}P + 4.74TP \quad (2)$$

$$Y_{SFE} = 41.47^{*} - 4.62^{*}T - 4.44^{*}T^{2} + 13.01^{*}P - 4.62^{*}P^{2} + 4.74TP \quad (3)$$

Both linear and quadratic models are predictive, and therefore it is suggested to apply them in the study of the process on larger scales, aiming to validate them for the process scale-up. However, in the quadratic model, the quadratic terms of the parameters (T and P) were also significant, influencing the process yield. In addition, when comparing the models, the regression coefficient of the second-order model (R^2 = 0.98, adjustment = 0.96) was higher, proving its superior predictive capacity. In both models (Equations 2 and 3) there was no significant interaction between P and T. P was the most significant factor (p = 0.0065), followed by T (p = 0.0076) in quadratic functions. The highest yield was obtained in the combination of high pressure and moderate temperature, which can be seen on the response surface generated by the second-order model (Figure 2B), where it is also observed that the effect of T can be negative in the process.

The response surface (Figure 2B) resulting from the quadratic model for the extraction yield shows the influence of the factors, and the optimum point can be seen in the region related to the highest P employed, where the solvent is found with greater density, carrying the oil particles. The less optimized points can be seen in the region with the highest T, where the solvent is less dense and, consequently, more volatile. Thus, it is concluded that the $scCO_2$ solvent acts in the extraction of rolled sunflower seeds more effectively when it is in high density.

Similar results were found by other authors. The different Ts influence the oil extraction process. Rai et al. (2016a) showed that, at 99.85 ºC, about 69 % of sunflower oil was extracted before the end of the transition period, while at 59.85 ºC, only about 54 % was extracted by the end of the same period. For T, Salgin et al. (2006) obtained an overlap between the extraction points with different T when P = 20 and 30 MPa. However, when using P higher than 30 MPa, the increase in extraction yield was proportional to the increase in the applied temperature.

Moreover, Rai et al. (2016a) and Salgin et al. (2006) showed that the yield of sunflower oil extraction is proportional to the increase in P, due to the increase in the solubility of the constituents of sunflower oil, especially TAGs. At 20 MPa, the amount of sunflower oil extracted is very small, due to the characteristics of CO_2, which reaches the liquid density with increasing P (RAI et al., 2016a). In addition, Salgin et al. (2006) reported that CO_2 solubility increased slightly with an increase in T and P above 30 MPa.

However, at 20 MPa, the increase in T caused a decrease in solubility of the solute, as in this research.

III.3.4. Extraction with pressurized ethanol (PLE)

III.3.4.1. Selection of variables for obtaining sunflower oil by PLE

When evaluated at 90 %, the selection of two variables is noted: the temperature (T) and the volume of solvent (VS) (Tables 6 and 7).

Normally, extractions under atmospheric pressure conditions are impacted by both the increase in T and the contact time between the matrix and the solvent, specifically, in the PLE of the sunflower oil this time did not significantly interfere.

The results obtained were partially opposite to those seen in conventional extraction, since in the extraction of sunflower oil by hexane, the diffusion coefficient is proportional to the increase in temperature, initial oil content, and its degree of establishment and the process yield increases with the contact time between the solvent and the matrix (PEREZ et al., 2011).

Table 6 – Yield obtained for selection of variables (2^3) on pressurized liquid extraction (PLE) yield of sunflower seed oil using ethanol

Run	Ct (min)	T (ºC)	RSV (%)	Total Yield (%)	Recovery[1] (%)
1	5	40	120	31.19	17.18
2	9	40	80	29.63	16.32
3	5	60	80	41.10	22.63
4	9	60	120	46.56	25.64
5	7	50	100	39.78	21.91
6	7	50	100	37.02	20.39
7	7	50	100	37.56	20.69

Ct = Contact time; T = Temperature; RSV = Rinse Solvent Volume; [1]= in relation to the ethereal extract (55.07 %). Source: own authorship

Table 7 – Analysis of variance for selections of variables (2^3) on pressurized liquid extraction (PLE) yield of sunflower seed oil using ethanol at 90 % significance

Factor	SS	df	MS	F test	p-value
(1) Contact time (min)	3.80	1	3.80	1.90332	0.261555
(2)Temperature (°C)	180.10	1	180.10	90.14607	0.002477
(3) Rinse Solvent Volume (%)	12.32	1	12.32	6.16675	0.089010
Error	6.00	3	2.00		
Total SS	202.21	6			

R-sqr=.97036; Adj:.94072; MS Residual=1.997829.

SS = square sum; df = degress of freedom; MS = mean square. Source: own authorship.

PLE enables fast extraction, ranging in this study from 5 to 9 min, a difference of 4 min between the minimum and the maximum, which may be the reason for the non-interference in the process.

III.3.4.2. Yield of obtaining sunflower oil by PLE

In this study, up to 93.93 % of the total oil contained in the seed was recovered under conditions of higher temperature (T) and central value of the rinse solvent volume (VS) (test 6, Table 5). The lowest recovered content was 75.37 %, under the conditions of lower T and central VS value (test 5, Table 5). In some tests, the yield of extracts obtained via PLE were higher than those obtained by SFE, this is expected, since ethanol, being a polar solvent at high pressure, solubilizes polar compounds together with the oil.

Also, in conventional sunflower seed extraction (Soxhlet), ethanol is capable of obtaining a higher total yield than hexane, however presenting similar oil phase (Baümler et al., 2016). Using ethanol as a solvent in the extraction of sunflower oil, Baümler et al. (2016; 2017) claimed to obtain 70 % less crystallizable waxes and at least 38 % more tocopherols and phospholipids when compared to hexane extraction. From sugars, more than 75 % of the total was extracted, mainly raffinose and sucrose.

In the statistical analysis of the yield of the extracts obtained by PLE in function of the process variables (Table 5), specifically in the analysis of the main effects of T and VS on the oil yield (Y), the only variable that had a significant effect was the temperature (T) as can be seen in the Pareto diagram (Figure 3A).

The generated models, both linear (Equation 4) and quadratic (Equation 5), showed excellent adjustment to the experimental data for the yield of sunflower oil by

PLE, with an adjusted coefficient of variation of 0.97 in both models The model adjustments are shown in the ANOVA for the models in Appendix II.

For both models (Equations 4 and 5), the only significant coefficient was the linear coefficient for temperature, indicating that the linear model is sufficient to describe the variation in yield with temperature.

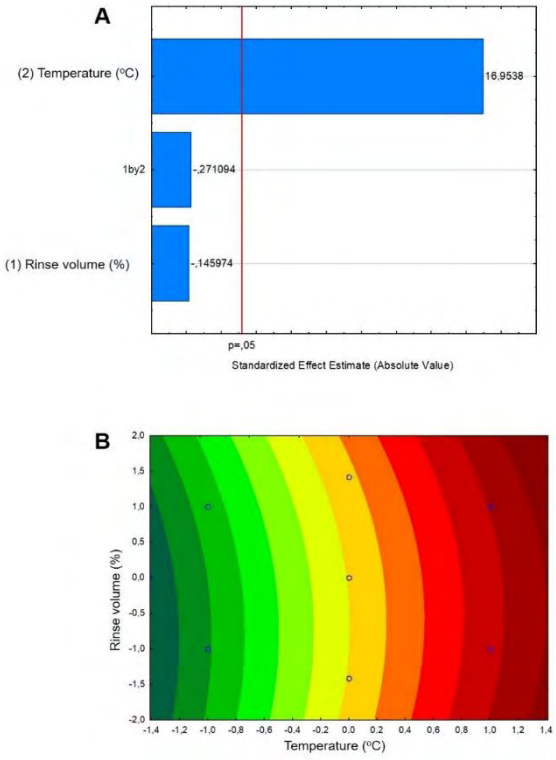
Figure 3B shows the contour line generated by the quadratic model (Equation 5) where it is possible to visualize the tendency of increasing the extraction yield with the increase of T for the entire range of the rinse solvent volume applied.

The water content added to the solvent influences the solubility of sunflower oil in ethanol, in an inversely proportional way (CUEVAS et al., 2009). Thus, in this study, the process was optimized using pure ethanol.

$$Y_{PLE} = 47.06^{*} - 0.04VS + 4.07^{*}T - 0.07VS \quad (4)$$

$$Y_{PLE} = 47.06^{*} + 3.84^{*}T - 0.21T^{2} + 0.24VS + 0.18VS^{2} - 0.06TVS \quad (5)$$

F**igure 3 –** Pareto chart for estimated effects of the linear model (A) and adjusted response surface in the quadratic model (B) for yield in obtaining sunflower oil by pressurized ethanol



Source: own authorship

The material obtained from sunflower seeds by PLE - ethanol, in addition to nonpolar compounds (oil) also contains polar compounds since ethanol is a polar solvent. According to Baümler et al. (2016; 2017), the oil obtained from sunflower seeds using ethanol as the solvent has crystallizable wax, phospholipids, pigments, and sugars contents lower than the oil obtained by hexane. The authors indicate that the insoluble fraction of this material can be refined and used as sunflower lecithin, and consider the possibility of removing raffinose, an indigestible sugar, from the oil and thereby improving its nutritional quality.

Due to the type of process, other factors may influence the yield of the process. For example, according to Sineiro et al. (1998), the pulsed extraction increased the extraction yield of sunflower oil using ethanol as a solvent by up to 8.7%. In this process, most of the oil was extracted at the beginning, reducing over time since the extraction is controlled by the diffusion of the solute to the particle surface, regardless of the flow rate. As a result, after 95 min of extraction with 96% ethanol, at 50 ºC, 50.3% of extractable oil was obtained in pulsed ethanol, while 41.6% of the oil was obtained by the nonpulsed method.

III.3.5. Tocopherols content

The total tocopherol content ranged from 15.84 to 91.17 mg of total tocopherols per 100 g of sunflower oil in the extracts obtained via SFE and from 40.30 to 83.16 in the oil obtained via PLE-ethanol (Table 8).

Therefore, the highest levels obtained by SFE and PLE were 91.17 and 83.16 mg of tocopherols/100 g of sunflower oil, respectively. Of these, 88.00 (SFE) and 76.99 (PLE) mg/100 g correspond to the content of α-tocopherol (Table 8). The SFE was more efficient in the extraction of tocopherols and showed a great amplitude in the levels obtained for different conditions of extraction, while in the PLE the contents were smaller as well as the amplitude.

Using hexane as a solvent, Nimet et al. (2011) and Calvo et al. (1994) obtained, respectively, α-tocopherol content of 79.0 mg/100 g and 121.9 mg/100 g in sunflower oil obtained by SFE, and 12.0 mg/100 g and 30.2 mg/100 g of oil. A significant variation that also demonstrates the efficiency of SFE in the extraction of tocopherols when compared to conventional extraction with hexane.

Table 8 – Influence of quadratic designs on tocopherol content (mg / 100g) in the extraction of sunflower oil by supercritical carbon dioxide (SFE) and by pressurized ethanol (PLE)

	SFE					PLE				
Run	α	β	γ	δ	Total	α	β	γ	δ	Total
1	65.42	4.97	0.72	0.24	71.35	59.97	4.19	1.61	0.17	65.06
2	62.66	0.32	0.32	<0.02	63.3	46.23	3.72	1.89	< 0.13	50.82
3	29.06	0.82	0.47	0.31	30.66	49.3	3.62	1.87	0.17	54.10
4	22.19	0.40	0.30	<0.02	22.89	52.05	3.75	1.47	< 0.13	56.64
5	55.84	0.59	0.37	0.22	57.02	76.99	5.36	1.59	0.20	83.16
6	14.92	0.46	0.39	0.07	15.84	36.32	3.02	1.58	< 0.13	40.30
7	88.00	1.56	1.20	0.41	91.17	73.51	3.76	1.23	0.19	78.06
8	42.41	0.66	0.50	0.43	44.00	62.9	3.55	1.55	0.17	67.24
9[c]	49.66	0.24	0.38	0.09	49.90	70.34	4.13	0.54	0.22	75.23
10[c]	49.53	0.19	0.38	0.08	49.72	68.89	3.62	0.63	0.19	73.33
11[c]	48.63	0.13	0.37	0.08	48.76	68.73	3.78	0.59	0.19	73.29
RSO	66.14	2.7	13.02	2.67	84.53					
C.Al.	40.3-93.5	<4.5	<3.4	<0.7						
CV%	3.22	4	5.4	4.89	3.5	2.86	3.89	5.44	5.35	2.73

RSO = Refined sunflower oil; C.Al. = *Codex alimentarius* (FAO, 1999); c = central points; CV% = coefficient of variation. Source: own authorship

III.3.5.1. Analysis of tocopherol content in sunflower oil as a function of SFE process variables

In the analysis of the concentration of tocopherols, it is important to note that, of the nine tests performed in this study, only three (33.3%) had a lower α-tocopherol content than that established by Codex Alimentarius (Codex Stan 210-1999) (FOOD AND AGRICULTURE ORGANIZATION [FAO], 1999), which stipulates the α-tocopherol content between 40.3 and 93.5 mg/100g of sunflower oil. As for the other forms of tocopherol, a maximum value normally found in sunflower oils is established and one of the tests (test 1, Table 8) was able to overcome this limit for β-tocopherol. Commercial oil stood out in terms of γ- and δ-tocopherol levels, being the only one to present higher values than those established by Codex Alimentarius (FAO, 1999) in these parameters (Table 8).

Comparing to the commercial version of sunflower oil, the extraction with SFE with central T and lower P (test 7, Table 8) resulted in a higher content of α-tocopherol, while tests 1 and 2 showed values similar to the commercial oil in addition to surpassing it in the β-tocopherol content.

Given the association with the process yield, the parameters used for the extraction of test 2 (Tables 5 and 8) proved to be the most promising to be applied in a commercial SFE of tocopherol-rich sunflower oil.

This condition allows 86.96 % of oil recovery with values equal to or greater than α and β-tocopherol. In contrast, test 7 (Table 8), despite the high content of α-tocopherol, shows a yield of only 12.95%, being economically unfeasible.

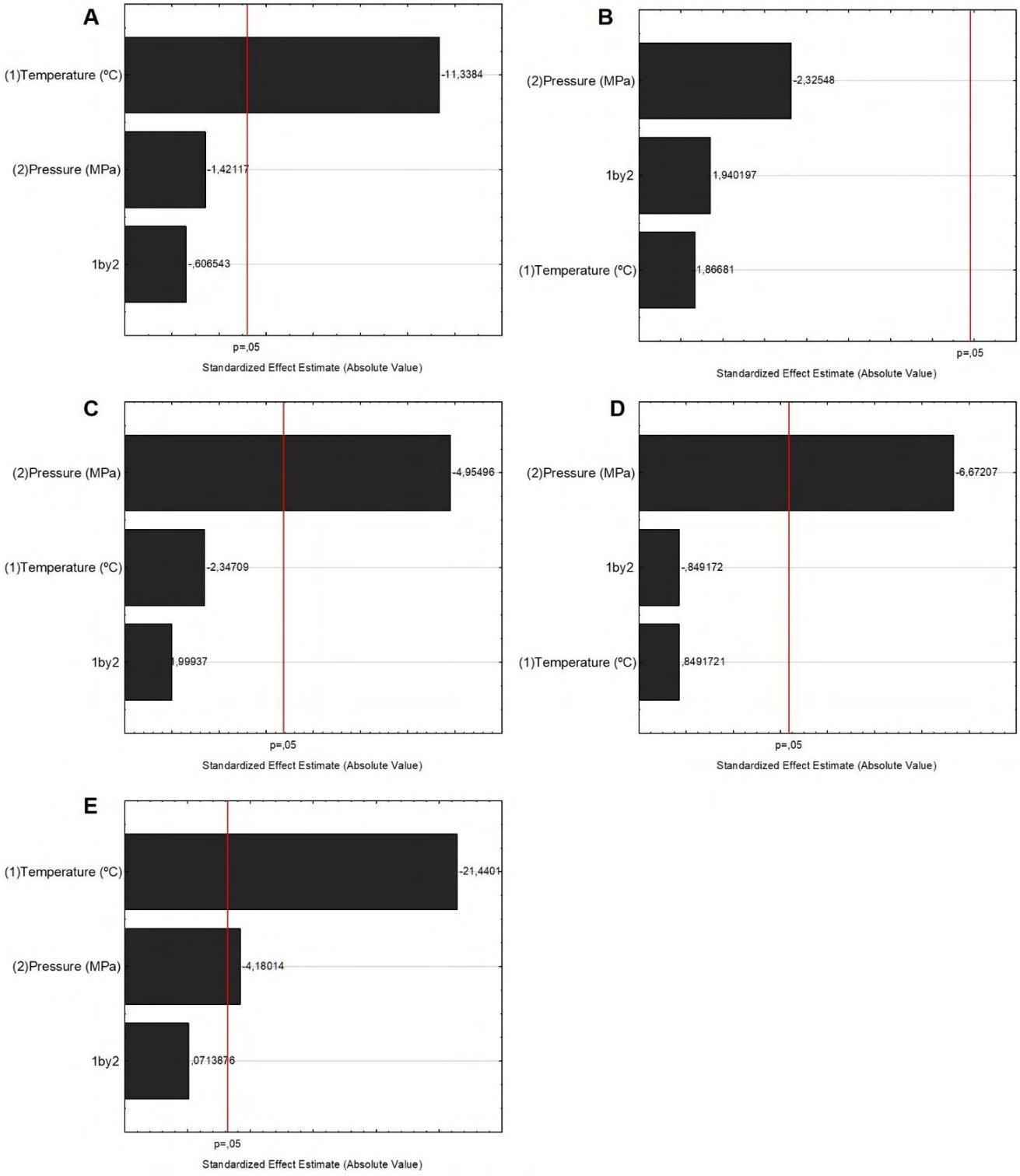
When evaluating the influence of the process on the concentration of tocopherols, analysis of the main effects showed the increase of T which negatively influenced the levels of α and δ-tocopherol ($p \leq 0.05$), although this factor was not significant for the levels of β and γ-tocopherol ($p \leq 0.05$).

Del Valle et al. (2020) correlated the solubility of α and δ tocopherol in $scCO_2$ (from 40 to 60 ºC, and from 9.8 to 33.6 MPa). The authors reported that, under equivalent conditions, the solubility of α-tocopherol was 3 to 4 times lower than that of δ-tocopherol.

In the yield analysis (Figure 2), the temperature was not significant, therefore, in this temperature range studied, if the increase in temperature negatively influences the extraction of tocopherols, lower T's can be applied without compromising the yield of the extract.

T was the most influential parameter in obtaining α-tocopherol (Figure 4A), while P was the determinant for γ and δ-tocopherol (Figures 4C and 4D). For the total content of tocopherols, both parameters were significant (Figure 4E). The interaction between the parameters was not significant for any of the tocopherols.

Figure 4 - Pareto chart for estimated effects of the linear model for α-tocopherol (A), β-tocopherol (B), γ-tocopherol (C), δ-tocopherol (D) and total tocopherols (E) in sunflower oil by supercritical oil extraction



Source: own authorship

In $scCO_2$ extraction, the denser the fluid, the greater is its solubilizing power. Density is directly related to pressure since the higher the pressure, the greater the density of $scCO_2$. However, the increase in temperature decreases the density, causing the vapor pressure (Pv) of the compounds to increase, facilitating their volatilization and, consequently, the migration of these compounds to the solvent (light phase). The results show that the pressure facilitated the oil extraction yield (Figure 2), acting positively, showing that the higher the solvent density, the better the extraction. Pressure and temperature negatively influenced the extraction of tocopherols, indicating that the optimum extraction conditions are probably in use, since the fact that the increase in temperature decreases the density is more significant than its increase in facilitating the volatility of these components.

The behavior of SFE in oils and their minority compounds is particular and depends heavily on the raw material. Davarnejad et al. (2010) studied the content of tocopherols in palm oil obtained via SFE and found that the increase in T positively influences the solubility of α-tocopherol, as well as the increase in P. In this case, both the high density of the fluid increased the solubility tocopherols as the temperature that facilitated the migration of these compounds to the solvent.

Another factor that positively influences the solubility of tocopherols is the high solubility of TAGs which, in extraction with $scCO_2$, which can act as cosolvents and increase the solubility of tocopherols (Davarnejad et al., 2010). This behavior was observed for sunflower seed oil, as high pressures solubilized TAGs providing higher yields (Figure 2). Besides, as TAGs act as cosolvents of tocopherols they provide a higher yield of these compounds.

Using coded variables, in the statistical analysis of a model to predict the behavior of P and T in the levels of tocopherols in sunflower oil obtained by SFE, the ANOVA of the linear model (Figure 4; Appendix II) indicates that at least one of the variables was significant (*) to obtain α, γ, and δ-tocopherol, in addition to total tocopherols (Equations 5, 9, 11, and 13). However, the parameters are influenced negatively in obtaining the compounds, both individually (T and P), as well as their interaction (TP) for some models.

When analyzing the estimated regression coefficients that compose the quadratic model, it is observed that all parameters (α, β, γ, δ, and total) showed less significance (*) at the level of 5% (Equations 7, 9, 11, 13, and 15) (R^2 = 0.88; 0.73; 0.85; 0.59; and 0.90, respectively) when compared to the linear model (R^2 = 0.98; 0.83; 0.89; 0.97; and 0.99, respectively) as seen in ANOVA (Appendix I).

In assessing the influence of process variables on the tocopherol content, linear and quadratic models were generated. However, in the second-order model, none of the quadratic terms was significant (Equations 7, 9, 11, 13, and 15) except for γ-tocopherol (Equation 11). Linear models can be used to predict the behavior of the content of α-tocopherol (Equation 6), β-tocopherol (Equation 8), and δ-tocopherol (Equation 12) in addition to the total of these components (Equation 14). Also, considering that the R^2 for the linear model that describes the influence of variables on the γ-tocopherol content (Equation 10) was greater than the quadratic model, and then the linear model can be used to predict the behavior of all tocopherols as a function of T and P.

For example, when evaluating the models generated for α-tocopherol, the determination coefficient (R^2) equal to 0.98, and the adjusted determination coefficient equal to 0.96 for the linear model (Equation 6) were higher than the same coefficients of the quadratic model (Equation 7), which was expected, since the quadratic terms of the second-order model (Equation 7) are not significant.

$$Y\alpha = 45.54^{*} - 19.21^{*}T - 2.41P - 1.03TP \quad (6)$$

$$Y\alpha = 46.51^{*} - 16.87^{*}T - 6.98\,T^2 - 9.27^{*}P + 8.02\,P^2 - 1.03\,TP \quad (7)$$

Considering that the quadratic models generated for the other tocopherols (Equations 9, 11, 13, and 15) presented the same behavior as those generated for α-tocopherol, the discussion on the behavior of the enrichment of the extracts in these compounds will be carried out based on the linear models (Equations 8, 10, 12, and 14).

Analyzing the content of the different tocopherols, the linear model proved to be less predictive for obtaining β-tocopherol, in comparison with the other tocopherols, since it presented $R^2 = 0.83$ (Equation 8) inferior to the others. For γ-tocopherol, for example, the correlation coefficient was higher, $R^2 = 0.89$ (Equation 10), but still lower than δ-tocopherol.

$$Y\beta = 1.07 - 1.02T - 1.27P + 1.06TP \quad (8)$$

$$Y\beta = 0.32 - 0.53\,T + 0.30\,T^2 - 0.79\,P + 0.60\,P^2 + 1.06\,TP \quad (9)$$

$$Y\gamma = 0.44^{*} - 0.07T - 0.14^{*}P + 0.06TP \quad (10)$$

$$Y\gamma = 0.42^{*} - 0.03\,T - 0.06\,T^2 - 0.19^{*}P + 0.17^{*}P^2 + 0.06\,TP \quad (11)$$

Although it is a minority tocopherol, the linear model proved to be quite predictive for the δ-tocopherol content, with a determination coefficient of $R^2 = 0.97$ (Equation 12).

$$Y\delta = 0.12^{*} + 0.02T - 0.14^{*}P - 0.02TP \quad (12)$$

$$Y\delta = 0.10 - 0.02\,T - 0.02\,T^2 - 0.06\,P + 0.12\,P^2 - 0.02\,TP \quad (13)$$

The linear model was also predictive for the total tocopherol content (Ytoc = α + β + γ + δ) (Equation 14), but in this case, the quadratic model also showed a good fit to the experimental data. When assessing the total tocopherol content, the coefficients that were significant in the quadratic model for the extraction yield (Equation 5), were also for the total tocopherol content (Equation 15).

$$Ytoc = 47.17^{*} - 20.27^{*}T - 3.95^{*}P + 0.07TP \quad (14)$$

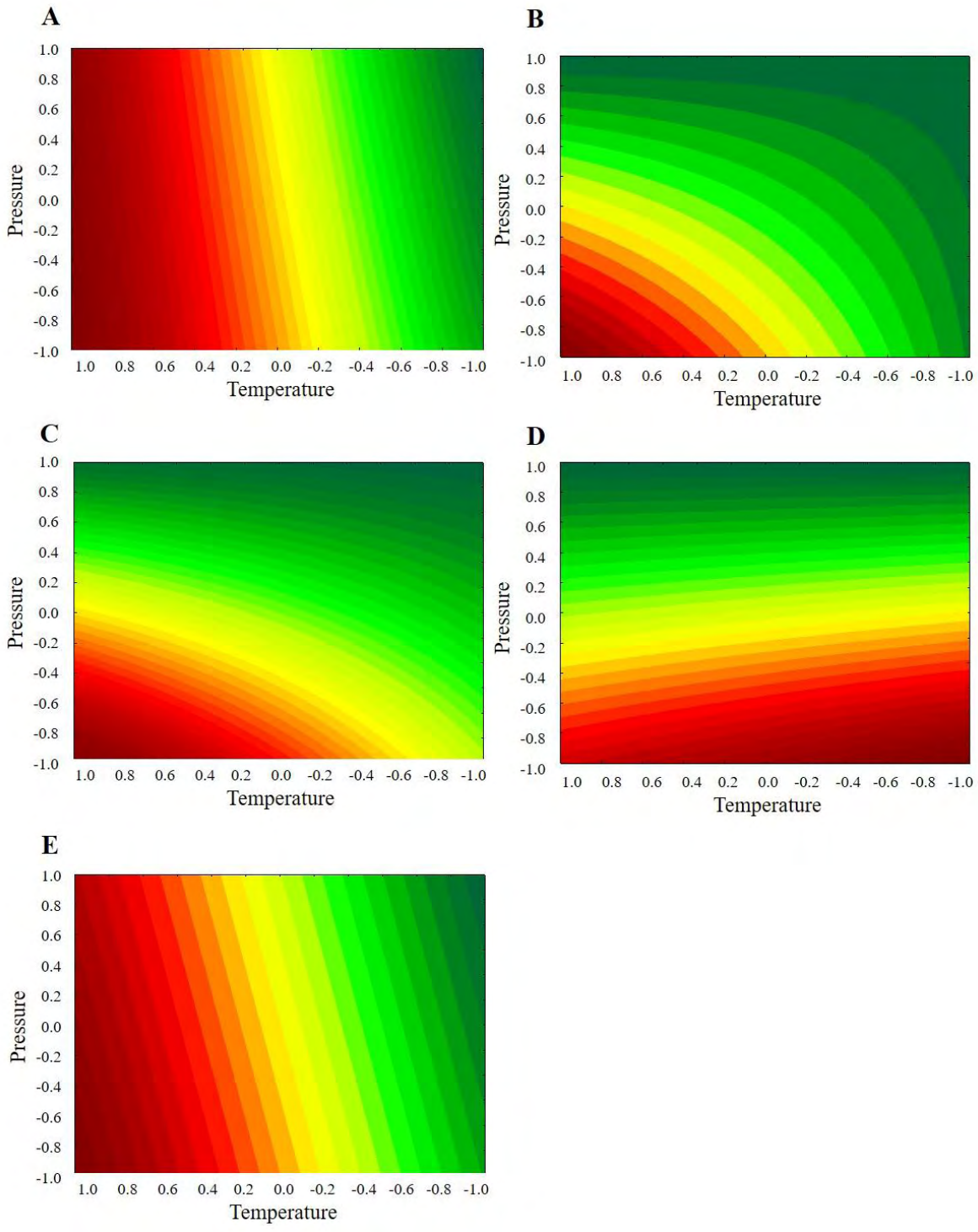
$$Ytoc = 47.35^{*} - 17.45^{*}T - 6.75T^2 - 10.32^{*}P + 8.92P^2 + 0.07TP \quad (15)$$

The contour surfaces generated by the linear models show the tendency to obtain higher concentrations of the compounds β, γ, and δ-tocopherols when lower values of P are used (Figures 5B, 5C, and 5D). However, when observing the behavior of the levels of α-tocopherol with P and T, high values are noted for the entire range of P studied (Figure 5A). Obviously, with α-tocopherol in greater concentration than the others when assessing the behavior of the concentration of total tocopherols with P and T (Figure 5E), the same profile is expected.

Extraction with $scCO_2$ allows us to fractionate compounds. From the moment that a material rich in a specific component is desired, knowing in which operational condition (P vs. T) this occurs is fundamental for the best solubilization of this compound. For ex ample, if only the content of total tocopherols was analyzed (higher P), within the studied range, the concentrations of β, γ, and δ-tocopherols would probably be lower, so the oil would be poor in these other compounds.

Thus, the use of supercritical fluid to obtain sunflower oil with tocopherol content proved to be a viable technology, considering the combination of the parameters evaluated.

Figure 5 – Response surface of the effect of the independent variables Temperature and Pressure on the content of α-tocopherol (A), β-tocopherol (B), γ-tocopherol (C), δ-tocopherol (D) and total tocopherols (E) in sunflower oil obtained by supercritical carbon dioxide using the linear model



Source: own authorship

III.3.5.2. Analysis of tocopherol content in sunflower oil as a function of PLE process variables

Comparing with the commercial version of sunflower oil, it is noted that extraction with PLE using the lowest T and the central values of VS (56 ºC, 110%, test 5, Table 8) resulted in a total tocopherol content very close to that observed in the commercial oil (83.16 and 84.56 mg/100 g, respectively). However, when evaluating only α-tocopherol, it is noted that tests 5, 7, and the central points had a higher content than the commercial one (Table 8). This suggests that, although commercial oil may contain a higher content of total tocopherols, it is possible to obtain tocopherols of greater biological value (better nutritional quality) through PLE extraction.

In the extraction of sunflower oil by ethanol by Baümler et al. (2017), the effective diffusion coefficient (De) for tocopherols was higher than that of phospholipids (independent of the temperature in the analyzed range), besides that, both the sugar mass transfer rate and the extraction yield increased with T (50 and 60 ºC).

None of the main effects of the variables T and VS on the content of the different tocopherols had significant effects (Table 8; Figure 6). As a result, it was not possible to generate predictive models that describe the behavior of the content of the different tocopherols in the oil as a function of these PLE variables.

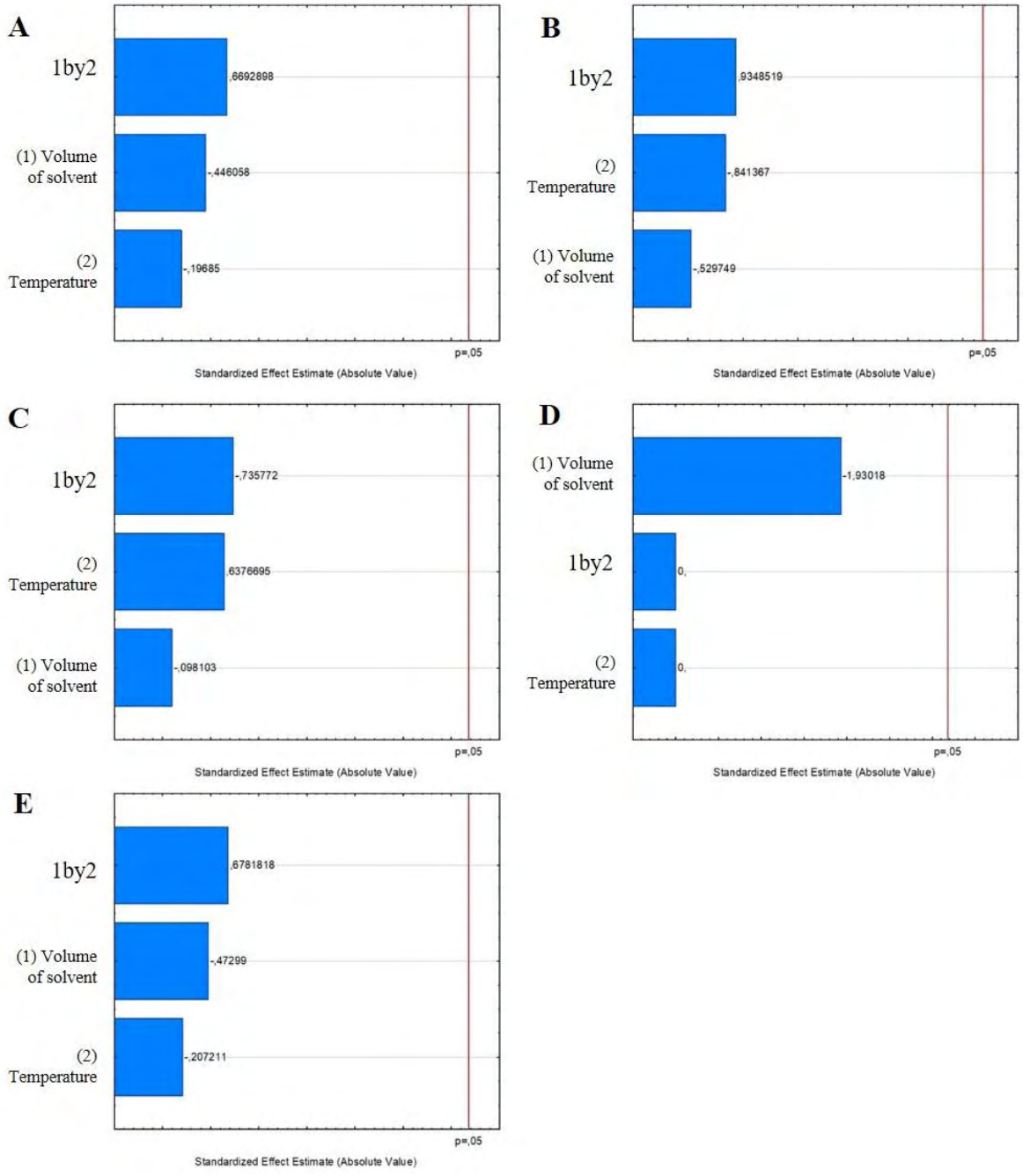
Although both the linear and quadratic models have been shown to be quite predictive for the extraction yield by PLE ($R^2 = 0.99$ and adjusted $R^2 = 0.97$), the models are not predictive for tocopherols (Figure 6). Types α, β, γ, δ, and totals presented $R^2 =$ 0.19; 0.38; 0.24; 0.55; and 0.19, respectively, in the linear model.

Despite the lack of adjustment, only one sample had a lower α-tocopherol content than that stipulated by Codex Alimentarius (FAO, 1999) (Table 8, test 7). Besides, the highest tocopherol content found was 83.16 mg/100 g, which corresponds to the test that obtained the lowest total yield, with 75.37 % recovery of the total extract.

Therefore, among the parameters obtained in this study, the most viable condition for obtaining rich sunflower oil with tocopherol by PLE is when using 70 ºC for a rinse volume of solvent (VS) of 110% of the volume of the fixed bed extractor (test 7).

Obtaining a sunflower oil that provides both high yield and high tocopherol content is possible by combining the second highest content of α-tocopherol (73.51 mg/100 g) with a total of oil recovered of approximately 85%, with the lowest rinse volume of solvent (82%, test 7, Tables 5 and 8), providing greater savings and sustainability.

Figure 6 - Pareto chart for estimated effects of the linear model for α-tocopherol (A), β-tocopherol (B), γ-tocopherol (C), δ-tocopherol (D) and total tocopherols (E) in sunflower oil by pressurized ethanol



Source: own authorship

III.3.6. Fatty acid and triacylglycerol profile

There was no difference in profile between the oils obtained by PLE, the SFE optimized, and the commercial (Table 9).

Note that this study obtained values very close to those available in the literature (NIMET et al., 2011; RAI et al., 2016b), regardless of the extraction methodology, suggesting that the quality of the oil is maintained when using alternative extraction techniques. Nimet et al. (2011) reported the same, who evaluated the extraction of sunflower oil by $scCO_2$ between 30 and 60 ºC, and 8 to 25 MPa, and observed that the amount of fatty acids was similar between the oil extracted by $scCO_2$ and by propane.

There is no difference in the fatty acid composition of the oil extracted with $scCO_2$ and hexane, and in the oil a high source of linoleic acid is observed (RAI et al., 2016b).

In addition, Velez et al. (2012) also present the fatty acid composition of commercial sunflower oil, with a fatty acid composition of 85 % oleic acid (w/w), 14 % palmitic acid (w/w), and 1 % stearic acid (w/w).

As for the probable composition of TAGs, this study obtained results very close to those obtained by Cuevas et al. (2009), who also applied statistic approach for TAG determination, with the following exceptions: Cuevas et al. (2009) also reported the groups 44:1, 46:1, 54:7, not observed in this study, where it was observed 58:3, not reported by the authors mentioned. All the other ten groups were compatible (Table 10).

Table 9 – Fatty acid profile (FA) of sunflower extracts obtained by pressurized ethanol (PLE), of the optimized oil obtained by supercritical carbon dioxide (SFE), and the refined commercial oil

FA (%)		PLE1	PLE 2	PLE 3	PLE 4	PLE 5	PLE 6	PLE 7	PLE 8	PLE 9	PLE 10	PLE 11	SFE 8	COM	M ± DP
C16:0	Palmitic	6.95	7.21	6.78	6.72	6.94	7.34	6.75	7.19	7.42	6.67	6.73	6.91	7.22	6.99 ± 0.26
C18:0	Stearic	5.28	5.49	5.03	5.02	5.30	5.66	5.08	5.05	5.51	5.03	5.01	5.03	5.49	5.23 ± 0.23
C18:1	Oleic	22.58	22.26	22.68	22.6	22.32	24.88	22.16	22.81	24.17	22.59	22.59	22.6	21.65	22.76 ± 0.81
C18:2	Linoleic	64.43	64.26	64.79	64.91	64.64	61.2	65.25	64.25	62.04	64.96	64.91	64.77	64.73	64.24 ± 1.25
C22:0	Behenic	0.77	0.79	0.72	0.75	0.8	0.92	0.76	0.7	0.85	0.76	0.76	0.69	0.91	0.78 ± 0.06

COM = refined commercial oil. Source: own authorship

Table 10 – Triacylglycerols (TAGs, %) groups estimation of sunflower extracts obtained by pressurized ethanol (PLE), of the optimized oil obtained by supercritical carbon dioxide (SFE), and the refined commercial oil

Groups	TAG	PLE 1	PLE 2	PLE 3	PLE 4	PLE 5	PLE 6	PLE 7	PLE 8	PLE 9	PLE 10	PLE 11	SFE 8	COM
50:2	PLP	1.13	1	0.88	0.86	0.92	0.99	0.88	0.98	1.02	0.85	0.87	0.91	1.01
52:2	PLS	3.08	2.63	2.39	2.36	2.49	2.98	2.35	2.56	2.90	2.35	2.36	2.43	2.58
54:2	OOS	1.61	1.36	1.23	1.23	1.3	1.63	1.22	1.25	1.51	1.23	1.22	1.23	1.32
52:3	POL	8.47	7.06	6.78	6.71	6.84	7.69	6.65	7.18	7.64	6.66	6.72	6.89	6.94
54:3	SOL	7.37	6.08	5.82	5.79	5.95	7.03	5.72	5.86	6.66	5.8	5.78	5.79	5.89
58:3	OLB	0.78	0.64	0.6	0.62	0.65	0.80	0.62	0.58	0.72	0.63	0.63	0.56	0.72
52:4	LLP	12.14	10.24	9.73	9.68	9.95	9.51	9.83	10.17	9.86	9.62	9.70	9.91	10.42
54:4	LLS	20.45	16.62	16.54	16.51	16.56	18.00	16.33	16.49	17.5	16.53	16.49	16.46	16.3
58:4	LLB	1.11	0.93	0.85	0.89	0.95	0.99	0.92	0.82	0.93	0.91	0.91	0.81	1.09
54:5	LLO	11.34	27.17	28.19	28.21	27.61	27.62	27.94	27.85	27.55	28.23	28.2	28.06	26.83
54:6	LLL	32.53	26.28	26.98	27.14	26.78	22.76	27.56	26.27	23.69	27.19	27.14	26.94	26.89

P = palmitic acid; L = linoleic acid; S = stearic acid; O = oleic acid; B = behenic acid; COM = refined commercial oil. Source: own authorship

III.4. CONCLUSION

This study shows relevant results regarding the extraction of sunflower oil by two green technologies, supercritical extraction (SFE) with carbon dioxide and extraction with pressurized liquid (PLE) using ethanol as a solvent. These technologies are emerging due to the use of solvents that are safe for health and therefore promising to replace the conventional extraction that uses hexane. Ethanol and CO_2 are renewable solvents and are therefore environmentally friendly extraction techniques.

Both technologies are promising for obtaining sunflower oil with good levels of tocopherol of high biological value using green solvents

The greatest recovery of crude oil obtained by SFE was 87.6%, and 93.9% by PLE. The greatest content of tocopherols in SFE oil was observed at 60 ºC and 18 MPa (91.17 mg of tocopherols/100 g). The oil obtained via PLE presented up to 83.16 mg/100 g of tocopherols.

PLE provides facilitated technology, with less time of execution and low solvent usage. In about 30 min, the process was able to recovery from 75.37 to 93.93 % (m/m) of the oil in the seed. Considering the intermittent process, the solvent consumption is considerably lower than conventional methods. Convection stage predominates, indicating that the solvent solubilizes the components on the surface of the particles, as discussed in Chapter IV.

However, there were differences in tocopherol content: in oils obtained by SFE, temperature affected α-tocopherol content, while pressure affected γ-tocopherol and δ-tocopherol. Therefore, the total tocopherol content was affected by these two variables. In oils obtained by PLE, no variables affected tocopherol content.

Based on the probable composition of TAGs, the products obtained by SFE and PLE proved to be equivalent to the commercial product, already consolidated in the Brazilian market. This is considered a good indication, since none of the processes that use green technology and solvents that are safe for health interfere with the lipid composition of the oil, maintaining its characteristics.

REFERENCES

ANDREO, D.; JORGE, N. Antioxidantes naturais: técnicas de extração. **Boletim do Centro de Pesquisa de Processamento de Alimentos**, v. 24, n. 2, p. 319-336, 2006. doi:10.5380/cep.v24i2.7489.

ANTONIOSI FILHO, N. R.; MENDES, O. L.; LANÇAS, F. M. Computer prediction of triacylglycerol composition of vegetable oils by HRGC. **Chromatographia**, v. 40, n. 9, p. 557-562, 1995.doi:10.1007/BF02290268.

AOAC. Official Methods of Analysis of AOAC International, 18th ed. Gaithersburg: AOAC International; 2005.

BAÜMLER, E. R.; CARRÍN, M. E.; CARELLI, A. A. Diffusion of tocopherols, phospholipids and sugars during oil extraction from sunflower collets using ethanol as solvent. **Journal of Food Engineering**, v. 194, p. 1-8, 2017. doi:10.1016/j.jfoodeng.2016.09.003.

BAÜMLER, E. R.; CARRÍN, M. E.; CARELLI, A. A. Extraction of sunflower oil using ethanol as solvent. **Journal of Food Engineering**, v. 178, p. 190-197, 2016. doi:10.1016/j.jfoodeng.2016.01.020.

BOUTIN, O.; DE NADAÏ, A.; PEREZ, A. G.; FERRASSE, J. H.; BELTRAN, M.; BADENS, E. Experimental and modelling of supercritical oil extraction from rapeseeds and sunflower seeds. **Chemical Engineering Research and Design**, v. 89, n. 11, p. 2477-2484, 2011. doi:10.1016/j.cherd.2011.02.032.

Brasil - Ministério da Agricultura, Pecuária e Abastecimento. Regras Para Análise de Sementes. Brasilia: MAPA, 2009.

BRUNNER, G. Supercritical fluids: technology and application to food processing. **Journal of food engineering**, v. 67, n. 1-2, p. 21-33, 2005. doi:10.1016/j.jfoodeng.2004.05.060.

COCERO, M. J.; CALVO, L. Supercritical fluid extraction of sunflower seed oil with CO_2-ethanol mixtures. **Journal of the American Oil Chemists' Society**, v. 73, n. 11, p. 1573-1578, 1996. doi:10.1007/BF02523527.

CORNELIO-SANTIAGO, H. P.; BODINI, R. B.; MAZALLI, M. R.; GONÇALVES, C. B.; RODRIGUES, C. E.; DE OLIVEIRA, A. L. Oil extraction from pequi (*Caryocar brasiliensis Camb.*) and sacha inchi (*Plukenetia huayllabambana sp. Nov.*) almonds by pressurized liquid with intermittent purge: The effects of variables on oil yield and composition. **The Journal of Supercritical Fluids**, p. 105527, 2022.

CORREIA, I. M. S.; SOUZA, M. J. B.; SOUZA, E. M. D. B. Extração e caracterização do óleo de semente de girassol (*Helianthus annus* L.) utilizando o método de prensagem a frio e extração por solvents. In… Congresso Brasileiro de Engenharia Química, 18th ed, Foz do Iguaçu, 2010.

CUEVAS, M. S.; RODRIGUES, C. E. C; MEIRELLES, A. J. A. Effect of solvent hydration and temperature in the deacidification process of sunflower oil using ethanol. **Journal of food engineering**, v. 95, n. 2, p. 291-297, 2009. doi:/10.1016/j.jfoodeng.2009.05.009.

DAVARNEJAD, R.; AHMAD, Z.; SATA, S. A.; MORAVEJI, M. K.; AHMADLOO, F. Mutual solubility study in supercritical fluid extraction of tocopherols from crude palm oil using CO_2 solvent. **International journal of molecular sciences**, v. 11, n. 10, p. 3649-3659, 2010. doi:10.3390%2Fijms11103649.

DEL VALLE, J. M.; REVECO-CHILLA, A. G.; VALENZUELA, L. M.; JUAN, C. Estimation of the solubility in supercritical CO2 of α-and δ-tocopherol using Chrastil'model. **The Journal of Supercritical Fluids**, v. 157, p. 104688, 2020. doi:10.1016/j.supflu.2019.104688.

DUNFORD, N. T.; KING, J. W.; LIST, G. R. Supercritical Fluid Extraction in Food Engineering. In: TZIA, C.; LIADAKIS, G. (editors). Extraction optimization in Food Engineering. USA: Marcel Dekker, 2003.

EGGERS, R.; JAEGER, P. Extraction Systems. In: TZIA, C.; LIADAKIS, G. (editors). Extraction optimization in Food Engineering. USA: Marcel Dekker, 2003.

FIORI, L. Supercritical extraction of sunflower seed oil: Experimental data and model validation. **The Journal of Supercritical Fluids**, v. 50, n. 3, p. 218-224, 2009.

FOOD AND AGRICULTURE ORGANIZATION [FAO]. Codex standard for named vegetable oils, 4th ed. Rome: FAO, 1999.

GUNSTONE, F. D. Vegetable Oils in Food Technology: Composition, Properties and Uses. United Kingdom: Wiley-Blackwell, 2011.

Hartman, L.; Antoniassi, R.; Freitas, S. P. Characterization of five sunflower varieties cultivated in Brazil. Curitiba: CEPPA, 1999.

KAUFMANN, B.; CHRISTEN, P. Recent extraction techniques for natural products: microwave-assisted extraction and pressurised solvent extraction. **Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques**, v. 13, n. 2, p. 105-113, 2002. doi:10.1002/pca.631.

KEMPER, T. G. Oil Extraction. In: SHAHID, F. (editor). Bailey's industrial oil and fat products, 6th ed. New Hersey: Hoboken, 2005.

LEO, L.; RESCIO, L.; CIURLIA, L.; ZACHEO, G. Supercritical carbon dioxide extraction of oil and α-tocopherol from almond seeds. **Journal of the Science of Food and Agriculture**, v. 85, n. 13, p. 2167-2174, 2005. doi:10.1002/jsfa.2244.

MOURA, P. M.; PRADO, G. H. C. D.; MEIRELES, M. A. D. A.; PEREIRA, C. G. Supercritical fluid extraction from guava (Psidium guajava) leaves: global yield, composition and kinetic data. **The Journal of Supercritical Fluids**, v. 62, p. 116-122, 2012. doi:10.1016/j.supflu.2011.11.014.

NIMET, G.; DA SILVA, E. A.; PALÚ, F.; DARIVA, C.; DOS SANTOS FREITAS, L.; NETO, A. M.; CARDOZO FILHO, L. Extraction of sunflower (*Heliantus annuus L.*) oil with supercritical CO_2 and subcritical propane: Experimental and modeling. **Chemical Engineering Journal**, v. 168, n. 1, p. 262-268, 2011. doi:10.1016/j.cej.2010.12.088.

OLIVEIRA, A. L.; DESTANDAU, E., FOUGÈRE, L.; LAFOSSE, M. Isolation by pressurised fluid extraction (PFE) and identification using CPC and HPLC/ESI/MS of phenolic compounds from Brazilian cherry seeds (*Eugenia uniflora L.*). **Food chemistry**, v. 145, p. 522-529, 2014.doi:10.1016/j.foodchem.2013.08.065.

PEREIRA, C. G.; MEIRELES, M. A. A. Supercritical fluid extraction of bioactive compounds: fundamentals, applications and economic perspectives. **Food and Bioprocess Technology**, v. 3, n. 3, p. 340-372, 2010. doi:10.1007/s11947-009-0263-2.

PEREZ, E. E.; CARELLI, A; A.; CRAPISTE, G. H. Temperature-dependent diffusion coefficient of oil from different sunflower seeds during extraction with hexane. **Journal of Food Engineering**, v. 105, n. 1, p. 180-185, 2011. doi:10.1016/j.jfoodeng.2011.02.025.

RAI, A.; MOHANTY, B.; BHARGAVA, R. Fitting of broken and intact cell model to supercritical fluid extraction (SFE) of sunflower oil. **Innovative Food Science and Emerging Technologies**, v. 38, p. 32-40, 2016. doi:10.1016/j.ifset.2016.08.019.

RAI, A.; MOHANTY, B.; BHARGAVA, R. Supercritical extraction of sunflower oil: A central composite design for extraction variables. **Food chemistry**, v. 192, p. 647-659, 2016a. doi:10.1016/j.foodchem.2015.07.070.

ROSTAGNO, M. A.; PALMA, M.; BARROSO, C. G. Pressurized liquid extraction of isoflavones from soybeans. **Analytica Chimica Acta**, v. 522, n. 2, p. 169-177, 2004. doi:10.1016/j.aca.2004.05.078.

SALGIN, U.; DÖKER, O.; ÇALIMLI, A. Extraction of sunflower oil with supercritical CO_2: Experiments and modeling. **The Journal of Supercritical Fluids**, v. 38, n. 3, p. 326-331, 2006. doi:10.1016/j.supflu.2005.11.015.

SINEIRO, J.; DOMÍNGUEZ, H.; NÚÑEZ, M. J.; LEMA, J. M. Ethanolic extraction of sunflower oil in a pulsing extractor. **Journal of the American Oil Chemists' Society**, v. 75, n. 6, p. 753-754, 1998. doi:10.1007/s11746-998-0220-7.

TZIA, C. Optimization. In: TZIA, C.; LIADAKIS, G. (editors). Extraction optimization in Food Engineering. USA: Marcel Dekker, 2003.

VELEZ, A.; SOTO, G.; HEGEL, P.; MABE, G.; PEREDA, S. Continuous production of fatty acid ethyl esters from sunflower oil using supercritical ethanol. **Fuel**, v. 97, p. 703-709, 2012. doi:10.1016/j.fuel.2012.02.024.

WAKELYN, P. J.; WAN, P. J. Solvent extraction: safety, health, and environmental issues. In: TZIA, C.; LIADAKIS, G. (editors). Extraction optimization in Food Engineering. USA: Marcel Dekker; 2003.

WANG, L.; WELLER, C. L. Recent advances in extraction of nutraceuticals from plants. **Trends in Food Science & Technology**, v. 17, n. 6, p. 300-312, 2006.doi:10.1016/j.tifs.2005.12.004.

ZENEBON, O.; PASCUET N. S.; TIGLEA, P. Normas analíticas do Instituto Adolfo Lutz: métodos físico-químicos para análise de alimentos. São Paulo: Instituto Adolfo Lutz, 2008.

APPENDIX I

Table I-1. Regression coefficients, p-value and analysis of variance (ANOVA). Linear and quadratic models for oil extraction yield and tocopherols content of sunflower oil obtained with supercritical fluid extraction (SFE)

Linear Model (Ftab = 9.28)						
Variation Source		SS	FD	MS	Fcalc	p-value
Regression	Yield	925.75	3	308.58	9.340	0.0495
	α-tocopherol	1503.12	3	501.04	56.829	0.0038
	β-tocopherol	15.04	3	5.01	1.642	0.3468
	λ-tocopherol	0.112675	3	0.04	8.0651	0.0601
	Δ-tocopherol	0.078075	3	0.03	31.635	0.0090
	Total tocopherol	1706.4	3	568.80	93.415	0.0018
Residue	Yield	99.061	3	33.02		
	α-tocopherol	26.45	3	8.82		
	β-tocopherol	3.05313	3	1.02		
	λ-tocopherol	0.014011	3	0		
	Δ-tocopherol	0.002468	3	0		
	Total tocopherol	18.267	3	6.09		
Lack of fit	Yield	94.319	1			
	α-tocopherol	4.691	1			
	β-tocopherol	2.90086	1			
	λ-tocopherol	0.001811	1			
	Δ-tocopherol	0.002001	1			
	Total tocopherol	0.134	1			
Pure error	Yield	4.7418	2			
	α-tocopherol	21.759	2			
	β-tocopherol	0.15227	2			
	λ-tocopherol	0.0122	2			
	Δ-tocopherol	0.000467	2			
	Total tocopherol	18.133	2			
Total	Yield	1024.8	6			
	α-tocopherol	1529.569	6			
	β-tocopherol	18.0938	6			
	λ-tocopherol	0.126686	6			
	Δ-tocopherol	0.080543	6			
	Total tocopherol	1724.671	6			
R^2; R^2 adjusted	Yield	0.90; 0.81				
	α-tocopherol	0.98; 0.96				

Continues…

...continuation

	β-tocopherol	0.83; 0.66
	λ-tocopherol	0.89; 0.78
	Δ-tocopherol	0.97; 0.94
	Total tocopherol	0.99; 0.98

Quadratic Model (Ftab = 5.05)

Variation Source		SS	DF	SQM	Fcalc	p-value
Regression	Yield	1794.7	5	358.95	60.027	0.0002
	α-tocopherol	3850	5	770	7.6539	0.0217
	β-tocopherol	13.90571	5	2.78	2.7178	0.1483
	λ-tocopherol	0.573181	5	0.11	5.7992	0.0382
	Δ-tocopherol	0.137952	5	0.03	1.4529	0.3459
	Total tocopherol	4258.203	5	851.64	9.3199	0.0143
Residue	Yield	29.899	5	5.9798		
	α-tocopherol	503.01	5	100.6		
	β-tocopherol	5.11651	5	1.02		
	λ-tocopherol	0.098837	5	0.02		
	Δ-tocopherol	0.094539	5	0.02		
	Total tocopherol	45.6894	5	91.38		
Lack of fit	Yield	25.157	3			
	α-tocopherol	481.25	3			
	β-tocopherol	4.96424	3			
	λ-tocopherol	0.086637	3			
	Δ-tocopherol	0.094072	3			
	Total tocopherol	438.761	3			
Pure error	Yield	4.742	2			
	α-tocopherol	21.759	2			
	β-tocopherol	0.15227	2			
	λ-tocopherol	0.0122	2			
	Δ-tocopherol	0.000467	2			
	Total tocopherol	18.133	2			
Total	Yield	1824.6	10			
	α-tocopherol	4353.005	10			
	β-tocopherol	19.02222	10			
	λ-tocopherol	0.672018	10			
	Δ-tocopherol	0.232491	10			
	Total tocopherol	4715.097	10			
R^2; R^2 adjusted	Yield	0.98; 0.96				
	α-tocopherol	0.88; 0.77				

Continues...

...continuation

β-tocopherol	0.73; 0.46
λ-tocopherol	0.85; 0.70
Δ-tocopherol	0.59; 0.19
Total tocopherol	0.90; 0.81

Statistical significance $p < 0.05$. SS = sum of squares; DF = degress of freedom; MS = mean square.

APPENDIX II

Table II-1. Regression coefficients, p-value and analysis of variance (ANOVA). Models for oil extraction yield (linear and quadractic models) and tocopherols content (linear model) of sunflower oil obtained with pressurized liquid extraction (PLE) using ethanol as a solvent

Linear Model (Ftab = 9.28)						
Variation Source		SS	FD	MS	Fcalc	p-value
Regression	Yield	119.046	3	39.68	68.81	0.003
	α-tocopherol	104.06	3	186.44	1.23	0.435
	β-tocopherol	0.19	3	0.06	0.21	0.886
	λ-tocopherol	0.04	3	0.04	0.91	0.531
	Δ-tocopherol	0.03	3	0.01	1.24	0.431
	Total tocopherol	111.34	3	37.11	0.24	0.863
Residue	Yield	1.73	3	0.58		
	α-tocopherol	455.28	3	151.76		
	β-tocopherol	0.31	3	0.10		
	λ-tocopherol	0.12	3	0.04		
	Δ-tocopherol	0.02	3	0.01		
	Total tocopherol	459.70	3	153.23		
Lack of fit	Yield	1.04	1			
	α-tocopherol	444.50	1			
	β-tocopherol	0.00	1			
	λ-tocopherol	0.05	1			
	Δ-tocopherol	0.02	1			
	Total tocopherol	442.57	1			
Pure error	Yield	0.69	2			
	α-tocopherol	10.78	2			
	β-tocopherol	0.31	2			
	λ-tocopherol	0.08	2			
	Δ-tocopherol	0.00	2			
	Total tocopherol	17.13	2			
Total	Yield	120.77	6			
	α-tocopherol	559.33	6			
	β-tocopherol	0.50	6			
	λ-tocopherol	0.16	6			
	Δ-tocopherol	0.05	6			
	Total tocopherol	571.03	6			

Continues…

…continuation

R^2; R^2 adjusted	Yield	0.99; 0.97
	α-tocopherol	0.19; 0.00
	β-tocopherol	0.38; 0.00
	λ-tocopherol	0.24; 0.00
	Δ-tocopherol	0.55; 0.11
	Total tocopherol	0.19; 0.00

Quadratic Model (Ftab = 5.05)

Variation Source	Yield	SS	DF	MS	Fcalc	p-value
Regression		119.05	5	23.81	68.815	0.0001
Residue		1.73	5	0.35		
Lack of fit		1.04	3			
Pure error		0.69	2			
Total		120.77	10			
R^2; R^2 adjusted		0.99; 0.97				

Statistical significance $p < 0.05$. SS = sum of squares; DF = degress of freedom; MS = mean square.

CHAPTER IV: SUNFLOWER OIL EXTRACTION KINETICS BY PRESSURIZED ETHANOL

IV.1. INTRODUCTION

Sunflower seed obtained by unconventional processes is rich in high quality oil (VICENTINI-POLETTE et al., 2021). However, the particle size used can influence the extraction process (FIORI et al., 2008) as a whole.

The observation of the kinetics of oil extraction by unconventional processes has been recurrent (RAI et al., 2016; CAVALCANTI et al., 2013; EIKANI et al., 2011), and allows observing differences in the extraction process. Rai et al. (2016) observed the process in sunflower seed, where the extracted oil in each time step was weighed and recorded as kinetic extraction data at that time, in a similar way to that performed in this study. The authors state that the extraction efficiency increases with temperature, pressure, solvent mass flow, and addition of co-solvent (ethanol). In addition, particle size has an inverse effect on extraction yield. Cavalcanti et al. (2013) stated that the kinetic behavior of the global extraction curve starts with a constant extraction rate, then decreases, and a mechanism combining diffusion and convection is proposed. Eikani et al. (2011) evaluated the behavior of pomegranate seed oil extraction by supercritical fluid. Acoording to the authors, at higher flow rates, the extraction of seed oil was too fast, meaning that the mass transfer of the seed oil from the surface of the small solid particles to the solvent phase regulates most of the extraction process. However, using higher flow rates resulted in more diluted extracts.

No studies have been found reporting the kinetics of sunflower seed oil extraction by pressurized ethanol.

The collection of experimental data on the kinetics of sunflower oil extraction via PLE in the optimized extraction condition, for different particle sizes, will be used in the modeling of the intermittent extraction process in future studies.

IV.2. OBJECTIVES

The sunflower oil extraction kinetics using pressurized ethanol as solvent were determined for the continuous and intermittent processes using rolled and grounded

sunflower seeds. The behaviors of the extractions were compared to indicate the profile of the process.

IV.3. MATERIALS AND METHODS

The sunflower oil extractions in a continuous process using pressurized ethanol were performed in four repetitions, using 10 g of rolled (R) and grounded (G) sunflower seeds (particle diameter < 2 mm). Seeds were packaged in a 22 mL fixed bed extractor. The pressure during the extraction was kept constant at 10.34 MPa and the temperature at 84 ºC, according to the parameters established in previous studies.

For the analysis of the extraction kinetics of the continuous process with R seeds, only one extraction cycle (C = 1) was applied at a time, with 0 % rinsing volume of solvent (VS). In this case, the volume of solvent was the one used to pressurize the cell to 10.34 MPa and 84 °C. Static times (St) were applied, corresponding to the contact time between the matrix and solvent for 5 and 10 min, so that the first seven extract collections occurred after 5 min and the remaining five extract collections after 10 min. The total solvent used in this extraction was greater than 320 mL. The oleic extracts were stored in glass flasks, previously weighed. After extraction, the ethanol was recovered using a rotary evaporator at 40 ºC. The same methodology was applied for G seeds, in triplicate.

The extractions in the intermittent process with pressurized ethanol were carried out, in triplicate, using 10 g of R sunflower seeds (R) and 110 % of the cell volume as a rinse volume (24.2 mL). The rinse volume was chosen on previous studies (Chapter III). The total volume of pressurized ethanol used in this process was about 70 mL per replicate. In the intermittent process, a static time (St) of 5 min was applied in 4 cycles (C). Thus, in each cycle, the rinse volume was 6.05 mL. The extracted oils were stored in glass flasks, previously weighed on an analytical scale and, after the extraction, the ethanol was recovered in a rotary evaporator at 40 ºC. The same procedures of the intermittent process for rolled seeds were applied to grounded seeds, in triplicate (IG). Tukey's test at 5% significance was applied to analyze the processes.

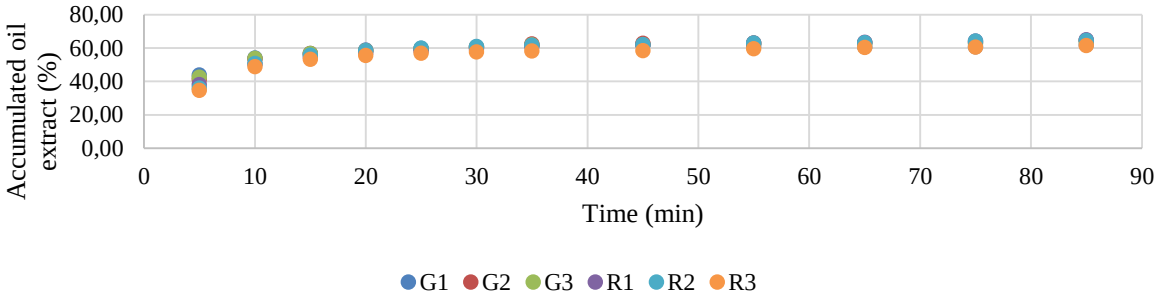
IV.4. RESULTS

The kinetic behavior of the global extraction curves starts with a constant extraction rate in which convection predominates as a mass transfer mechanism. Then, there is a decrease in this rate and the diffusion mechanism combined with convection is

presented. Finally, there is a period of controlled diffusion in which the diffusion mechanism prevails (CAVALCANTI, 2013).

In kinetics, it was observed the constant accumulation of masses (g) of sunflower oils extracted over time. In the continuous process, the controlled diffusion phase (*plateau*) was reached after 30 min of extraction, as shown by the kinetic curves (Figure 1). Considering the constant and decreasing extraction phases (30 min of extraction), maximum utilization of 59.40 % of extracted oil was achieved. When the G seeds were used, a maximum yield of 54.42 % was obtained in the same period, resulting in a statistical difference between the processes ($p \leq 0.05$), where at 30 min of extraction the R seeds achieved greater yield than the G one.

Figure 1 – Masses of sunflower oil extracted from rolled (R) and grounded (G) seeds accumulated over time in a continuous process by PLE at 84 ºC and 0 % RV



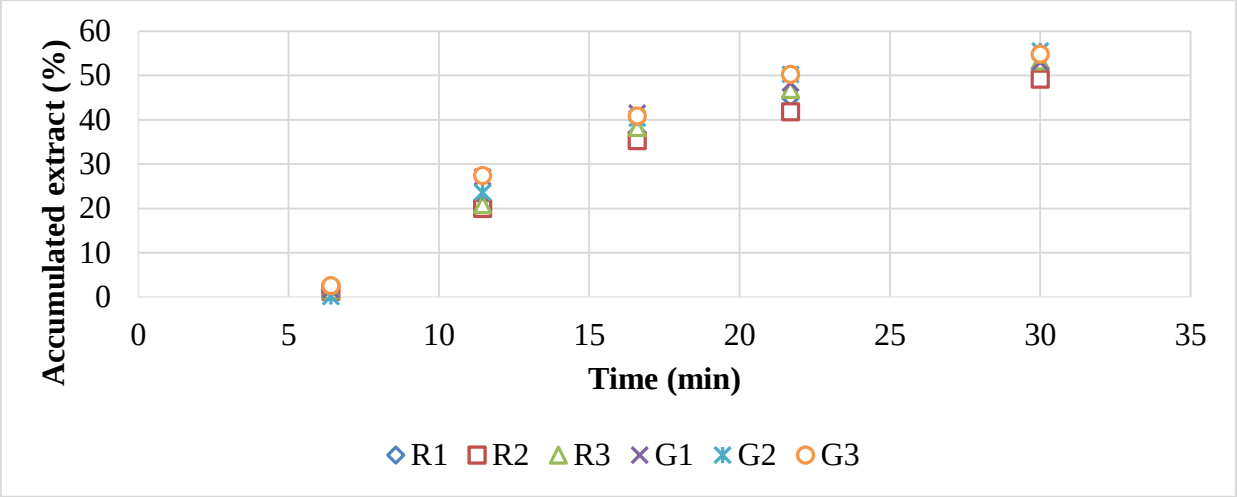
Source: own authorship

In the intermittent process, the mass of extracted oil when using R seeds was up to 51.34 % in a total period of 1800 seconds (30 min) in the triplicate performed (Figure 2). Meanwhile, the G seeds presented maximum accumulation equal to 54.42 % in the same period (Figure 2), also showing a statistical difference between the processings ($p \leq 0.05$).

In this case, as the extraction domain occurs in the constant extraction phase, the constant diffusion phase is not reached. Therefore, lower yields were obtained. When comparing the particle sizes, it was noted that when the seeds were G (smaller), the yields were higher. The granulometry interferes with the constant extraction phase, a phase in which the extract on the surface of the particles is solubilized by the solvent. Therefore, smaller particles have larger contact areas and allow for better extraction (EIKANI et al., 2011), although R are the most advantageous in the continuous process.

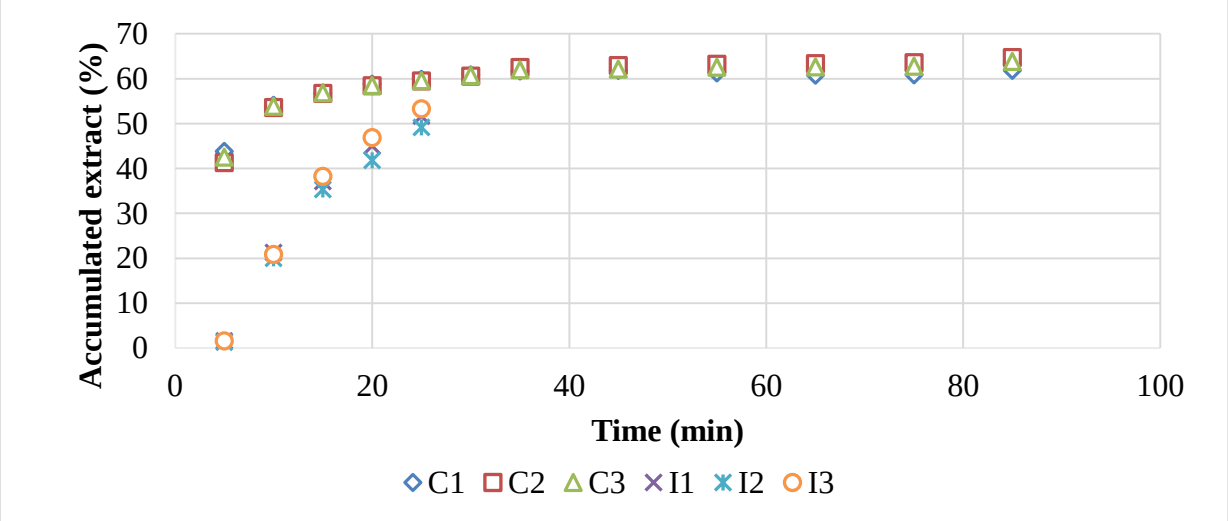
The intermittent extraction process, as well as the continuous one, results in the same accumulated oil mass in 30 min, however, in the intermittent one, the line inclination for constant extraction showed another angle (Figure 3). In the intermittent process, the yield obtained was slightly lower when compared to the continuous process; however, the amount of solvent was much lower.

Figure 2 – Masses of sunflower oil extracted from rolled (R) and grounded (G) seeds accumulated over time in the intermittent process by PLE at 84 ºC and 110 % of VS



Source: own authorship

Figure 3 – Masses of sunflower oil extracted from rolled seeds (R) accumulated over time in the continuous (C) and intermittent (I) process by PLE at 84 ºC



Source: own authorship

IV.5. CONCLUSIONS

The yield of extracted oil was different for the different processes. In the continuous process, the yield was about 60 %, not differentiating concerning the size of the particles rolled (R) or grounded (G). In the intermittent process, the extraction occurred in the decreasing phase, not reaching the diffusion phase, reaching 51.34 % (R) and 54.42 % (G). However, the solvent consumption was considerably lower. In both processes, convection predominates, which indicates that the solvent solubilizes the components on the surface of the particles. The kinetics and cell size will be analyzed in further studies.

REFERENCES

CAVALCANTI, R. N. **Extração de antocianinas de resíduo de jabuticaba (Myrciaria cauliflora) utilizando liquido pressurizado e fluido supercrítico: caracterização química, avaliação econômica e modelagem matemática**. 2013. 197 f. Tese (Doutorado em Engenharia de Alimentos)- Universidade Estadual de Campinas, Campinas, São Paulo, 2013.

COLIVET, J.; OLIVEIRA, A. L.; CARVALHO, R. A. Influence of the bed height on the kinetics of watermelon seed oil extraction with pressurized ethanol. **Separation and Purification Technology**, v. 169, p. 187-195, 2016.

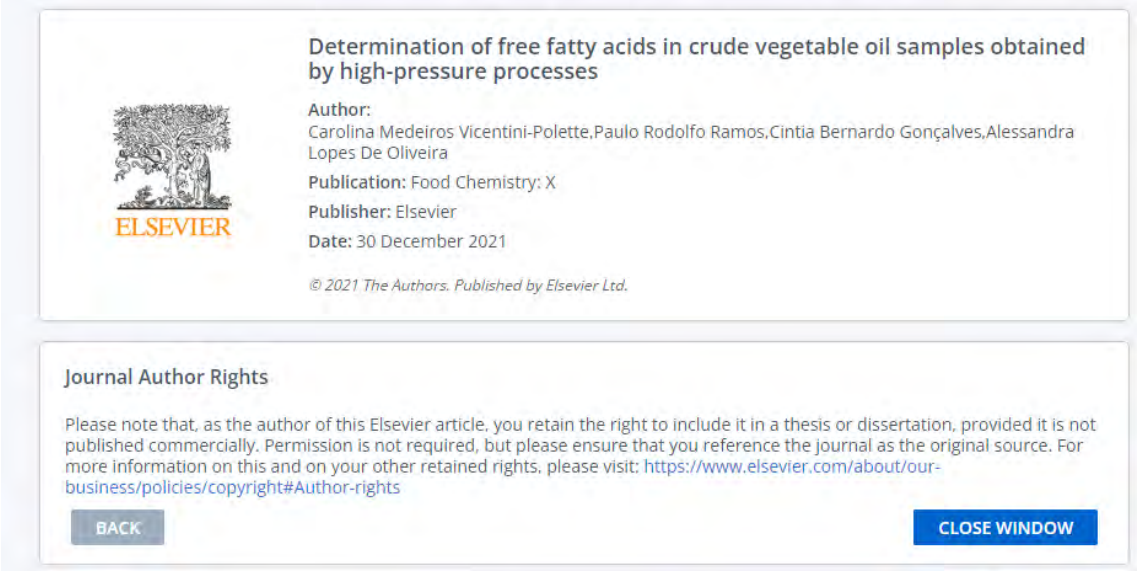
EIKANI, M. H., GOLMOHAMMAD, F., HOMAMI, S. S. Extraction of pomegranate (*Punicagranatum L.)* seed oil using superheated hexane**. Food and Bioproducts Processing**, v. 90, p. 32- 36, 2011.

FIORI, L.; BASSO, D.; COSTA, P. Seed oil supercritical extraction: Particle size distribution of the milled seeds and modeling. **The Journal of Supercritical Fluids**, v. 47, n. 2, p. 174-181, 2008.

RAI, A.; MOHANTY, B.; BHARGAVA, R. Fitting of broken and intact cell model to supercritical fluid extraction (SFE) of sunflower oil. **Innovative food science & emerging technologies**, v. 38, p. 32–40, 2016.

VICENTINI-POLETTE, C.M.; RAMOS, P. R.; GONÇALVES, C. B.; OLIVEIRA, A. L. de. Determination of free fatty acids in crude vegetable oil samples obtained by high-pressure processes. **Food Chemistry: X**, v. 12, p. 100166, 2021.

CHAPTER V: DETERMINATION OF FREE FATTY ACIDS IN CRUDE VEGETABLE OIL SAMPLES OBTAINED BY HIGH-PRESSURE PROCESSES



Determination of free fatty acids in crude vegetable oil samples obtained by high-pressure processes

Author:
Carolina Medeiros Vicentini-Polette,Paulo Rodolfo Ramos,Cintia Bernardo Gonçalves,Alessandra Lopes De Oliveira

Publication: Food Chemistry: X

Publisher: Elsevier

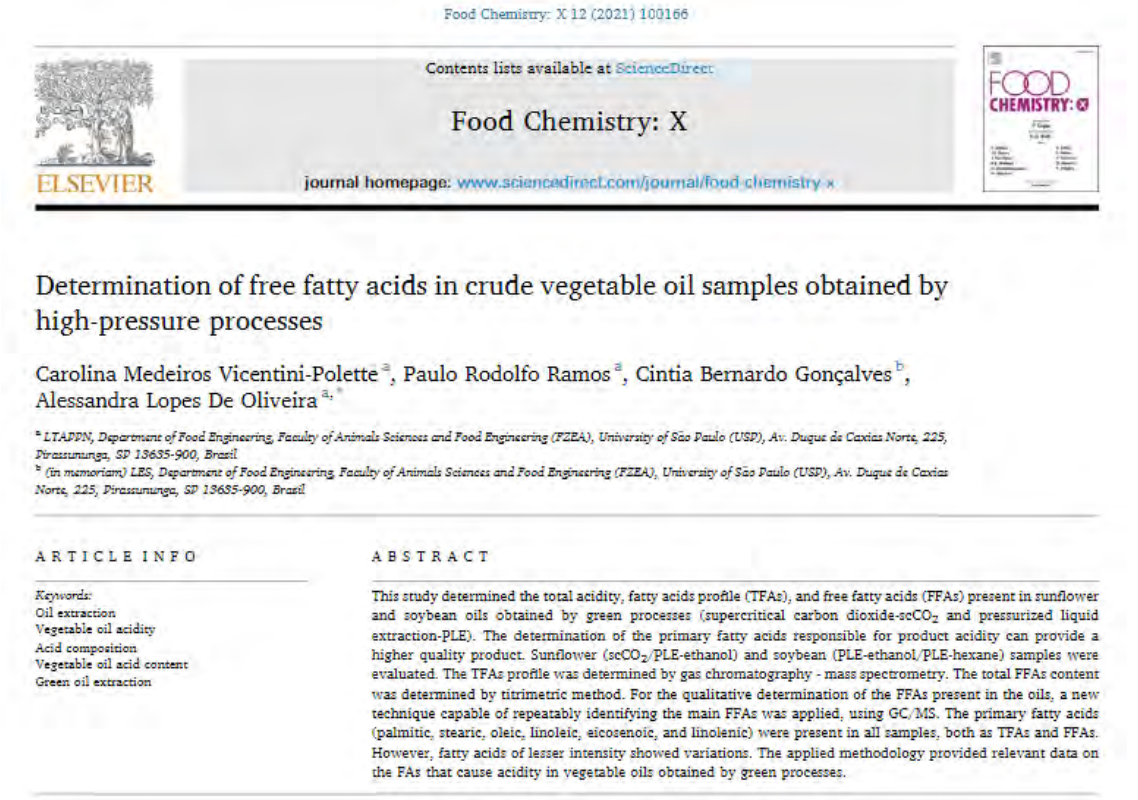
Date: 30 December 2021

© 2021 The Authors. Published by Elsevier Ltd.

Journal Author Rights

Please note that, as the author of this Elsevier article, you retain the right to include it in a thesis or dissertation, provided it is not published commercially. Permission is not required, but please ensure that you reference the journal as the original source. For more information on this and on your other retained rights, please visit: https://www.elsevier.com/about/our-business/policies/copyright#Author-rights

BACK CLOSE WINDOW



Food Chemistry: X 12 (2021) 100166

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x

Determination of free fatty acids in crude vegetable oil samples obtained by high-pressure processes

Carolina Medeiros Vicentini-Polette [a], Paulo Rodolfo Ramos [a], Cintia Bernardo Gonçalves [b], Alessandra Lopes De Oliveira [a,*]

[a] LTAPPN, Department of Food Engineering, Faculty of Animals Sciences and Food Engineering (FZEA), University of São Paulo (USP), Av. Duque de Caxias Norte, 225, Pirassununga, SP 13635-900, Brazil
[b] (in memoriam) LES, Department of Food Engineering, Faculty of Animals Sciences and Food Engineering (FZEA), University of São Paulo (USP), Av. Duque de Caxias Norte, 225, Pirassununga, SP 13635-900, Brazil

ARTICLE INFO

Keywords:
Oil extraction
Vegetable oil acidity
Acid composition
Vegetable oil acid content
Green oil extraction

ABSTRACT

This study determined the total acidity, fatty acids profile (TFAs), and free fatty acids (FFAs) present in sunflower and soybean oils obtained by green processes (supercritical carbon dioxide-scCO_2 and pressurized liquid extraction-PLE). The determination of the primary fatty acids responsible for product acidity can provide a higher quality product. Sunflower (scCO_2/PLE-ethanol) and soybean (PLE-ethanol/PLE-hexane) samples were evaluated. The TFAs profile was determined by gas chromatography - mass spectrometry. The total FFAs content was determined by titrimetric method. For the qualitative determination of the FFAs present in the oils, a new technique capable of repeatably identifying the main FFAs was applied, using GC/MS. The primary fatty acids (palmitic, stearic, oleic, linoleic, eicosenoic, and linolenic) were present in all samples, both as TFAs and FFAs. However, fatty acids of lesser intensity showed variations. The applied methodology provided relevant data on the FAs that cause acidity in vegetable oils obtained by green processes.

This chapter consists in the article published by Carolina Medeiros Vicentini-Polette, Paulo Rodolfo Ramos, Cintia Bernardo Gonçalves (*in memoriam*), and

Alessandra Lopes De Oliveira at Food Chemistry: X Journal, in November 2021 (VICENTINI-POLETTE et al., 2021). Therefore, the manuscript is present in its original format, including references.

ABSTRACT

This study determined the total acidity, fatty acids profile (TFAs), and the free fatty acids (FFAs) present in sunflower and soybean oil obtained by green processes (supercritical carbon dioxide, $scCO_2$ and pressurized liquid extraction, PLE). The determination of the primary fatty acids responsible for product acidity can provide a higher quality product. Sunflower ($scCO_2$ / PLE-ethanol) and soybean (PLE-ethanol / PLE-hexane) samples were evaluated. The TFAs profile was determined by gas chromatography - mass spectrometry. The total FFAs content was determined by titrimetric method. For the qualitative determination of the FFAs present in the oils, a new technique capable of repeatably identifying the main FFAs was applied, using gas chromatography - mass spectrometry. The primary fatty acids (palmitic, stearic, oleic, linoleic, eicosenoic, and linolenic) were present in all samples, both as TFAs as FFAs. However, fatty acids of lesser intensity showed variations. The applied methodology provided relevant data on the fatty acids that cause acidity in vegetable oils obtained by green processes.

Keywords: oil extraction; vegetable oil acidity; acid composition; vegetable oil acid content; green oil extraction.

V.1. INTRODUCTION

Hexane has been used for decades in the conventional extraction of vegetable oils and fats. This solvent consists of a mixture of hydrocarbons that generally boils between 65 and 69°C. Its commercial version contains approximately 65% of normal hexane, with

the remaining 35% comprising cyclopentane and hexane isomers (Bailey and Shahidi, 2005).

However, new extraction alternatives are currently being investigated, such as processes that use renewable or "green" solvents to obtain vegetable oils. A conventional, more rustic extraction technique (mechanical pressing) has also been used, providing vegetable oils without petroleum-derived organic solvent residues but lower yields. Green and health-safe solvents have been employed in high-pressure systems, such as extractions using solvents in supercritical states (Supercritical fluid extraction, SFE) and pressurized solvents (Pressurized liquid extraction, PLE). SFE and PLE are considered clean extraction techniques since they also do not leave toxic solvent residues in the extracted vegetable oils and because they require smaller amounts of solvent (in some processes) and shorter periods of time to perform.

Supercritical fluid extraction is based on the interaction between a sample and a supercritical fluid. The fluid becomes supercritical under conditions of pressure (P) and temperature (T) above its critical point, defined as the T and P in which the gaseous and liquid phases become indistinguishable. When a substance is heated and compressed above its critical point, its "supercritical phase" is formed. Carbon dioxide (CO_2) is the most used solvent in supercritical extraction processes of vegetable oils since it presents the advantage of having low values of relative critical temperature (31.1ºC) and pressure (7.4 MPa) (Dunford et al., 2003). Extraction with $scCO_2$ is indicated for lipophilic compounds and apolar substances. The combination of relative low temperature and high pressure allows good separation of phytosterols and vitamin E, more efficiently, purely, and with lower waste compared to the conventional process, since tocopherol (vitamin E) is fat-soluble (Asl et al., 2020; Maul et al., 1996).

Recently, studies based on extraction using supercritical CO_2 have been directed towards some unconventional vegetable oils, such as wheat germ (Bojanić et al., 2019), palm (Promraksa et al., 2020), and "bacaba-de-leque" – a Brazilian palm tree (Cunha et al., 2019). Benito-Román et al. (2018) evaluated the quinoa oil extraction using CO_2, proving that the oil obtained by $scCO_2$ presented higher antioxidant capacity and tocopherol content than the oil extracted with hexane, and also that the extraction rate may be strongly controlled by the pressure rate (20–40 MPa).

Obtaining conventional oils using this technology has also been evaluated, such as canola oil (Sun et al., 2021) and coconut oil (Torres-Ramón et al., 2021). In addition, the technique also has excellent potential for extracting oil contained in byproducts, including olive oil cake (Durante et al., 2020), raspberry seeds (Pavlić et al., 2020), and coffee grounds (Muangrat and Pongsirikul, 2019).

PLE with intermittent extract purge is an efficient extraction method that was developed in the 1980s for analytical purposes to extract compounds at low concentrations from samples (Richter et al., 1996). The technique started to be used to obtain extracts with active principles, including "araçá" (Bittencourt et al., 2019) and "candeia" (Santos et al., 2019) extract, and to optimize the conditions for the extraction of matrices, such as coffee grains (N. A. de Oliveira et al., 2018). This process enables extractions in shorter times and the use of renewable solvents, such as ethanol, for example. High temperatures (T) decrease solvent viscosity, facilitating its penetration and diffusion without solute degradation, while high pressure (P) maintains the solvent in the liquid state under T conditions, in which it would otherwise be in the gaseous state at atmospheric pressure (Wang and Weller, 2006). The optimization of the PLE of vegetable oils using pressurized alcohols, such as ethanol and isopropanol, has been the subject of

research by the current research group (Bittencourt et al., 2019; Okiyama et al., 2018; Oliveira et al., 2018).

The determination of free fatty acid (FFA) content is an important analysis for evaluating the quality of raw material and its degradation during storage, and throughout the shelf life of several vegetable oils, such as soybean, sunflower, rice, and canola. Also, the oxidation process may confers different quality and sensorial attributes to the oils (Coppin and Pike, 2001; Franco et al., 2018). The influence of temperature on oil degradation was assessed by Frega et al. (1999) in their study on oxidative stability.

The analysis of free fatty acids (FFAs) present in crude oils obtained by different extraction processes (conventional, PLE, and SFE) was standardized in the present study.

Two widespread analyses performed on oils are the determination of the total fatty acid (TFA) profile, which identifies the individual fatty acids that make up the triacylglycerols present in the oil, and the acidity index (AI), used to determine the FFA content. Thus, high acidity levels indicate high concentrations of FFAs in the oil (Santos et al., 2021).

Gonçalves and Meirelles (2004) determined the FFAs and TFAs in palm oil. According to the authors, although lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, and arachidic (eicosanoic) acid esters were found in the TFA profile, only lauric, palmitic, stearic, oleic, linoleic, and arachidic acid were observed in the FFA.

According to the studies by Nimet et al. (2011) and Rai et al. (2016), who described the use of gas chromatography to determine the TFA profile in sunflower oil extracted using supercritical carbon dioxide ($scCO_2$), the extraction method did not influence the TFA profile, as was observed in coffee oil extraction (Cornelio-Santiago et al., 2017; De Oliveira et al., 2014). However, in Brazil nuts, Cornelio-Santiago et al. (2021) found differences in fatty acid content depending on how the oil was extracted.

Acidity can be determined by volumetric titration and can be expressed as a percentage by mass of lauric, palmitic, erucic, or oleic acid, depending on the oil's origin. This method is widely used for refined oils and presents good repeatability (Varona et al., 2021).

Although the identification of the TFA profile and the determination of titrimetric acidity are highly relevant for the characterization of oils, there is still no standardized methodology for the determination of FFAs in vegetable oils without the occurrence of triacylglycerol breakdown. Determining the acidity index and acid content does not allow for the individual identification of the free fatty acids. From a practical standpoint, the results of this study might provide insights leading to better understanding of the free fatty acids in crude vegetable oils extracted using green technologies.

Therefore, this study aims mainly at the development and detailing of a methodology for identifying FFAs in sunflower and soybean crude oils obtained by green processes, and stating the TFAs profile and total acidity levels for such oils as well as their qualitative FFAs profiles using the new methodology, determining eventual differences in FFA composition regarding the extraction method.

V.2. MATERIAL AND METHODS

V.2.1. Reagents

In order to obtain the crude oils, 99.5% absolute ethanol (Dinâmica, Indaiatuba, Brazil), 99% carbon dioxide [CO_2] (Linde, Sertãozinho, Brazil), and n-hexane P.A. ACS (Êxodo Científica, Hortolândia, Brazil) were used.

Sample acidity was determined using ethyl ether P.A. (Dinâmica, Indaiatuba, Brazil), phenolphthalein P.A. (Synth, Diadema, Brazil), and 99% sodium hydroxide (Êxodo Científica, Hortolândia, Brazil).

For the determination of the free fatty acid content, the following reagents were used: 99% sodium hydroxide [NaOH] (Êxodo Científica, Hortolândia, Brazil), n-hexane P.A. ACS (Êxodo Científica, Hortolândia, Brazil), chromatographic grade n-hexane (VWR, Fontenay-sous-Bois, France), 14% boron trifluoride [BF_3] in methanol (Aldrich, St. Louis, USA), 98.5% hexane (Êxodo Científica, Sumaré, Brazil), and 99% anhydrous sodium sulfate P.A. [Na_2SO_4] (Synth, Diadema, Brazil); 99.9% compressed nitrogen (Messer, Barueri, Brazil) was used for drying the samples

V.2.2. Methods

Four vegetable oil samples obtained by alternative processes were analyzed, two sunflower oils and two soybean oils.

To determine the free fatty acids, the AOAC method 969.33 (AOAC, 2005) has been modified as an analytical basis so, instead of the total content, only the free fatty acids present in the crude oils would be determined.

V.2.2.1. Obtaining the crude oils

The sunflower oils were obtained by pressurized liquid extraction (PLE), with intermittent solvent purge, using 99.5 % ethanol as a solvent at a temperature (T) of 84 °C, and a rinse volume (RV) in each cycle corresponding to 110 % of the 100 mL extraction cell volume (110 mL). The rinse volume was divided into four cycles (N), each lasting 5 min, constituting the static time (St) of contact between the matrix and the solvent in each cycle. The extraction was conducted under optimized conditions, in which it was possible to recover 93.93 % of oil. The optimized conditions were determined in previous studies of the extraction process using rotated central compound delineation (CCRD) design. In the supercritical extraction using carbon dioxide ($scCO_2$), also

performed under optimized conditions (60 ºC; 32.1 MPa; flow rate of 10 g/s for 4 h), it was possible to recover 87.58 % of oil.

Meanwhile, the crude soybean oils were obtained by PLE, with ethanol and hexane as pressurized solvents. Upon extraction using 99.5 % ethanol, the optimized conditions (80 °C; RV corresponding to 60 % of the extraction cell volume of 100 mL, or 60 mL) were applied. The RV was divided into three cycles (N), with a St of 12 min; soybean oil recovery under this condition was 94.40 %. As for the PLE-hexane, also performed under optimized conditions (85 °C; RV of 60 % of the extr [eq. 1] olume, divided into 3 cycles (N); St of 13 min), the recovery rate was 86.16 % of oil.

V.2.2.2. Determination of the total titratable acidity

The total titratable acidity was determined for all samples according to the Ca 5a-40 method (AOCS, 2004). Approximately 2.0 g of oil were used, dissolved in 25 mL of ether-alcohol solution (2:1), as well as two drops of 1 % phenolphthalein indicator in ethanol. Titration was performed using 0.1 M sodium hydroxide solution in deionized water until the appearance of pink color, with a minimum persistence of 30 s (Moretto and Fett, 1986). The FFA content was calculated according to Equation 1.

$$\% FFA = \frac{(V.M.28.2)}{m}$$

where % FFA represents the percentage of free fatty acids, V is the volume of solvent used, M corresponds to the Molarity of the NaOH solution, and m is the mass of the crude oil sample.

The results were expressed in oleic acid content (% m/m), and all analyses were conducted in triplicate.

V.2.2.3. Identification of total fatty acids (TFA)

To the total fatty acid profile determination, the crude oil samples were saponified and esterified using hexane (method 969.33, AOAC, 2005), then diluted to 10 % (in hexane) and injected into the chromatograph.

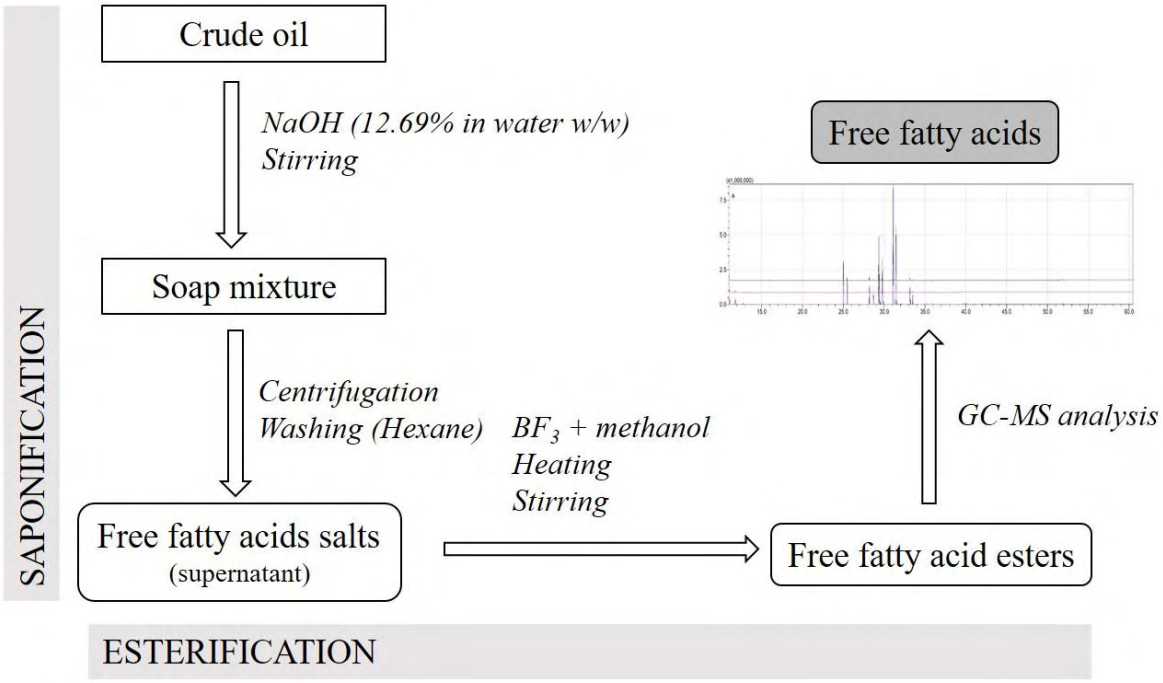
The profile was obtained using a capillary column used was the SP 2560 (100 m x 0.25 mm x 0.2 μm), with helium as the carrier gas, at a flow rate of 1.59 $mL.min^{-1}$, by Gas Chromatography coupled to the Mass Spectrometer (GC-MS), equipped with a Split injector (1:40) (Shimadzu, GCMS-2010), with an automatic injector (model AOC-5000) at 250 ºC and P = 300 kPa. The oven temperature was programmed to go from 100 to 250 °C, initially remaining at 100 °C for 1 min and gradually increasing by 5 $°C.min^{-1}$ until reaching 195 °C. Next, the ramp was adjusted to gradually increase by 2 °C until it reached 250 °C, where it remained for 13 min for completion. Readings were taken between minutes 11 and 60.5, with a mass range of 40 to 350 m/z.

The obtained data were evaluated using the GC-MS Solutions software (v. 2.5, USA), coupled to the gas chromatograph and the mass spectrometer. The detector provided the ion chromatograms and molecular mass spectra for each sample. Identification was carried out by comparing the mass spectra of the compounds with those found in the NIST Library (National Institute of Standards and Technology, v. 11, USA), integrated in the detector. Peaks with an area ≥ 0.5% were considered.

V.2.2.4. Saponification

The saponification and esterification processes are outlined in Figure 1.

Figure 1. Diagram of the processes used to determine the free fatty acid.



A total of 3.0 g of crude vegetable oil were used. This mass was established considering the acidity of the crude sunflower and soybean vegetable oils used in the analysis (from 2.1 to 3.6 g/100 g oleic acid), estimating the amount required for detection by gas chromatography-mass spectrometry (GC-MS).

In order to determine the FFAs, it was necessary to ensure that all the FFAs present in the oil were completely neutralized. The present analysis was based on the study by Gonçalves and Meirelles (2004), who highlighted the use of an excess of 10 % of NaOH solution for complete acid neutralization. Since there was no prior knowledge of the acids contained in the oils, it was not possible to determine the molar mass of the compounds. Therefore, the used volume was estimated based on the aforementioned study.

Five milliliters of NaOH solution (12.69 %, m/m) in deionized water were added to each sample at room temperature, followed by vigorous stirring in a Vortex shaker (model Genius 3, IKA, Staufen, Germany) for 15 min. The mixture of NaOH with the oil results in the saponification of free fatty acids, *i.e.*, the formation of fatty acid salts. This

reaction was performed at room temperature (Choe and Min, 2006) and using low concentration NaOH solution to avoid the tracylglycerol breakdown / hydrolysis.

Subsequently, the mixtures were centrifuged (Excelsea II 206-BL centrifuge, Fanem, Guarulhos, Brazil) at 5000 rpm for 10 min to separate the soap. Following centrifugation, three phases were observed. By tilting the tube and with the aid of a glass Pasteur pipette, the aqueous and oil phases were drained. Then, the white solid (soap) phase was carefully transferred to a new tube. In this step, it is crucial to avoid the transfer of neutral oil together with the soap. Therefore, the solid phase was carefully dried with absorbent paper.

V.2.2.5. Separation of the fatty acid salts

The soap was then washed with P.A. hexane. To this end, 3 mL of the solvent was added, vortexed for 15 s, and then centrifuged for 10 min. This process was repeated five times, carefully collecting the upper phase (hexane), where the salts are found.

The collected hexane was accumulated in a 5 mL vial. Subsequently, the solution containing the fatty acid salts was dried under N_2 flow in a sample concentrator (Marconi, Piracicaba, Brazil).

V.2.2.6. Esterification

Upon obtaining the fatty acid salts, esterification was conducted to volatilize their compounds. After drying, 3 mL of boron trifluoride (BF_3) in methanol were added to the samples at a concentration of 14 %. In a water bath, the vials were heated to 100 °C for 2 min, followed by rapid cooling in running water. For convenience and safety, the glass vials were placed in 50 mL screw-capped plastic tubes for heating.

Next, 6 mL of chromatographic grade hexane were added to the vials, which were then vortexed. After phase separation, the supernatant phase (hexane + fatty acid esters)

was collected into a new glass flask, while the lower phase (BF_3 + methanol) was discarded.

Approximately 0.2 g of Na_2SO_4 was added to the collected phase, aiming at capturing possible water molecules that could be present. The vials were then shaken, after which phase separation was awaited.

Using a glass Pasteur pipette, approximately 1.5 mL of the supernatant phase were carefully transferred to the GC-MS injection vials.

V.2.2.7. Determination of free fatty acids

The free fatty acids in the oils were analyzed by gas chromatography-mass spectrometry (QP 2010 Plus, Shimadzu, Tokyo, Japan) in a device equipped with a Split/Splitless injector (AOC-5000, Shimadzu, Tokyo, Japan) with an automatic injector (model AOC-5000). A capillary column (100 m × 0.25 mm id × 0.20 μm df, SP-2560 Supelco, Bellefonte, USA) was used as a stationary phase, with helium as the carrier gas, at a flow rate of 1.59 mL/min. The samples were injected into an injector with a Split ratio of 1:40 and a temperature of 250 °C; the pressure in the column was 300 kPa.

The oven temperature and data treatment were applied similarly to the TFA analysis, however, this analysis considered peaks with an area ≥ 0.4 % in at least two of the three replicates of each sample.

The values were shown as the percentage of the total area detected in the analysis. Since this study proposed a qualitative analysis of the free fatty acids, the values were not normalized. Therefore, the actual mean values detected during the analysis were shown.

V.3. RESULTS AND DISCUSSION

The extracted crude oils were stored in a freezer at -20 °C, in the absence of light, until the moment of analysis.

V.3.1. Titratable acidity

The crude sunflower oils extracted by the green processes exhibited an acidity of 3.59 ± 0.010 and 3.17 ± 0.025 g oleic acid / 100 g oil when obtained by $scCO_2$ extraction and PLE using ethanol as a solvent, respectively. In turn, the soybean oils presented an acidity of 2.10 ± 0.001 and 2.11 ± 0.008 g/100 g oleic acid in the PLE using ethanol and hexane as solvents, respectively.

An increase in the acidity index of an oil is an indication of the breakdown of triacylglycerols and the degradation of the product, given that the greater an oil's acidity, the greater its concentration of free fatty acids (Santos et al., 2021).

Some physical parameters, such as temperature, humidity, exposure to light, or the action of lipolytic enzymes, can accelerate the process of breaking down triacylglycerols into FFAs. Frega et al. (1999) used an accelerated oxidative test to analyze the influence of temperature (110 °C) on the oxidation of various oils and concluded that there is an increase in oil acidity with increasing duration of exposure to high temperatures.

The presence of light also accelerates the oxidation process and is one of the most common problems in the stocking and storage of oils and lipid-rich foods. Thode Filho et al. (2014) analyzed the deterioration rates of soybean, sunflower, canola, and corn oil in the presence and absence of light. After measuring oil acidity for 70 days, the authors noted that the acidity levels were higher in oils exposed to light as compared to those that remained in the absence of light.

Refined sunflower oil has 0.13% equivalent in oleic acid, while soybean oil, 0.09% (Jorge et al., 2005). This result indicates that sunflower oil has a higher content of free fatty acids than soybean oil, even after refinement.

According to Correia et al. (2014), the authors found, in crude sunflower oil obtained by pressing and filtering, an acidity of 3.09 % in oleic acid. This value has the same order of magnitude as that observed herein, indicating that the crude sunflower oil has an acidity greater than 3 % in oleic acid; minor differences may be related to both the extraction process and seed variety.

Avramiuc (2013) evaluated the variation of the acidity index in sunflower oil extracted using a conventional method during storage under different conditions. The oil was stored in the presence of light and in the absence of light, at 4 ºC and 20 ºC. The author reported that the fresh oil had 4.5 mg KOH/g (2.26 g oleic acid/100 g oil). Based on the author's findings, in the presence of light at 4 ºC, the acidity remained at 4.5 mg KOH/g (2.26 g oleic acid/100 g oil) on the 5^{th} day of storage, increasing until 12.8 mg KOH/g (6.44 g oleic acid/100 g oil) on the 60^{th} day; in the absence of light at 4 ºC, the acidity ranged from 4.5 to 13.8 mg KOH/g (2.26 to 6.94 g oleic acid/100 g oil) in the same period. Meanwhile, at 20 ºC, the authors observed a variation in acidity from 9.7 to 31.8 mg KOH/g (4.88 to 16.01 g oleic acid/100 g oil) in the presence of light and 10.5 to 30.3 mg KOH/g (5.29 to 15.25 g oleic acid/100 g oil) in the absence of light, on the 5^{th} and 60^{th} day, respectively. Therefore, it is evident that the correct storage of extracted oils is essential since the increase in acidity results from the degradation of triacylglycerols present in these oils, *i.e.*, loss of neutral oil. In the present study, the seeds were stored at -20 °C, in the absence of light, avoiding the acidification increase in oils, and the acidity observed in both treatments is close to the acidity reported by Avramiuc (2013) in the first 5 days.

In turn, Thode Filho et al. (2014) evaluated the variation in the acidity index of commercial soybean, sunflower, canola, and corn oil obtained by conventional extraction in the presence and absence of light for 70 days, kept in their plastic cartons and at a

temperature of 30 °C. According to the authors, an increase in the acidity of the analyzed oils was observed both in the absence and presence of light in all the studied oils. Also, Nodar et al. (2002) claimed that soybean oil obtained by $scCO_2$ has higher total acidity when compared to oil obtained by conventional extraction (hexane).

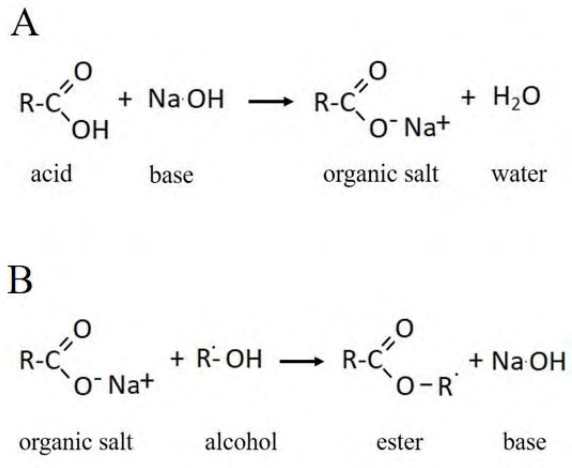
Nodar et al. (2002) reported acidity of 0.8 % (oleic acid) in crude soybean oil extracted by $scCO_2$ (300 bar, 40 ^oC).

V.3.2. FFAs

In order to determine the FFA, we ensured that all necessary steps were carried out so that the fatty acids could be saponified, separated, and esterified, enabling their detection via GC-MS.

The performed saponification procedure was equivalent to an acid-base neutralization reaction, in which an acid is neutralized by a base, forming salt and water. In oils and fats, the FFAs are neutralized by NaOH, forming water and organic salts, the latter being used in the next steps of the analysis. Figure 2 shows the chemical equation that represents the neutralization reaction.

Figure 2. Schematic of the neutralization reaction for the formation of fatty acid salts (A) and their esterification (B).



Triacylglycerols present in these oils can be degraded into FFAs by hydrolysis, also called hydrolytic or oxidative rancidity, a phenomenon that confers unpleasant odor and taste to the oils. The oxidation process is initiated by the breakage of the bond between hydrogen and the α-carbon, forming the first free radical. This newly formed radical reacts with oxygen, forming peroxides, which, in turn, steal hydrogen from another lipid molecule, restarting the cycle (Coppin and Pike, 2001).

The neutralization reaction was conducted at room temperature to avoid the breakdown of tracylglycerol molecules, since the increase in temperature favors the thermal degradation of triacylglycerol molecules into free fatty acids (Choe and Min, 2006). In addition, the temperature rise increases the agitation of the molecules and atoms forming the chemical bonds (Sobrinho and Paula e Souza, 2006).

The greater agitation of the molecules would favor the occurrence of shock between the triacylglycerols molecules and the hydroxyl of the base used for the neutralization reaction. The greater agitation/vibration of the atoms makes the bond more unstable and favors its breaking (Sanibal and Filho, 2002). Therefore, a lower reaction temperature disfavors the degradation of the triacylglycerol as well as the neutralization reaction.

In addition, free fatty acids have O-H bonds (acid group). Oxygen has a higher electronegativity than hydrogen, so the bond atoms between these two compounds are closer to oxygen than to hydrogen. This lower electron density around hydrogen causes the free hydroxyl molecules (OH-) to be more attracted to the hydrogen of the free acid molecules than to the O-C bonds of the triacylglycerol, since oxygen and carbon have similar electronegativities.

For these reasons, associated with the small amount of base used (in low concentration) there is the favorable neutralization of only the free fatty acids in the reaction medium and not the breakdown of triacylglycerols.

Therefore, the breakdown of triacylglycerol molecules was avoided. Thus, the salts were formed with the free fatty acids present in the crude oil. These hexane-soluble FFAs were collected by sequential washing, followed by drying in nitrogen. In this step, it is crucial that the supernatant phase be carefully collected in order to avoid carrying the neutral oil onto the following stages.

In GC-MS analyses, it is essential that the compounds of interest be volatile. Therefore, after the final drying step, the collected salts underwent esterification, aiming to obtain free fatty acid esters. To this end, BF_3 in methanol (alcohol) was added to the samples, followed by heating, the addition of hexane, and stirring. These steps ensure phase separation, allowing the collection of esters without the presence of secondary compounds. The esterification reaction is outlined in Figure 2.

The previously described steps enabled the qualitative analysis of the free fatty acids present in the crude oils. However, further studies addressing their quantitative analyses are required.

Figures 4 and 5 show the repeatability of the method for obtaining the different oils by different extraction techniques. The repetition of peaks was observed in all three replicates, indicating that the retention time (r't) (x-axis) is a reliable identification factor when applying the exact methodology described in this study.

Although this technique is not suitable for quantifying free fatty acids, it is possible to observe that oleic and linoleic acid stood out proportionally regarding the other acids, as was expected. This result was observed for both sunflower oil (Figure 3) and soybean oil (Figure 4), regardless of the extraction method used.

Figure 3. Chromatograms for sunflower oil extracted by supercritical CO_2 (2) and pressurized ethanol (B).

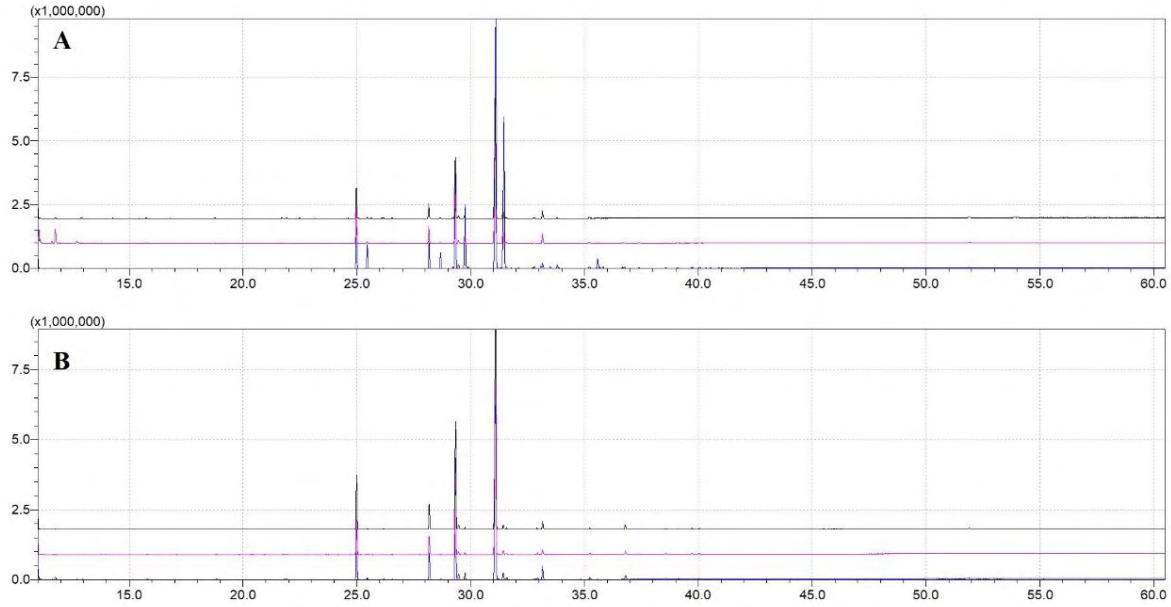


Figure 4. Chromatograms for soybean oil extracted by pressurized ethanol (A) and hexane (B).

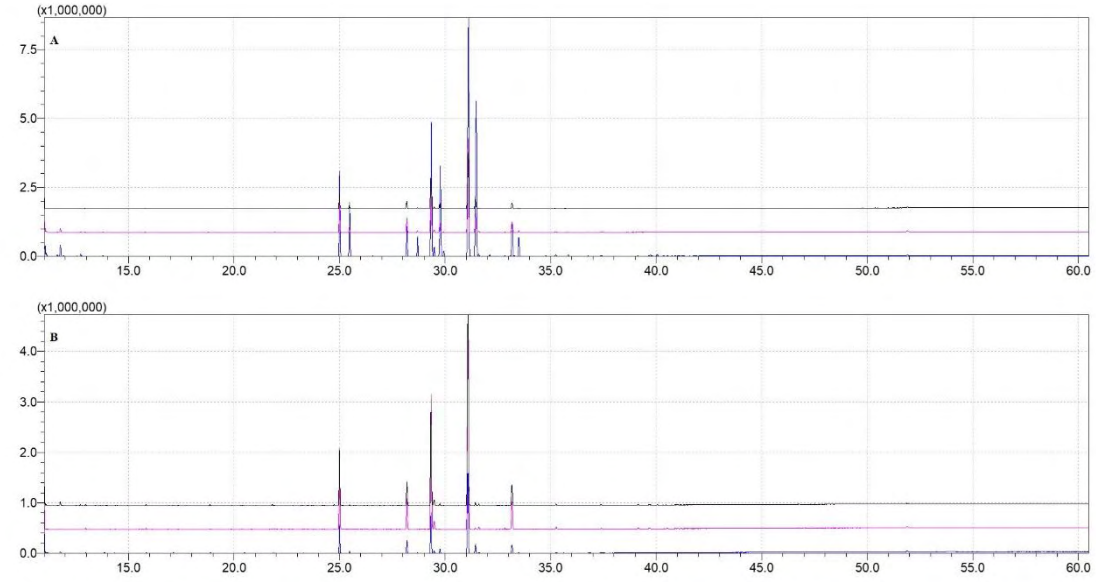
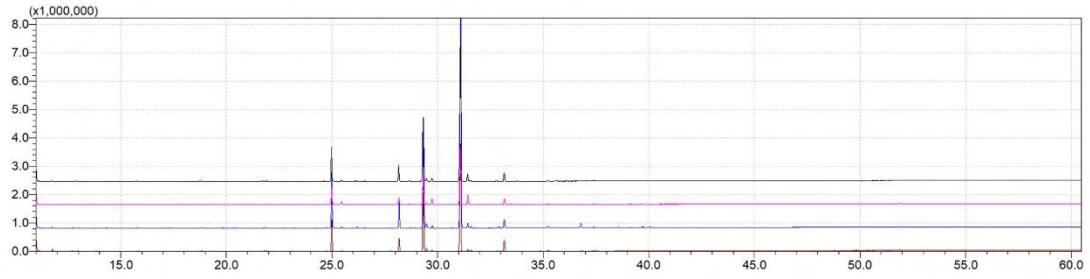


Figure 5. Superimposition of the fatty acid esters in sunflower and soybean oil extracted using green technologies



In the two Figures, it can also be noted that, in addition to the major compounds, the minor ones were also reproduced, indicating method reliability.

Although slight differences were observed between the minor compounds of the different crude oils analyzed, Figure 5 shows the superimposition of the four samples, indicating similarities between the free fatty acids of the vegetable oils obtained using green technologies.

The extraction method influenced the free fatty acid composition of the vegetable oils (Table 1), although only regarding minor fatty acids. The most present FFAs were equivalent among the samples, corroborating reports in the literature concerning TFAs.

Although this technique does not allow quantification, when using the criterion for selecting peaks with at least 0.4 % of total area in at least two of the three replicates per sample, it was noted that in sunflower oil, eicosanoic and elaidic acid were found when extraction with $scCO_2$ was applied, but not by PLE-ethanol (Table 1). This result is consistent with the observed acidity index, in which the acidity of the sunflower oil obtained by SFE-CO_2 was more pronounced. Thus, it is likely that the proportion of these fatty acids in the oil obtained by PLE-ethanol became undetectable or that their values were null or irrelevant in the whole.

When extracted by PLE-ethanol, the presence of arachidonic and elaidic acid was also observed, although no differences were found in the acid content of these oils extracted using different solvents.

Table 1. Free fatty acid composition in sunflower and soybean oil extracted using green processes.

Matrix	Sunflower oil		Soybean oil	
	$scCO_2$	PLE-ethanol	PLE-ethanol	PLE-hexane
FA ester (group)	Mean peak area (%) ± SD			
Palmitic (C16:0)	10.21 ± 0.31	9.06 ± 0.74	11.38 ± 1.03	12.27 ± 0.49
Eicosanoic (C20:0)	0.40 ± 0.06		1.93 ± 0.02	
Stearic (C18:0)	5.18 ± 0.14	4.65 ± 0.09	5.13 ± 0.30	5.22 ± 0.14
Arachidonic (C20:4)			0.65 ± 0.03	
Oleic (C18:1)	21.93 ± 0.53	22.80 ± 0.47	20.48 ± 0.85	25.05 ± 0.17
Elaidic (*trans*C18:1)	1.51 ± 0.65		3.95 ± 0.17	
Linoleic (C18:2)	48.75 ± 3.20	56.25 ± 1.81	41.08 ± 1.06	47.75 ± 0.22
Eicosenoic (C20:1)	3.10 ± 0.60	1.05 ± 0.13	7.09 ± 0.20	2.59 ± 1.93
Linolenic (C18:3)	3.09 ± 0.04	1.89 ± 0.47	4.15 ± 0.01	5.17 ± 0.14

*FA = Fatty Acid; SFE = Supercritical Fluid Extraction; PLE = Pressurized Liquid Extraction;

The proportions shown in Table 1 were not normalized since there was a possibility that other fatty acids of lesser intensity, undetectable by this methodology, could be present.

In bleached and refined palm fat, Gonçalves and Meirelles (2004) observed the following fatty acids in the TFA profile: lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, and arachidic acid. Linolenic acid was found only in the bleached oil. However, in the FFA content of both treatments, only lauric, palmitic, stearic, oleic, linoleic, and arachidic acid were observed. Those found in the FFAs had the highest mass

percentage, except for lauric acid, which presented a mass of 0.67 % and 0.49 % in the bleached and refined oils, respectively.

V.3.3. TFAs

In general, the results observed in this study are close to the ones available in relevant literature (Table 2).

Table 2. Total fatty acid profile (TFA) of sunflower (SF) and soybean (SB) oils obtained by optimized green processes (SFE and PLE), compared to relevant literature.

Matrix	Process	C16:0 Palmitic	C18:0 Stearic	C18:1 Oleic	C18:2 Linoleic	C18:3 Linolenic	C22:0 Behenic
SF (this study)	CO_2	6.91	5.03	22.6	64.77	*nd*	0.69
SF (this study)	Ethanol	7.34	5.66	24.88	61.2	*nd*	0.92
SB (this study)	Hexane	11.62	4.71	24.83	49.45	6.29	*Nd*
SB (this study)	Ethanol	12.84	7.49	24.49	39.06	8.15	*Nd*
SF (Rai et al., 2016b)	CO_2	4.91-6.97	3.13-4.54	51.83-56.43	33.31-36.18	*nd*	0.36-1.04
SF (Correia et al., 2014)	Cold pressing	4.00	1.47	49.02	45.36	*nd*	*Nd*
SB (Jokic et al., 2013)	CO_2	9.02-11.67	4.14-6.80	21.24-25.39	50.98-55.76	5.21-6.31	0.18-0.73
SB (Ivanov et al., 2011)	CO_2	16.95	5.15	16.02	47.57	12.11	0.39
SF* (Cuevas et al., 2009)	Ethanol	6.80	4.16	22.75	63.83	0.82	0.71
SF (Chowdhury et al., 1970)	*ng*	6.52	1.98	45.39	46.02	0.12	*Nd*
SB (Chowdhury et al., 1970)	*ng*	14.04	4.07	26.27	52.18	5.63	*Nd*

* degummed; ng = not given; nd = not detected.

Information on the total fatty acid profile of sunflower oil extracted by PLE or SFE can be found in the literature (Nimet et al., 2011; Rai et al., 2016a), but not on the FFAs.

Velez et al. (2012) determined the composition of the total fatty acids (TFAs) found in commercial sunflower oil, which comprised 85 % in weight of oleic acid, 14 % in weight of palmitic acid, and 1 % in weight of stearic acid. Also regarding TFAs, higher concentrations of linoleic acid were observed, ranging from 33.3 to 68.7 %, and oleic acid, between 17.3 and 56.4 % (Cuevas et al., 2009; Nimet et al., 2011; Rai et al., 2016a). Correia et al. (2014) reported concentrations of 49.02 and 45.35 % of oleic and linoleic acids, respectively, in sunflower oil obtained by pressing. However, no information on the FFAs was provided.

The composition of the TFAs in the soybean oil determined herein corroborates the data obtained by several authors (Chowdhury et al., 1970; Ivanov et al., 2011; Jokic et al., 2013), who found higher concentrations of linoleic and oleic acid (52.18 and 23.27 %, respectively), in addition to similar concentrations of palmitic acid (11.74 %), stearic acid (5.15 %), and linolenic acid (6.24 %). Information on the FFAs of the soybean oil was also unavailable.

These results suggest that the profiles of TFA and FFAs are similar for major compounds, although minor compounds may vary. In the present study, the acids that generated the smallest peaks (area, Tables 1 and 2) varied among the samples.

Modern deacidification techniques based on the removal of oleic acid are under development, such as the method described by Ilgen and Dulger (2016). The authors used zeolite 13X as an adsorbent to remove the acid in sunflower oil, managing to desorb up to 91.6 % of the acidity present in the samples. The present study could corroborate the development of specific deacidification methodologies, as the one described by Ilgen and Dulger (2016), increasing its efficiency.

However, the various studies discussed herein reveal that, sometimes, the predominant oil found in TFAs is linoleic, suggesting the need for specific applications

for each matrix, depending on the FFAs observed. Also, the present study could corroborate the development of specific deacidification methodologies, thus increasing the efficiency of currently used techniques.

V.4. CONCLUSION

It can be concluded that the used methodology rendered satisfactory results for the determination of free fatty acids in crude sunflower and soybean oil extracted using green technologies.

The primary free fatty acids observed in both oils were linoleic, oleic, palmitic, and stearic acid, listed in ascending order for proportion regarding the FFAs found.

Furthermore, it can be suggested that there are similarities between the major total fatty acids and free fatty acids among the different treatments, although the minor FFAs may vary. Thus, the present study provides relevant information on the determination of the FFAs in crude vegetable oils.

Acknowledgments

Funding: This work was supported by the Brazilian agency Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - Process nº 2018/18024-7); and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – Process nº 304573/2019-1). C.M. Vicentini-Polette and P.R. Ramos thanks the Brazilian agency Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship (Process nº 001).

Declarations of interest: none

REFERENCES

AOAC. (2005). *Official Methods of Analysis of AOAC International* (18th ed.). AOAC International.

AOCS. (2004). *Official methods and recommended practices of theAmerican Oil Chemists' Society*. American Oil Chemists' Society.

Asl, P. J., Niazmand, R., & Yahyavi, F. (2020). Extraction of phytosterols and tocopherols from rapeseed oil waste by supercritical CO_2 plus co-solvent: A comparison with conventional solvent extraction. *Heliyon*, *6*(3), e03592. https://doi.org/10.1016/j.heliyon.2020.e03592

Avramiuc, M. (2013). The acidity index evolution of maize and sunflower crude oils under storage conditions. *Genetica Si Biologie Moleculara*, *14*(4), 43–46.

Bailey, A. ., & Shahidi, F. (2005). *Bailey's industrial oil & fats products*. John Wiley & Sons.

Benito-Román, O., Rodríguez-Perrino, M., Sanz, M. T., Melgosa, R., & Beltrán, S. (2018). Supercritical carbon dioxide extraction of quinoa oil: Study of the influence of process parameters on the extraction yield and oil quality. *Journal of Supercritical Fluids*, *139*(March), 62–71. https://doi.org/10.1016/j.supflu.2018.05.009

Bittencourt, G. M., Firmiano, D. M., Fachini, R. P., Lacaz-Ruiz, R., Fernandes, A. M., & Oliveira, A. L. (2019). Application of Green Technology for the Acquisition of Extracts of Araçá (*Psidium grandifolium Mart. ex DC.)* Using Supercritical CO_2 and Pressurized Ethanol: Characterization and Analysis of Activity. *Journal of Food Science*, *84*(6), 1297–1307. https://doi.org/10.1111/1750-3841.14584

Bojanić, N., Teslić, N., Rakić, D., Brdar, M., Fišteš, A., Zeković, Z., Bodroža-Solarov, M., & Pavlić, B. (2019). Extraction kinetics modeling of wheat germ oil supercritical fluid extraction. *Journal of Food Processing and Preservation*, *43*(9), 1–12. https://doi.org/10.1111/jfpp.14098

Choe, E., & Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. *Comprehensive reviews in food science and food safety*, *5*(4), 169-186. https://doi.org/10.1111/j.1541-4337.2006.00009.x

Chowdhury, K., Banu, L., Khan, S., & Latif, A. (1970). Studies on the Fatty Acid Composition of Edible Oil. *Bangladesh Journal of Scientific and Industrial Research*, *42*(3), 311–316. https://doi.org/10.3329/bjsir.v42i3.669

Coppin, E. A., & Pike, O. A. (2001). Oil stability index correlated with sensory determination of oxidative stability in light-exposed soybean oil. *JAOCS, Journal of the American Oil Chemists' Society*, *78*(1), 13–18. https://doi.org/10.1007/s11746-001-0212-4

Cornelio-Santiago, H. P., Bodini, R. B., & de Oliveira, A. L. (2021). Potential of Oilseeds Native to Amazon and Brazilian Cerrado Biomes: Benefits, Chemical and Functional Properties, and Extraction Methods. *JAOCS, Journal of the American Oil Chemists' Society*, *98*(1), 3–20. https://doi.org/10.1002/aocs.12452

Cornelio-Santiago, H. P., Gonçalves, C. B., de Oliveira, N. A., & de Oliveira, A. L. (2017). Supercritical CO_2 extraction of oil from green coffee beans: Solubility, triacylglycerol composition, thermophysical properties and thermodynamic modelling. *Journal of Supercritical Fluids*, *128*(May), 386–394. https://doi.org/10.1016/j.supflu.2017.05.030

Correia, I. M. S., Araújo, G. S., Paulo, J. B. A., & Sousa, E. M. B. D. De. (2014). Avaliação das potencialidades e características físico- químicas do óleo de Girassol (*Helianthus annuus L.)* e Coco (*Cocos nucifera L.*) produzidos no Nordeste brasileiro. *Scientia Plena*, *10*, 1–7.

Cuevas, M. S., Rodrigues, C. E. C., & Meirelles, A. J. A. (2009). Effect of solvent hydration and temperature in the deacidification process of sunflower oil using ethanol. *Journal of Food Engineering*, *95*(2), 291–297. https://doi.org/10.1016/j.jfoodeng.2009.05.009

Cunha, V. M. B., Silva, M. P. da, Sousa, S. H. B. de, Bezerra, P. do N., Menezes, E. G. O., Silva, N. J. N. da, Banna, D. A. D. da S., Araújo, M. E., & Carvalho Junior, R. N. de. (2019). Bacaba-de-leque (*Oenocarpus distichus Mart.*) oil extraction using supercritical CO2 and bioactive compounds determination in the residual pulp. *Journal of Supercritical Fluids*, *144*(August 2018), 81–90. https://doi.org/10.1016/j.supflu.2018.10.010

De Oliveira, P. M. A., De Almeida, R. H., De Oliveira, N. A., Bostyn, S., Gonçalves, C. B., & De Oliveira, A. L. (2014). Enrichment of diterpenes in green coffee oil using supercritical fluid extraction - Characterization and comparison with green coffee oil from pressing. *Journal of Supercritical Fluids*, *95*, 137–145. https://doi.org/10.1016/j.supflu.2014.08.016

Dunford, N. T., King, J. W., & List, G. R. (2003). Extraction optimization in food engineering. In C. Tzia & G. Liadakis (Eds.), *Supercritical fluid extraction in food*

engineering (pp. 57–93). Marcel Dekker Inc.

Durante, M., Ferramosca, A., Treppiccione, L., Di Giacomo, M., Zara, V., Montefusco, A., Piro, G., Mita, G., Bergamo, P., & Lenucci, M. S. (2020). Application of response surface methodology (RSM) for the optimization of supercritical CO_2 extraction of oil from patè olive cake: Yield, content of bioactive molecules and biological effects in vivo. *Food Chemistry*, *332*(April), 127405. https://doi.org/10.1016/j.foodchem.2020.127405

Franco, D., Rodríguez-Amado, I., Agregán, R., Munekata, P. E. S., Vázquez, J. A., Barba, F. J., & Lorenzo, J. M. (2018). Optimization of antioxidants extraction from peanut skin to prevent oxidative processes during soybean oil storage. *LWT - Food Science and Technology*, *88*, 1–8. https://doi.org/10.1016/j.lwt.2017.09.027

Frega, N., Mozzon, M., & Lercker, G. (1999). *S11746-999-0239-4*. *76*(3), 325–329.

Gonçalves, C. B., & Meirelles, A. J. A. (2004). Liquid-liquid equilibrium data for the system palm oil + fatty acids + ethanol + water at 318.2 K. *Fluid Phase Equilibria*, *221*(1–2), 139–150. https://doi.org/10.1016/j.fluid.2004.05.002

Ilgen, O., & Dulger, H. S. (2016). Removal of oleic acid from sunflower oil on zeolite 13X: Kinetics, equilibrium and thermodynamic studies. *Industrial Crops and Products*, *81*, 66–71. https://doi.org/10.1016/j.indcrop.2015.11.050

Ivanov, S., Lević, J., & Sredanović, S. (2011). Fatty acid composition of various soybean products. *Food and Feed Research (Serbia)*, *37*(2), 65–70.

Jokic, S., Sudar, R., Svilovic, S., Vidovic, S., Bilic, M., Velic, D., & Jurkovic, V. (2013). Fatty acid composition of oil obtained from soybeans by extraction with supercritical carbon dioxide. *Czech Journal of Food Sciences*, *31*(2), 116–125. https://doi.org/10.17221/8/2012-cjfs

Jorge, N., Soares, B. B. P., Lunardi, V. M., & Malacrida, C. R. (2005). Alterações físico-químicas dos óleos de girassol, milho e soja em frituras. *Química Nova*, *28*(6), 947–951. https://doi.org/10.1590/s0100-40422005000600003

Maul, A. A., Wasicky, R., & Bacchi, E. M. (1996). Extração por fluido supercrítico. *Revista Brasileira de Farmacognosia*, *5*(2), 185–200.

Moretto, E., & Fett, R. (1986). *Óleos e gorduras vegetais: processamento e análises*. UFSC.

Muangrat, R., & Pongsirikul, I. (2019). Recovery of spent coffee grounds oil using supercritical CO_2: extraction optimization and physicochemical properties of oil. *CYTA - Journal of Food*, *17*(1), 334–346.

https://doi.org/10.1080/19476337.2019.1580771

Nimet, G., da Silva, E. A., Palú, F., Dariva, C., Freitas, L. dos S., Neto, A. M., & Filho, L. C. (2011). Extraction of sunflower (*Heliantus annuus L.*) oil with supercritical CO2 and subcritical propane: Experimental and modeling. *Chemical Engineering Journal*, *168*(1), 262–268. https://doi.org/10.1016/j.cej.2010.12.088

Nodar, M. D., Molero Gómez, A., & Martinez De La Ossa, E. (2002). Characterisation and process development of supercritical fluid extraction of soybean oil. *Food science and technology international*, *8*(6), 337-342.

Okiyama, D. C. G., Soares, I. D., Cuevas, M. S., Crevelin, E. J., Moraes, L. A. B., Melo, M. P., Oliveira, A. L., & Rodrigues, C. E. C. (2018). Pressurized liquid extraction of flavanols and alkaloids from cocoa bean shell using ethanol as solvent. *Food Research International*, *114*(July), 20–29. https://doi.org/10.1016/j.foodres.2018.07.055

Oliveira, N. A. de, Cornelio-Santiago, H. P., Fukumasu, H., & Oliveira, A. L. de. (2018). Green coffee extracts rich in diterpenes – Process optimization of pressurized liquid extraction using ethanol as solvent. *Journal of Food Engineering*, *224*, 148–155. https://doi.org/10.1016/j.jfoodeng.2017.12.021

Pavlić, B., Pezo, L., Marić, B., Tukuljac, L. P., Zeković, Z., Solarov, M. B., & Teslić, N. (2020). Supercritical fluid extraction of raspberry seed oil: Experiments and modelling. *Journal of Supercritical Fluids*, *157*. https://doi.org/10.1016/j.supflu.2019.104687

Promraksa, A., Siripatana, C., Rakmak, N., & Chusri, N. (2020). Modeling of Supercritical CO_2 Extraction of Palm Oil and Tocopherols Based on Volumetric Axial Dispersion. *Journal of Supercritical Fluids*, *166*, 105021. https://doi.org/10.1016/j.supflu.2020.105021

Rai, A., Mohanty, B., & Bhargava, R. (2016a). Fitting of broken and intact cell model to supercritical fluid extraction (SFE) of sunflower oil. *Innovative Food Science and Emerging Technologies*, *38*, 32–40. https://doi.org/10.1016/j.ifset.2016.08.019

Rai, A., Mohanty, B., & Bhargava, R. (2016b). Supercritical extraction of sunflower oil: A central composite design for extraction variables. *Food Chemistry*, *192*, 647–659. https://doi.org/10.1016/j.foodchem.2015.07.070

Richter, B. E., Jones, B. A., Ezzell, J. L., Porter, N. L., Avdalovic, N., & Pohl, C. (1996). Accelerated solvent extraction: A technique for sample preparation. *Analytical Chemistry*, *68*(6), 1033–1039. https://doi.org/10.1021/ac9508199

Sanibal, E. A. A., & Filho, J. M. (2002). Alterações físicas, químicas e nutricionais de óleos submetidos ao processo de fritura. *Food Ingredients South America*, *1*(3), 64-71.

Santos, K. A., de Aguiar, C. M., da Silva, E. A., & da Silva, C. (2021). Evaluation of favela seed oil extraction with alternative solvents and pressurized-liquid ethanol. *Journal of Supercritical Fluids*, *169*(December). https://doi.org/10.1016/j.supflu.2020.105125

Santos, K. A., Klein, E. J., da Silva, C., da Silva, E. A., & Cardozo-Filho, L. (2019). Extraction of vetiver (*Chrysopogon zizanioides*) root oil by supercritical CO_2, pressurized-liquid, and ultrasound-assisted methods and modeling of supercritical extraction kinetics. *Journal of Supercritical Fluids*, *150*, 30–39. https://doi.org/10.1016/j.supflu.2019.04.005

Sobrinho, A.A. & Paula e Souza, G.M. (2006). *Física Térmica teórica e experimental*. Natal: CEFET/RN.

Sun, Q., Shi, J., Scanlon, M., Xue, S. J., & Lu, J. (2021). Optimization of supercritical-CO_2 process for extraction of tocopherol-rich oil from canola seeds. *Lwt*, *145*(December 2020), 111435. https://doi.org/10.1016/j.lwt.2021.111435

Thode Filho, S., Cabral, G. B., Maranhão, F. D. S., Sena, M. F. M., & Silva, E. R. da. (2014). Deterioração De Óleos Vegetais Expostos a Diferentes Condições De Armazenamento. *Revista Eletrônica Em Gestão, Educação e Tecnologia Ambiental*, *18*(0), 7–13. https://doi.org/10.5902/2236117013802

Torres-Ramón, E., García-Rodríguez, C. M., Estévez-Sánchez, K. H., Ruiz-López, I. I., Rodríguez-Jimenes, G. C., Romero de la Vega, G., & García-Alvarado, M. A. (2021). Optimization of a coconut oil extraction process with supercritical CO_2 considering economical and thermal variables. *Journal of Supercritical Fluids*, *170*(December 2020), 105160. https://doi.org/10.1016/j.supflu.2020.105160

Varona, E., Tres, A., Rafecas, M., Vichi, S., Barroeta, A. C., & Guardiola, F. (2021). Methods to determine the quality of acid oils and fatty acid distillates used in animal feeding. *MethodsX*, *8*, 101334. https://doi.org/10.1016/j.mex.2021.101334

Velez, A., Soto, G., Hegel, P., Mabe, G., & Pereda, S. (2012). Continuous production of fatty acid ethyl esters from sunflower oil using supercritical ethanol. *Fuel*, *97*, 703–709. https://doi.org/10.1016/j.fuel.2012.02.024

Vicentini-Polette, C.M., Ramos, P. R., Gonçalves, C. B., & Oliveira, A. L. de (2021). Determination of free fatty acids in crude vegetable oil samples obtained by high-

pressure processes. *Food Chemistry: X*, 12, 100166. https://doi.org/10.1016/j.fochx.2021.100166

Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science and Technology*, *17*(6), 300–312. https://doi.org/10.1016/j.tifs.2005.12.004

CHAPTER VI: Goat milk yogurt enriched with sunflower oil extracted with supercritical fluid as a viable alternative to vitamin e intake

ABSTRACT

The sensory profile of goat milk yogurt enriched with vitamin E-rich sunflower oil obtained by supercritical CO_2 was evaluated during qualitative research that indicated potential consumers of goat milk yogurt enriched with sunflower oil. The yogurt was formulated with xylitol, commercial starter culture, and vitamin E-rich sunflower oil obtained via supercritical free extraction of any organic solvent residue. In the formulation it was considered that a portion of 200 g would supply 15% of the recommended values for vitamin E consumption. In order to guarantee food safety, microbiological analyses to identify some of the main pathogens common in food (*Escherichia coli*, Salmonella, total Listeria coliforms) as well as molds and yeasts were carried out. The color, acidity and oxidative stability were evaluated in the characterization of the product. To determine the sensory profile of the product developed, the Free Profile technique was used, characterizing two products, natural yogurt made with goat milk enriched with vitamin E. The evaluators were selected by interest, availability and residual error <15. The product proved to be microbiologically safe. The addition of oil did not influence the color or acidity of the product. There was a good correlation between the sensory attributes, and the presence of the oil was perceived by the evaluator, although the main attributes for flavor and aroma are related to the dairy characteristics, regardless of the oil. Thus, the enriched yogurt presented sensory difference from the natural one, but there was no statistical difference regarding the acceptance, which remained in the positive part of the scale, showing an environmentally and nutritionally friendly alternative for human consumption in the enriched yogurt.

Keywords: goat's milk; yogurt; tocopherols; sensory analysis; free profile.

VI.1. INTRODUCTION

There is a worldwide search for foods and processes that are environmentally friendly, quick to consume, inclusive for people on restrictive diets, and highly nutritious and tasty. This study sought to reconcile these trends into a single product.

The enrichment of food products using vegetable oil is present in the food industry, based on the extraction and addition of the oil in the product, changing its

properties and nutritional quality. The technique used for the extraction must be carefully selected, aimed at both a high yield and the obtaining of compounds of interest (MUKHOPADHYAY, 2000).

Improved techniques for extraction have been studied by the industry, including extraction by supercritical fluids. This fluid presents thermodynamic characteristics simultaneously similar to a gas and a liquid, as it has high density and diffusivity in addition to low viscosity (MAUL et al., 1996).

The main solvent used is supercritical carbon dioxide ($scCO_2$), due to its low toxicity, ease of separation of the material, high yield and preservation of several bioactive components, providing better nutritional quality. Thus, this environmentally friendly technology has been shown to be a promising replacement to conventional extraction processes (DANH et al., 2009).

In addition, $scCO_2$ has a high capacity to carry lipophilic substances and nonpolar chains, such as tocopherols (vitamin E) and phytosterols (ASL et al., 2020; MAUL et al., 1996).

A healthy diet consists of the diversification of foods to absorb different vitamins and minerals. Sunflower oil is a clear example, as its advantages include being an energy source and also being able to act in decreasing blood cholesterol levels and being rich in vitamin E of high biological value, or that which enable high use by the body, such as α-tocopherol (ROCHE, 2001).

An individual needs a daily dose of at least 15 mg/day of vitamin E for a stable level of metabolism and the absence can lead to peripheral neuropathy and pigmented retinopathy, among other disorders (NRC, 2006). However, the average consumption of vitamin E is 5.3 mg, and in Brazil it is much lower than indicated, suggesting the need for new means and products to insert this compound in the daily diet (TURECK et al., 2013).

Milk is considered a basic food for the formation of children and has become a supplement in the lives of adults. However, it is common to observe cases of intolerance or allergy to cow's milk. This is due to the partial or total loss of the ability to produce the enzyme that digests cow's milk protein, the lactase enzyme (FOX; THOMSON, 2007). After developing allergy and/or intolerance, total exclusion of milk and its derivatives is common and can cause problems in the body structure. Thus, it is suggested that cow's milk be replaced (MEDEIROS et al., 2004).

The choice for goat's milk comes from its high digestibility, due to the smaller fatty acid chains, combined with a low allergenic potential when compared to cow's milk. Thus, it is more suitable for consumption by the elderly and intolerant, facilitating the digestion and absorption of the product (HAENLEIN, 2004).

Lipid levels in goat's milk are lower than those in cow's milk. Furthermore, they are formed by medium and short chain fatty acids, providing better digestibility and avoiding problems caused by food residues in the intestine, such as fermentation, poor digestion and constipation (CRUZ et al., 2016), given their digestibility facilitated by lower fat globules compared to cow's milk globules (CORREIA et al., 2008). It is known that lipids affect the sensory characteristics, and can be something pleasant if well used (FROST et al., 2001).

One of the ways of including goat's milk in human food is through yogurt. This derivative is known for its nutritional value and for its pleasant and attractive sensory characteristics, besides presenting great popularity among consumers (ANNUNZIATA; VECCHIO, 2013).

In addition to being nutritious, it is important for the food to be palatable. Sucrose, common sugar, is one of the most widely used sweeteners because of its sweetening power. However, its high consumption can bring numerous adverse effects, such as obesity and type 2 diabetes. Thus, public policies and private industry seek to reduce the use of this sweetener (SCHULZE et al., 2004) by the use of alternative sweeteners, such as xylitol.

Xylitol has the same sweetening power as sucrose, but it is not metabolized by insulin. Thus, it is an excellent substitute for diabetics, who have difficulty producing insulin, in addition to its reduced calorie content (KUMAR et al., 2019). In addition, the sweetener has been observed for its medicinal potential, since xylitol cannot be metabolized by bacteria and is not an energy source, reducing the spread of some microorganisms (DASGUPTA et al., 2017). This characteristic is also important in the conservation of food products.

Thus, xylitol, as a natural sweetener, is seen as an alternative to conventional refined sugar. The sweetener is tasty and has a low caloric value, and is naturally found in fruits, vegetables and some mushrooms, in addition to also being produced by the human body during carbohydrate metabolism in an amount of approximately 5 to 15 grams per day (BÄR, 1986; MUSSATTO; ROBERTO, 2002). Its metabolism occurs by the pentose phosphate pathway, without depending on insulin and, therefore, not

increasing blood glucose levels (BÄR, 1986), as a sweetener indicated for people with diabetes.

However, it is important that the product developed is desired by the consumer and, for this objective to be achieved, the consumer public potential must be considered as a determining factor in the development of production and marketing strategies (ROSS, 2009).

Market research is already consolidated in the development of food products, such as vegan burgers (NAGAGATA et al., 2020), artisanal beers (BELLUCCI et al., 2018) and lunch boxes (VEIGA; REDIN, 2017). In this study, we opted for a broad research prior to the formulation of the product, in order to identify the consumer's perception and desire regarding the possibility of consuming a food with a strong health appeal.

In addition to the desire to buy, a new product must be studied from the perspective of sensory food analysis. This analysis allows a new product to be identified and evaluated through the sense of the human body, in order to define its characteristics. Thus, it is possible to predict acceptance by the public effectively.

Food sensory analysis is an extremely important science, and should be applied considering the most specific variations, as a way of presenting samples and relevant information to evaluators (VIDAL et al., 2018). Furthermore, it is important that the selected test is suitable for the study.

One of the methods of sensory analysis is the descriptive Free Profile test, which consists in the evaluators freely describing the samples presented, without the use of predetermined characteristics (WILLIAMS; LANGRON, 1984). The evaluator proposes a list with their perceptions and meanings of each attribute so that the evaluator is free to describe the product in the way they deem appropriate and realistic, being a reliable and pleasant technique (MACFIE, 2009). In addition, the free profile test allows the correlation between the attributes described by different evaluators, with intensity indicators by attribute.

The descriptive analysis of the free profile has been applied to different food products, such as buffalo milk mozzarella cheese (VERRUMA-BERNARDI; DAMÁSIO, 2004), hams (GUÀRDIA et al., 2010), lettuce (COVRE et al., 2020) and also in an apple genetic improvement program (DE CARVALHO et al., 2020).

Thus, this study aimed to describe the sensory profile of goat milk yogurt enriched with sunflower oil obtained by supercritical technology as an alternative to consumption of high digestibility dairy products rich in vitamin E.

VI.2. METHODOLOGY

VI.2.1. Consumer Research

An online questionnaire was applied to 204 volunteers over 18 years old to identify the perception of yogurt consumers, taking into account the healthiness of goat milk yogurt enriched with sunflower oil. Questions were asked about demographic characterization, consumption habits of dairy products and also questioned about which parameters the evaluators perceive or consider when they consume dairy products, healthy foods, enriched products, benefits of goat's milk, benefits of vitamins, and personal opinion regarding product development. For analysis of the questionnaire data, invalid answers were disregarded, such as those with incomprehensible terms (such as numbers or scores instead of words). Similar terms were grouped by convenience, and the data were analyzed qualitatively by descriptive analysis (DO AMARAL et al., 2012), with direct observation of the data using Microsoft Excel (2013), in which the frequency of answers to each question was raised, similar to the study by Paula et al. (2021).

VI.2.2. Supercritical extraction of sunflower oil enriched with vitamin E

The seeds used were kindly donated by Caramuru Alimentos. They are sunflower seeds (*Helianthus annuus*), variety Altis 99 (Atlântica Sementes S.A.). The characterization of this seed was performed in a previous study.

Sunflower oil was obtained via supercritical extraction (SFE) on Thar-SFC equipment produced by Thar Technologies Inc. (Pittsburgh, USA), using CO_2 (Linde, Sertãozinho, BR) as a supercritical solvent ($scCO_2$). The extraction system included high pressure pumps, heat exchangers, two separators, an extractor and a collector, kept in an ice bath. The sample (10 g of rolled sunflower seeds) was packaged in a fixed bed extractor (290 cm^3) of cylindrical stainless steel. The voids were filled with 5 mm glass beads. The optimized extraction condition was used with a temperature of 60 $^{\circ}C$, pressure of 32.1 MPa, flow rate of 10 g CO_2/min, during 4 h of extraction, which resulted in recovery of 87.6% of the oil contained in the seeds with tocopherol content of 44 mg/100 g of oil. This optimized condition was determined in previous studies of the extraction process. With these operational parameters it was possible to perform extraction in a condition that simultaneously provided a good yield and a high content of total tocopherols.

VI.2.3. Yogurt formulation

The goat's milk was obtained from the Department of Animal Reproduction of the School of Veterinary Medicine and Animal Science of the University of São Paulo (FMVZ-USP), and comes from Saanen goats, Alpine females and Saanen and Alpine crossbreds, between 1.5 and 6 years old, in the first to the fourth parturition, with daily milking. The goats were fed with local corn silage, Cross hay and concentrated with 20 % protein.

Two yogurts were prepared, both received 5 g of xylitol/100 mL of milk and the enriched sample included the addition of 2.25 mg of tocopherols (5.11 g of oil) per 100 mL of pasteurized and sweetened milk. The amount of oil added represents 15% of the minimum daily amounts of vitamin E consumption per 200 mL portion, characterizing the product as enriched.

Milk pasteurization occurred at 60 °C for 30 min, followed by heating at 90 °C for 10 min (SILVA et al., 2017). Then, 1 L of pasteurized goat's milk was transferred to a domestic yogurt maker with stainless steel container (Naturalle Cadence, China), properly sanitized, where 50 g of xylitol was added, followed by homogenization.

The container was kept closed at room temperature up to 45 ºC. Then, 1 sachet of starter culture (BioRich ®, Horsholm, Denmark) was added, according to the manufacturer's guidelines (45 °C, 10 g/L of milk). The mixture was incubated at 45°C, in the closed yogurt maker, for 8 h uninterrupted. Then, for the preparation of the enriched yogurt, 5.11 g of sunflower oil per 100 mL of yogurt was added. In the natural yogurt there was no addition of oil or other ingredients.

After formulation, the yogurt was matured for 24 h. Microbiological, physical, chemical and sensory analyses were performed between 24 and 48 hours after yogurt production.

VI.2.4. Microbiological analysis

The Compact Dry^{TM} SL (Nissui Pharmaceutical Co., Japan) was used for detection of Salmonella spp, for qualitative detection, that is, presence and absence (detectable by yellowish color in case of presence). As such, a solution enriched with 25 mL of sample in 225 mL of enterobacteria enrichment broth (EE Broth Mossel, HIMEDIA, Mumbai) was prepared, and this solution was incubated at 35 °C for 24 h. Then, 0.1 mL of culture solution and 1 mL of sterile water were pipetted into the dry plate, and the plates were incubated inverted at 41 °C for 24 h.

The Compact Dry^{TM} EC kit (Nissui Pharmaceutical Co., Japan) was used for detection of total coliforms, and *E. coli*, qualitative detection, (detectable by red/pink color for total coliforms except *E. coli*, and blue/purple color for *E. coli*). The samples were diluted in 0.1% peptone water in a ratio of 1:10 (10^{-1}), and sequentially the dilution 10^{-2} was obtained. Dilutions were applied directly to the dry plate (1 mL), and then incubated inverted at 35 ºC for 24 h.

The presence of Listeria spp. was determined using the Compact Dry^{TM} LS kit (Nissui pharmaceutical Co., Japan). As such, the samples were diluted in peptone water 0.1 % in a 1:10 proportion. The solution rested at room temperature for 1 h, and then 1 mL was applied to the dried plates, followed by incubation at 35 °C for 24 h. Blue colonies indicate the presence of listeria. The tests (dry kits) were performed in duplicate per sample and, when appropriate, by dilution.

The analysis of molds and yeasts was carried out in triplicate per sample and dilution (10^{-1}, 10^{-2} and 10^{-3}), using potato glucose agar 2 % acidified with tartaric acid 10 % to pH 3.5. 0.1 mL of the selected dilutions was inoculated on the dry surface of potato agar, and incubated at 35 °C for 72 h. The results were expressed in CFU / mL.

VI.2.5. Analysis of acidity, color and oxidation

Yogurts were evaluated for total titratable acidity (AOAC, 2005), that is, the amount of lactic acid of a given sample quantity that reacts with a base of known concentration (0.1 N NaOH) was determined, and its results expressed in % lactic acid. The acidity in yogurts is related to their sensory and microbiological quality, and their variation may indicate changes in the product during storage. Furthermore, the acidity of the final product can be influenced by the addition of crude sunflower oil, that is, without industrial refinement to decrease acidity.

The color of a product may be indicative of changes in its properties and quality, and is closely linked to the consumer's acceptance of a product, since it is the first attribute observed in the purchase or consumption comment. The color analysis of the yogurts was performed using the Miniscan XE colorimeter (HunterLab, with D65 and observer at 10º) and the Universal Software 3.2 (HunterLab Associates Laboratory). The equipment provides the CIELab color standard, that is, the luminosity (L*) from 0 (black) to 100 (white); a*, from green (-a) to red (+ a); and b*, from blue (-b) to yellow (+b). The chromaticity (Chroma) and Hue angle (Hue) indexes were determined from the instrumental data.

Lipids undergo oxidation by the action of heat, presence of oxygen and interference from the medium. Goat milk yogurt has a lipid content between 3.8 to 4.1 g / 100 g (UNICAMP, 2011; USDA, 2020), and in the formulation received 5.11 g of sunflower oil enriched with vitamin E / 100 g of yogurt. Due to the increase in lipid content in the product, lipid oxidation analysis was measured as a characterization parameter. To determine the accelerated oxidative stability, 3 g of sample at 120 °C was placed in contact with an air flow rate of 15 L/h in a Rancimat 873 biodiesel equipment (Metrohm, Switzerland). The analyses were performed in triplicate for yogurt with and without sunflower oil in its formulation (FARHOOSH, 2007).

VI.2.6. Determination of the caloric value of goat's milk yogurt

The increase in caloric value was calculated theoretically, considering 9 kcal / g of oil used (FOOD AND DRUG ADMINISTRATION, 2020), and 2.4 kcal / g of xylitol (MUSSATTO; ROBERTO, 2002).

VI. 2.7. Sensory test

VI.2.7.1. Sensory test conditions

The study was approved by the Ethics Committee of the University of São Paulo, number and date: 4.505.304 on 01/21/2021, CAAE 40406820.4.0000.5422.

Due to the current pandemic caused by COVID-19 (SARS-CoV-2), the evaluators were received at different times, with a minimum interval of two hours for the use of the same cabin, in addition to cleaning the surfaces with 70% ethanol and minimum distancing between the evaluators of 1.5 m. The evaluators were also asked to perform the correct hand hygiene, and remain wearing a mask throughout the journey and interaction with the team.

Sensory tests were performed at the Sensory Analysis Laboratory of the Faculty of Animal Science of Food Engineering of the University of São Paulo (FZEA-USP), in Pirassununga/SP, Brazil, in individual cabins and white light. All evaluators received a copy of the Informed Consent Form, in which they became aware of the product they would be evaluating, the conditions of the test and the non-obligation to perform it. The samples were served at 8 °C ± 1 °C in quantities around 30 mL in a clear disposable container with a capacity of 40 mL, in single form, coded with three digits and in randomized order. The evaluators were asked to consume the yogurts directly from the

container. Along with the samples, savory biscuits and drinking water were provided to clean the palate.

VI.2.7.2. Free profile descriptive analysis

The Free Profile technique was used for sensory analysis of the yogurts (WILLIAMS; LANGRON, 1984). Initially, 12 evaluators were selected, using an online questionnaire on personal data (age and gender) and yogurt consumption profile (frequency, form of consumption, period of consumption and amount consumed habitually).

The evaluators were chosen based on the frequency and profile of yogurt consumption (frequency, form of consumption, period of consumption and amount consumed habitually). The data were collected via an online questionnaire, and for the first stage those who: voluntarily agreed to the terms and conditions of the research; were over 18 years old; declared they habitually consumed yogurt; and were available to participate in the on-site sessions for the application of the sensory tests were accepted.

After applying the free profile technique, the evaluators who presented a residual error < 15 were selected, that is, variability less than 15% between the responses provided in the triplicates, leaving 9 evaluators.

The survey of yogurt attributes (natural goat's milk yogurt and goat's milk yogurt enriched with sunflower oil) was performed using the Rede method (MOSKOWITZ, 1983). The yogurts were presented to the evaluators and the evaluator was asked to note the similarities and differences between the samples. After the sessions of terms survey and discussion between the team leader and individually with each evaluator, the individual list was defined and the individual form was elaborated with intensity scales, using unstructured scales of 9 cm. The tests were performed in triplicate for each taster.

VI.2.7.3. Acceptance analysis

Thirty-six volunteers who consumed yogurt participated in the acceptance test, where each sample was asked to be evaluated for acceptance of the global impression using a seven-point facial scale (1=I disliked it very much and 7=I liked it very much), that is, the acceptance of the product considering all its characteristics (MEILGAARD et al., 2007). Samples were served in single form, in order of balanced and randomized presentation.

VI.3. RESULTS AND DISCUSSION

VI.3.1. Consumer Research

This is the first study to simultaneously present a product that meets the needs of lactose intolerants, cow's milk allergies, and people with vitamin E deficiency, in addition to evaluating the perception of the yogurt consuming public about this product.

VI.3.1.1. Characterization of participants

Of the 204 consumers who participated in the research, 122 declared themselves as female, 81 as male and one as gender-fluid. The predominant age group was 30 to 39 years (30.7%), and the one with the lowest participation was between 70 and 79 years (0.5%). It is noted that the age groups between 20 and 49 years concentrated 76.2% of the participants, while the representatives of the extremities were scarce (18 to 20 and 70 to 79 years) or null (above 80 years).

In an open questionnaire, 76.7% were classified as white, 14.4% as brown, 5.4% as black and 3.5% as yellow.

Regarding the place of residence, 98.5% lived in Brazil, while the other participants (1.5%) lived in the United States, Belgium or France.

Monthly household income was also observed, with 73.1% of participants declaring a monthly household income of at least three minimum wages (about BRL 1,000.00) and 51.0% of these receiving at least five minimum wages. At the other end, 3.9% of the participants declared to receive up to one minimum wage and, curiously, none of the 204 participants declared to have a household income between BRL 1,000.00 and BRL 2,000.00 per month.

Regarding level of education, 100% of the participants declare themselves as literate. Of these, 54.4% had a postgraduate degree (incomplete and complete) as their maximum degree. On the other hand, 4.9% declared that they had attended Basic Education or less.

Knowing the consumer profile is essential for the development of a product, considering that the choices are often based on social and economic factors. Thus, a consumer with a certain purchasing power may have a greater interest in a product with high added value, while a certain gender tends to be more or less concerned with health issues. Furthermore, the level of education may be related to a better understanding of the benefits of a particular product, impacting their preference beyond the sensory attributes.

VI.3.1.2. Consumption habits and perception of healthiness

Regarding yogurt consumption, 72.1% of participants declared they were consumers (Table 1).

In consumer research, when asked about which products are considered healthy, 467 answers were obtained. Of these, 20.3% were related to natural or fresh products, followed by fruits, organic foods, dairy products, vegetables, legumes and others with less frequency (Table 1). Of the 39 citations involving dairy products, 10 were related to yogurts.

Regarding the perception of healthy products, the valuation of natural food production was evaluated, where 68.6% of the participants declared to value a lot or value (26.0%), for all ranges of purchasing power. These results suggest the importance of the availability of products with natural appeal, and may represent a trend in the consumer market. Only 10 participants chose the neutral or negative part of the scale, and all of them are between 26 and 41 years old.

The valuation of the nutrient content concentrated 76.5% of the responses in the positive part of the scale (4 and 5), and only 5.88% in the negative part (1 and 2) (Table 1)

However, 80.4% of the participants said they valued food brands and labels, and 51.0% of the participants said they valued them very much.

The negative part of the scale concentrated 7.4% of the participants, and only 2.0% declared not to value this aspect (Table 1).

This suggests that, although the consumer wishes to consume natural products, there is a preference for already known and established brands, suggesting the importance of high quality industrialized products.

Table 1 – List of products considered healthy by 204 yogurt consumers

Products	%	Products	%

Natural or minimally processed products	20.3	Fish	0.9
Fruits	9.6	Products of animal origin or honey	0.9
Organic products	8.8	Zero or few calories	0.9
Dairy products	8.4	Promotion of health benefit	0.6
Leafy greens	8.4	Grains	0.6
Vegetables	6.6	Meals prepared at home	0.6
No additives	4.5	Few understandable ingredients or terms	0.6
Meat	3.6	Sanitized products	0.4
Plant products	3.4	Agro-ecological products	0.4
Reduction or absence of sugars	2.6	Not harmful to health	0.4
Cereals	2.1	Wholewheat biscuits or bars	0.4
Low fat	1.7	Fermented products	0.2
High nutritional value	1.7	Dried or freeze-dried fruit	0.2
Eggs	1.5	Açaí	0.2
Seeds	1.5	Kefir	0.2
Reduction or absence of salt	1.3	Non-conventional food plants	0.2
Nuts	1.1	Breads	0.2
Sources of protein	1.1	Gluten free	0.2
Whole products	1.1	Abundance of fibers	0.2
Juices	1.1	Healthy appetizers	0.2
Unsaturated oils	0.9		

Participants were asked to report terms related to healthy foods. A total of 435 entries were identified, the most cited being the terms related to natural, health, nutrients, vegetables and organic. Terms related to the absence or reduction of sugars, enriched, probiotic or functional, kefir or yogurt and rapid digestion, characteristics of the developed product were also referenced (Table 2).

Again, the consumer's association between healthy and natural foods is remarkable, showing a disparity between the search for products that are simultaneously natural, but that are produced by certain companies.

Table 2 – Terms related to healthy foods by 204 yogurt consumers

Relationship with healthy foods	%	Relationship with healthy foods	%
Natural products	11.3	Absence or reduction of salt	1.2
Health	9.9	Enriched, probiotic or functional	1.2
Nutrients or vitamins	9.0	Longevity	0.9
Leafy greens	8.5	Whole foods	0.9
Organic	6.5	Appearance	0.7
Fresh or not very industrialized	6.0	Lactose free	0.5
Fruits	5.8	Validity	0.5
Wellbeing	4.6	Fish or seafood	0.5
No additives	4.4	Gluten free	0.5
Absence or low sugar	3.7	Vegan	0.5
Absence or low fat	3.2	Water	0.5
Sustainable	2.8	Practicality and availability	0.5
Origin or brand	2.3	Quality	0.5
Flavor	1.8	Kefir or yogurt	0.5
Safety and hygiene	1.6	Quick digestion	0.2
Light food	1.6	Meat	0.2
Green or nature	1.4	Validity	0.2
Reduced calories	1.4	Aroma	0.2
Absence of hydrogenated or trans fat	1.4	Few ingredients	0.2
Average	1.4	Unawareness	0.2
Family / domestic production	1.4		

Enriched products are known to 74.5% of consumers, and 55.4% said they consume them. Although 43 participants claimed not to consume these products, and another 48 claimed not to know these products, only 60 participants (29.4%) selected the “not consumed” option when asked about frequency of consumption. Such variation suggests a possible confusion among the participants regarding the definition of such products. A total of 35.8% of participants claimed to consume enriched products once a week or more, while only 1.47% claimed to consume once every seven to 12 months. As yogurt is a frequently consumed product, the product developed in this study presents itself as a favorable alternative to increase the consumption of a food enriched with

vitamin E, especially by consumers between 20 and 49 years old, with complete higher education and a monthly income of at least two minimum wages.

Participants were also asked to report terms related to enriched products. 471 entries were observed, and 43.95% related the enriched product to the nutrient content, leading the ranking of terms. The second place was the term "health", with only 5.94% of entries, followed by ignorance (4.67%) and the best quality (4.67%) (Table 3). This result suggests that, although there may be confusion as to the types of products that can be considered enriched, there is a strong association of the term with the nutritional contents of the food.

Related to the possibility of consumption of enriched goat milk yogurt, the participant was allowed to express opinions on the product described. 209 entries were obtained and, of these, 36.36% declared that they believe that the product is "interesting".

Nutritional or general health advantage (18.18%) and advantage against gastrointestinal problems or allergies (3.35%) were also reported positively. However, several points of rejection or attention were observed: uncertainty about the taste of the final product (10.05 %); resistance to goat's milk (7.66 %); disinterest or general disapproval (7.66 %); uncertainty about the cost (4.31 %); resistance to sunflower oil (4.31 %); uncertainty about the texture (1.91 %); and uncertainty about the form of production (0.96 %). Also, 5.26% of the participants have no opinion on the product.

Interestingly, the negative opinions were mainly based on the possible sensory characteristics of the product.

Concerns have been reported about the possible pronounced flavor of goat's milk, and the oily texture that yogurt may have due to enrichment.

One participant reported concern about the reason for such enrichment, suggesting that adding oil could be an industry maneuver to increase yield and decrease product costs.

Thus, it is clear the importance of the effective dissemination of the benefits of products from goat's milk, and the importance of sensory aspects before any health benefit.

Table 3 – Terms related to enriched foods by 204 yogurt consumers

Relationship with enriched foods	%	Relationship with enriched foods	%
Nutrients	44.0	Diets, shakes, fitness	1.3
Health	5.9	Artificial	1.1
Unawareness	4.7	Suspicion	0.9
Better quality	4.7	Antioxidants	0.6
Processed foods	4.5	Reduced quality	0.6
Supplementation	4.5	Health problems or medications	0.6
Healthy	4.3	Natural products	0.6
Flour-based products	3.2	High calorie content	0.6
Dairy products	3.0	Enriched industrialized products	0.6
Whole grains and seeds	3.0	Safety	0.4
Functional food	2.3	Infant food	0.4
Wellbeing or longevity	1.7	Ammonia	0.2
Salts / sodium	1.7	Food for the elderly	0.2
High cost	1.5	Marketing	0.2
Additives	1.3	Water	0.1
Organic or sustainable	1.3		

VI.3.1.3. Possibility of consumption of enriched goat's milk yogurt

When asked whether they would consume a goat's milk yogurt enriched with sunflower oil, on a 5-point semi-structured scale where 1 = would not consume and 5 = would consume for sure, 30.4% of evaluators claimed that they would consume for sure. In general, 53.4 % of the entries are in the positive part of the scale, compared to only 17.2 % in the negative part. 29.4% of the entries were neutral. In addition, knowing that the product has a high content of vitamin E or antioxidants would encourage the purchase by 70.1% of the participants, and knowing that the product is more easily digestible would positively influence the purchase by 78.9% of the participants.

Furthermore, 69.6% of participants claimed to understand the benefits of vitamin E consumption, while only 38.2% understand the benefits of goat milk consumption. Thus, although 78.9 % of participants showed interest in a better digestibility product, only 38.2 % tend to associate this characteristic with goat's milk.

VI.3.2. Extraction of sunflower oil and production of goat's milk yogurt

The oil was extracted under optimized process conditions to obtain a product rich in vitamin E, the same week the yogurt was formulated. Extraction with $scCO_2$ is indicated for lipophilic compounds and nonpolar substances, such as tocopherols (MAUL et al., 1996). Producing extracts free of organic solvents, extraction with $scCO_2$ is known as clean technology and has been used for decades to obtain natural products that can be used in the food and pharmaceutical industry. In addition, $scCO_2$ has advantageous extraction characteristics when compared to conventional methods (UQUICHE et al., 2004). The oil obtained in the extraction showed a yellowish color and characteristic smell, and was added to the goat milk yogurt produced in this study.

Microbiological and sensory analyses were performed 24 hours after production. During this period, the yogurt was stored in a domestic refrigerator at a temperature of approximately 8 ˚C.

VI.3.3. Microbiological analysis

No colonies Salmonella, Listeria, *Escherichia coli* and total coliforms were observed in any sample, suggesting the high quality of the milk used, as well as its pasteurization.

In the analysis of molds and yeasts, only 0.4 CFU / g of natural yogurt (without oil) were observed in only one repetition of the dilution 10^{-1}, and 0.01 and 0.003 CFU / g of enriched yogurt in one repetition of each of the dilutions 10^{-2} and 10^{-3}, respectively. All other repetitions per sample did not present CFUs.

The legislation for fermented milk specifies that for every 5 samples: all present absence of salmonella and listeria / 25 mL; up to 2 present between 3 and 10 CFU E. coli / mL; and between 10^2 and 10^3 CFU of molds and yeasts / mL (BRASIL, 2019). The samples from this study are suitable for consumption.

VI.3.4. Analysis of acidity, color and oxidation

After the formulation and preparation of the yogurts, they were evaluated for instrumental color (Table 4), acidity and oxidative stability (Table 5). There were no statistical differences ($p \geq 0.05$) between the samples for instrumental color or titratable acidity (Table 4).

Table 4 – Instrumental color indexes for goat's milk yogurts enriched or not with vitamin E-rich sunflower oil obtained via SFE

Sample / parameter	L*	a*	b*	Chroma	Hue
No oil	67.14 ± 0.01b	-1.89 ± 0.1a	2.64 ± 0.06a	3.03 ± 0.17a	-54.50 ± 0.85a
With oil	67.17 ± 0.00a	-1.84 ± 0.07a	2.65 ± 0.03a	3.25 ± 0.33a	-55.27 ± 1.15a

Means followed by equal letters in the same column do not differ by the 5% probability Tukey test.

The variable L* observed in this study was considerably lower than that observed by Machado et al. (2017), who evaluated natural goat milk yogurt L* = 91.06 within 24 h after production. Although the authors observed a reduction in L* over 28 days, the lowest reported value was 90.85. As for the parameters a * and b*, the yogurts in this study fall within the yellow-green quadrant, but very close to the center of the scale, represented by the white color, suggesting that although with less luminosity, they presented low Chroma, that is, low color saturation, and the yogurts were whiter than that observed in the literature. The color of milk is very related to the food suffered by the lactating animal among other factors, so it is impossible to compare it with the results of other authors. The interesting thing to observe in this result is that the addition of sunflower oil did not influence the color of the yogurt when compared to the natural one (Table 4).

As for acidity, although unrefined oil was used in the formulation of yogurt enriched with vitamin E, no influence was observed on the total acidity of the product. Comparing the acidity of goat milk yogurt formulated in this experiment with the acidity of the same product prepared by Costa et al. (2016) values higher than 0.14% in natural goat milk yogurt are observed. This difference can be attributed to several factors such as the composition of the raw material, formulation, yeast, incubation, among others.

Generally speaking, regardless of the type of raw material, the acidity of yogurts must be at least 0.6%, expressed as lactic acid, according to Codex Alimentarius CXS 243-2003 (FAO, 2018), while Normative Instruction No. 46 of 23 October 2007 stipulates acidity between 0.6 and 1.5% (BRASIL, 2007). Thus, the yogurt formulated in this study is adequate to international standards for acidity.

Table 5 – Titratable total acidity and accelerated oxidative stability expressed in hours

Sample / parameter	Acidity %*	Induction time (120 °C, 15 L/h)
No oil	0.72 ± 0.02a	0.53 ± 0.21a
With oil	0.73 ± 0.02a	0.64 ± 0.14a

*in lactic acid. Means followed by equal letters in the same column do not differ by the 5% probability Tukey test.

Yogurts did not differ in oxidative stability analysis. Although the data obtained show that the addition of the oil provided a longer oxidative induction time, this difference was not significant (Table 5). This small increase in oxidation time could be attributed to the presence of tocopherols or other antioxidant compounds that sunflower crude oil could contain, but other experiments would be necessary for conclusive data.

VI.3.5. Caloric value of the yogurt

The protein content in raw goat milk ranges from 3.1 to 3.6 g/100 g; total carbohydrates range from 4.5 to 5.2 g/100 g; and total lipids represent 3.8 to 4.1 g / 100 g. As for the energy value, natural goat milk ranges from 66 to 69 kcal/100g (UNICAMP, 2011; USDA, 2020).

Considering that the production of yogurt does not significantly change the caloric content of the raw material (USDA, 2021b, 2021a), it is considered that the energy value of goat milk yogurt is close to that stipulated for its raw and whole version. Since 1 g of lipids, such as sunflower oil, represent 9 kcal, the theoretical energetic increase in enriched yogurt would be 46 kcal/100 g of yogurt. Xylitol has 2.4 kcal/g (MUSSATTO et al., 2006) and, therefore, in this study it would be responsible for the increase of 12 kcal/100 g, resulting in a product of approximately 126 kcal/100 g.

Lipids correspond to an important part of yogurt, and are responsible for much of its caloric value. Dos Santos et al. (2018) described the reduction of caloric value in goat milk yogurts with different levels of sucrose and mango pulp between 0 and 30 days. According to the authors, there is a reduction in lipid content in yogurts, which can reduce by up to 8 kcal/100 g in 30 days, probably due to hydrolysis triggered by exposure to light or oxygen.

A 200g portion of yogurt provides between 144.78 and 200.26 kcal, that is, between 7.24 and 10.01% of the recommended daily value for a 2,000 kcal diet (BRASIL, 2003). Thus, although goat milk yogurt enriched with tocopherols from sunflower oil

cannot be considered a product with reduced caloric value, an alternative to lactose and conventional sugar restrictive diets is shown, one that is formulated with xylitol, a sweetener suitable for groups of people with glucose restrictions and suchlike.

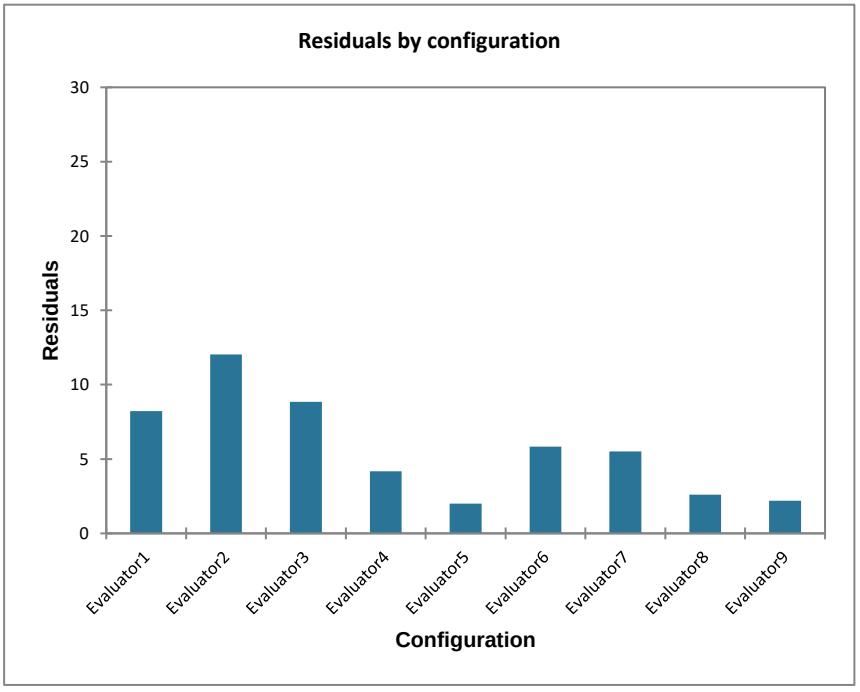
VI.3.6. Sensory Analysis

Goat milk yogurt is different due to its better digestibility when compared to cow's milk, and has a great potential for consumption by children, the elderly and people allergic to cow's milk. In this yogurt, its benefits were enhanced by the use of xylitol, a polyalcohol ($C_5H_{12}O_5$) with sweetening power similar to sucrose which, in addition to being better tolerated by diabetics when compared to sucrose, is a stable compound that does not undergo non-enzymatic darkening reaction (Maillard reaction), even helping to converse food better. Finally, the insertion of vitamin E through sunflower oil is a simple and natural way to promote improvements in human nutrition, respecting both nutritional needs and the environment.

VI.3.6.1. Free Profile Descriptive Analysis

The 9 selected evaluators are shown in Figure 1. All selected evaluators presented residual error <15%.

Figure 1 – Residual errors of the selected evaluators



The 9 selected evaluators were characterized according to age group, 33.3% of the evaluators are younger than 25 years; 44.4% between 25 and 35 years; and 22.2% between 36 and 50 years. As for sex, 44.4% declared themselves female, and 55.6% male. A total of 88.9% reported consuming dairy products more than once a week, and only 11.1% consumed them only once a week. No evaluator reported consuming less frequently.

Regarding the consumption of yogurt, 5 evaluators reported consuming the product between two and six times a week, while 2 declared consuming it daily, 1 declared consuming it weekly and 1 every two weeks, either as a snack between lunch and dinner (7 evaluators), or at breakfast (2). When asked about the amount consumed at a time, 5 evaluators reported consuming approximately 180 g (1 conventional unit pack), while 2 claimed consumption of 90 g at a time, and only 1 reported consuming 360 g at a time.

As for the form of consumption of yogurts, the 9 evaluators provided 24 responses, as follows: with fruits (7), pure or natural (5), with oats or granola (4), with sweeteners (4), as dessert (2), in smoothies (1) and with whey supplement (1). The main parameters that the evaluators consider at the time of purchase of yogurts were also verified. The 32 reports were grouped into: price (8), expiration date (7), type of yogurt (7), trademark (6) and packaging (4). Several reasons for consuming yogurts were cited, including: flavor; dairy product; easily digestible product; nutrients; health; and convenience of consumption.

Table 6 shows the attributes raised by the evaluators and their respective definitions, where it can be observed that for the citations of the appearance attributes, the most frequent were “fatty” and “white” (7 and 4 evaluators, respectively). For aroma, “characteristic” and “dairy aroma” stood out (7 and 5 evaluators, respectively). For flavor, the "dairy flavor" (6) was highlighted and, for texture, "homogeneity" and "consistency" (6). The attributes "characteristic aroma" and "characteristic flavor", often cited in the evaluation of goat milk yogurt (Table 6), suggest that the consumer considers these attributes similar between the products made from cow's milk and goat's milk, considering that all evaluators reported never having consumed goat milk yogurt prior to the research.

Table 6 – Definitions of sensory attributes reported by nine evaluators in analysis of goat milk yogurt enriched with sunflower oil

Attributes	Definition	Scale	Evaluators
	APPEARANCE		
Fatty	Fat droplets mixed with the drink	Slightly-Very	1,3,4,5,6,7,8
White color	Pure milk color (white), as opposed to yellowish color	Weak-Strong	6,7,8,9
Homogeneity	No separation of phases or coagulation	Slightly-Very	2,4,9
Creaminess	Consistency when shaken lightly	Slightly-Very	1,5,8
Shine	Shine of the sample, as opposed to opacity	Slightly-Very	2,4
	AROMA		
Characteristic	Characteristic of industrialized natural yogurt	Weak-Strong	2,3,4,5,6,7,8
Dairy Aroma	Intensity of the characteristic aroma	Weak-Strong	1,2,7,8,9
Sweet	Aroma that suggests sweet taste	Weak-Strong	8
	FLAVOR		
Dairy Flavor	Dairy flavor intensity	Slightly-Very	1,2,4,5,6,9
Acidity	Acidity characteristic of natural yogurts	Weak-Strong	4,6,7
Goat’s milk characteristic	Characteristic flavor of goat's milk	Weak-Strong	4,6,7
Intense aftertaste	Permanence of flavor in the mouth after swallowing	Weak-Strong	3,4,5
Characteristic of yogurt	Characteristic of industrialized natural yogurt	Slightly-Very	1,8
Sweet	Perception of sweeteners	Weak-Strong	3
Bitterness	Feeling of bitterness as in green fruit	Weak-Strong	2.8
Oily	Refers to the taste of vegetable oil	Weak-Strong	3
Cereal	Refers to the flavor of cereals	Weak-Strong	6
	TEXTURE		
Homogeneity	No separation of phases or coagulation	Slightly-Very	1,2,3,4,6,8
Consistency	Tongue involvement sensation	Slightly-Very	1,2,4,6,8,9
Viscosity	Refers to the effort for swallowing	Slightly-Very	5,6
Velvety	This refers to the feeling of softness	Slightly-Very	4
Characteristic of dairy drink	Characteristic of a natural industrialized dairy drink	Slightly-Very	7

The free profile test in dairy products is already known (VERRUMA-BERNARDI; DAMÁSIO, 2004). The results observed in the free profile analysis suggest that the presence of the oil is perceived by the evaluator (although there is no separation of phases), and that there is a comparison with a characteristic standard product (not shown) normally produced with cow's milk, although the yogurts formulated with cow's

and goat's milk differ in several physicochemical characteristics, gel firmness and whiteness (VARGAS et al., 2008).

The first dimension explained 80.50%, and the second dimension explained 11.79%, for both processes (Figure 2), and the sensory separation between the yogurts can be seen in Figure 3.

Goat milk yogurt enriched with sunflower oil proved to be distant from its version without enrichment (Figure 3), showing marked sensory differences between the beverages.

Figure 2 – Separation of descriptors for goat's milk yogurt enriched with sunflower oil

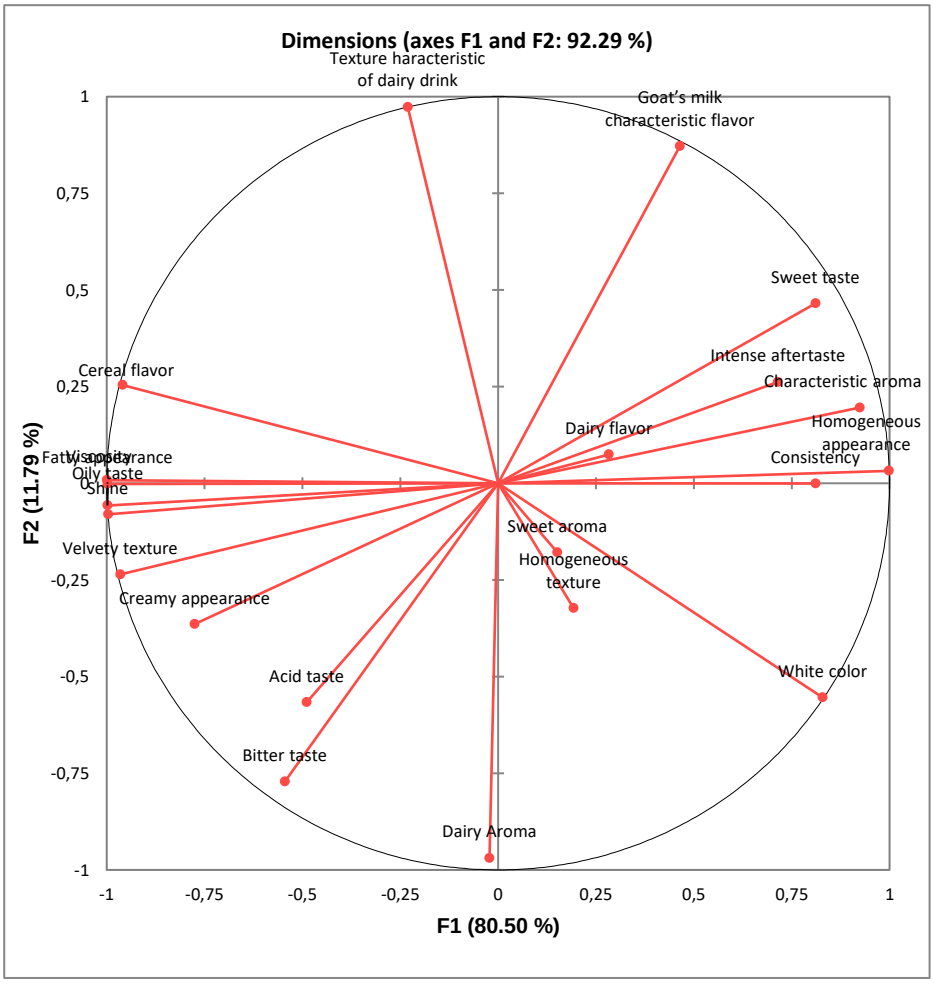
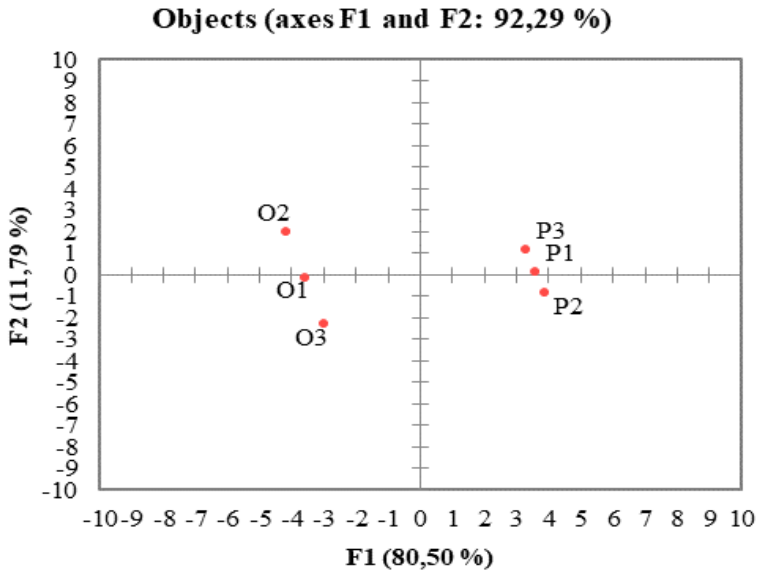


Figure 3 – Separation of the natural yogurts (P) or enriched with sunflower oil (O) according to the external preference map



The correlation coefficient (r) values between the attributes and the 1st dimension of the sample configuration were used for the presentation and discussion of the main characteristics perceived and reported by the evaluators, considering a correlation coefficient (r) greater than 0.50 ($|r| \geq 0.50$) (VERRUMA-BERNARDI; DAMÁSIO, 2004) in the two processes together (Table 7).

An explanation of 80.50% in the first dimension was observed, a value above those observed for Riesling vineyards (34%) by Benassi et al. (1998), but below that observed by (VERRUMA-BERNARDI; DAMÁSIO, 2004) in buffalo milk cheese, which reported, in three processes, explanations of 84.85, 81.60 and 88.29%. Benassi et al. (1998) reinforce the importance of separation between samples, despite the percentage explanation. In this study, both a high explanation and good separation were obtained (Figure 3), suggesting that sunflower oil significantly alters the sensory properties of goat milk yogurt.

Of the attributes with high correlation per evaluator in the first dimension (Table 7), there is a fatty appearance, an attribute with very high correlation in the citations of 77.8% of the evaluators; characteristic aroma, which presented high correlation for 55.6% of the evaluators; intense aftertaste, characteristic flavor of goat's milk and acid taste (33.3% each); and homogeneous texture (55.6%).

Table 7 – Attributes with the highest correlation ($|r| \geq 0.50$) for the evaluators in the evaluation of goat's milk yogurts in the 1st dimension

Attributes	
Evaluator 1	**Evaluator 5**
Fatty appearance (-0.95)	Fatty appearance (-0.87)
Milky aroma (-0.82)	Creamy appearance (-0.50)
Characteristic goat's milk flavor (-0.72)	Characteristic aroma (-0.68)
Homogeneous texture (0.50)	Intense aftertaste (0.93)
	Viscosity (-0.86)
Evaluator 2	**Evaluator 6**
Homogeneous appearance (1.0)	Fatty appearance (-0.99)
Shine (-0.85)	White (0.87)
Characteristic aroma (0.57)	Acid taste (0.89)
Milky aroma (0.50)	Cereal flavor (-0.96)
Homogeneous texture (0.74)	Homogeneous texture (0.86)
Consistency (0.62)	Consistency (0.61)
Evaluator 3	**Evaluator 7**
Fatty appearance (-0.96)	Fatty appearance (-1.00)
Characteristic aroma (0.94)	White (0.86)
Intense aftertaste (-0.79)	Milky aroma (0.66)
Sweet taste (0.81)	Acid taste (-0.79)
Oily flavor (1.0)	Characteristic goat's milk flavor (0.94)
Homogeneous texture (0.98)	
Evaluator 4	**Evaluator 8**
Fatty appearance (-1.00)	Fatty appearance (-0.99)
Homogeneous appearance (0.90)	White (-0.95)
Shine (-1.00)	Characteristic aroma (-0.64)
Characteristic aroma (0.98)	Milky aroma (-0.97)
Dairy flavor (0.96)	Bitter taste (-0.52)
Acid taste (-0,51)	Homogeneous texture (0.86)
Characteristic goat's milk flavor (0.93)	Consistency (-0.71)
Intense aftertaste (0.55)	
Homogeneous texture (-0.98)	**Evaluator 9**
Consistency (0.61)	Homogeneous appearance (1.00)
Velvety texture (-0.97)	White (0.86)
	Consistency (-0.52)

VI. 3.6.2. Acceptance testing

There was no statistical difference for acceptance between natural yogurts or yogurts with oil ($p \geq 0.05$). The natural yogurt presented a mean of 5.03 ± 1.6, while the enriched yogurt was scored as 4.61 ± 1.2, using a 7-point scale.

The average scores observed are in the positive part of the scale, suggesting that the product is accepted in this formulation. However, the insertion of other components, such as fruits, can increase their acceptance without prejudice to nutritional value. In addition, as a product aimed at an audience with dietary restrictions, the benefits of consumption may exceed the need for less healthy components (DOS SANTOS et al., 2018).

Furthermore, Vargas et al. (2008) reported that in a yogurt formulated with 50 % cow's milk and 50 % goat's milk, there is no sensory difference between the mixture and cow's milk in terms of flavor, flavor, whiteness, consistency and creaminess, suggesting that the use of goat's milk for yogurt formulation is promising both as the only type of milk employed and in mixed formulations.

VI.4. CONCLUSION

Consumer research has shown that the consumer public associates natural products with health, and that food enrichment is very related to the perception of nutrients in that product. However, while 70% of the participants declared they knew the benefits of vitamin E, only 38% declared the same about goat's milk, suggesting the need to disclose the beneficial properties of this milk of increasing importance, such as better digestion and lower allergenic factor.

Supercritical CO_2 proved to be a good option for obtaining sunflower oil for enrichment of goat's milk yogurt, as an excellent source of alpha-tocopherol (vitamin E with high biological value), without causing a significant increase in the acidity of the product, even though it did not undergo de-acidification processes and other refinements, as well as not influencing the oxidation induction time, maintaining the quality of the natural product.

Although the oil influenced the luminosity of the yogurt, it did not influence the color or shade. Even so, the evaluators noticed, visually, the presence of fat droplets mixed with the beverage, also related to the perception of homogeneity. Interestingly, the main attributes mentioned for aroma and flavor were related to the dairy characteristics of the product, and not to the oil, and no comments were made regarding the sweetener,

xylitol, suggesting that the sweetening function was achieved naturally, and the sweet taste was not perceived in a particular way, remaining an excellent option for people with restriction to the consumption of common sugar.

It can be concluded that goat milk yogurt enriched with vitamin E from the addition of sunflower oil obtained by supercritical technology can be presented as an environmentally and nutritionally friendly alternative for human consumption, in addition to having good sensory characteristics and causing interest in the consumer market.

REFERENCES

ANNUNZIATA, A.; VECCHIO, R. Consumer perception of functional foods: A conjoint analysis with probiotics. **Food Quality and Preference**, v. 28, n. 1, p. 348–355, 2013.

AOAC. **Official Methods of Analysis of AOAC International**. 18. ed. Gaithersburg: AOAC International, 2005.

ASL, P. J.; NIAZMAND, R.; YAHYAVI, F. Extraction of phytosterols and tocopherols from rapeseed oil waste by supercritical CO_2 plus co-solvent: A comparison with conventional solvent extraction. **Heliyon**, v. 6, n. 3, p. e03592, 2020.

BÄR, A. Xylitol. In: O'BREIN NABORS, L.; GELARDI, R. C. (Eds.). . **Alternative Sweeteners**. [s.l: s.n.]. p. 185–216.

BELLUCCI, C. F.; LAVARDA, R. B.; ROCZANSKI, C. R. M. A Prática Estratégica de Pesquisa de Mercado no Processo de Internacionalização de uma Pequena Cervejaria do Sul do Brasil. **REGEPE - Revista de Empreendedorismo e Gestão de Pequenas Empresas**, v. 8, n. 1, p. 24–51, 19 dec. 2018.

BENASSI, M. DE T.; DAMÁSIO, M. H.; CECCHI, H. M. Avaliação sensorial de vinhas riesling itálico nacionais utilizando perfil livre. **Ciência e Tecnologia de Alimentos**, v. 18, n. 3, p. 265–270, 1998.

BRASIL. **Instrução Normativa nº 46, de 23 de Outubro de 2007**. Diário Oficial da República Federativa do Brasil, , 2007. Available at: <https://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=24/10/2007&jornal=1&pagina=4&totalArquivos=96>

CORREIA, R. T. P.; DOS ANJOS MAGALHÃES, M. M.; DA SILVA PEDRINI, M. R.; DA CRUZ, A. V. F.; CLEMENTINO, I. Sorvetes elaborados com leite caprino e bovino: Composição química e propriedades de derretimento. **Revista Ciencia Agronomica**, v. 39, n. 2, p. 251–256, 2008.

COVRE, E. A.; BORBA, K. R.; FERREIRA, M. D.; SPOTO, M. H. F.; SALA, F. C., VERRUMA-BERNARDI, M. R. Caracterização físico-química e sensorial da alface Brunela. **Agrarian**, v. 13, n. 48, p. 265–272, 20 jul. 2020.

CRUZ, A. G.; ZACARCHENCO, P. B.; OLIVEIRA, C. A. F.; CORASSIN, C. H. **Química, bioquímica, análise sensorial e nutrição no processamento de leite e derivados.** 1. ed. Rio de Janeiro: Elsevier, 2016.

DANH, L. T.; MAMMUCARI, R.; TRUONG, P.; FOSTER, N. Response surface method applied to supercritical carbon dioxide extraction of *Vetiveria zizanioides* essential oil. **Chemical Engineering Journal**, v. 155, n. 3, p. 617–626, 2009.

DASGUPTA, D.; BANDHU, S.; ADHIKARI, D. K.; GHOSH, D. Challenges and prospects of xylitol production with whole cell bio-catalysis: A review. **Microbiological Research**, v. 197, p. 9–21, 2017.

DE CARVALHO, M. A.; KITZBERGER, C. S. G.; SARTORI, A. V. DE S.; BENASSI, M. DE T.; SCHOLZ, M. B. DOS S.; DA SILVA, C. M. Free choice profiling sensory analysis and principal component analysis as tools to support an apple breeding program. **AIMS Agriculture and Food**, v. 5, n. 4, p. 769–784, 2020.

DO AMARAL, D. S.; CARDOSO, D. S. G.; PESSOA, T.; DE MOURA NETO, L. G. Perfil dos consumidores da carne de sol comercializada nos municipios de em Caic E Currais Novos - RN. **Acta Veterinaria Brasilica**, v. 6, n. 4, p. 302–311, 2012.

DOS SANTOS, Y. M. G.; DE OLIVEIRA, E. N. A.; FEITOSA, B. F.; DA COSTA SANTOS, D.; FEITOSA, R. M.; ALMEIDA, F. L. C. Goat milk mango yoghurts: Physicochemical stability during or storage. **Ciencia Animal Brasileira**, n. 19, p. 1–12, 2018.

DOS SANTOS, Y. M. G.; DE OLIVEIRA, E. N. A.; FEITOSA, B. F.; DA COSTA SANTOS, D.; FEITOSA, R. M.; ALMEIDA, F. L. C. Identificação Do Perfil Do Consumidor Da Carne Ovina Na Região Sudeste Por Meio De Questionário Online. **SINERGIA - Revista do Instituto de Ciências Econômicas, Administrativas e Contábeis**, v. 25, n. 2, p. 21–32, 2021.

FAO. Standard for Fermented Milks. In: **Codex Alimentarius**. 4. ed. [s.l.] FAO, 2018.

FARHOOSH, R. The effect of operational parameters of the Rancimat method on the determination of the oxidative stability measures and shelf-life prediction of soybean oil. **JAOCS, Journal of the American Oil Chemists' Society**, v. 84, n. 3, p. 205–209, 2007.

FOOD AND DRUG ADMINISTRATION, U. S. A. **Interactive Nutrition Facts Label - Total Fat**. Available at: <https://www.accessdata.fda.gov/scripts/interactivenutritionfactslabel/assets/InteractiveNFL_TotalFat_March2020.pdf>.

FOX, A. T.; THOMSON, M. Adverse reactions to cow's milk. Paediatrics and Child Health. **Metabolic Medicine**, v. 17, n. 7, p. 288–294, 2007.

FROST, M. B.; DIJKSTERHUIS, G. B.; MARTENS, M. Sensory perception of fat in milk. **Food Quality and Preference**, v. 12, p. 327–336, 2001.

GUÀRDIA, M. D.; AGUIAR, A. P. S.; CLARET, A.; ARNAU, J.; GUERRERO, L. **Sensory characterization of dry-cured ham using free-choice profilingFood Quality and Preference**, 2010.

HAENLEIN, G. F. W. Goat milk in human nutrition. **Small Ruminant Research**, v. 51, n. 2, p. 155–163, 2004.

KUMAR, V.; SANDHU, P. P.; AHLUWALIA, V.; MISHRA, B. B.; YADAV, S. K. Improved upstream processing for detoxification and recovery of xylitol produced from corncob. **Bioresource Technology**, v. 291, n. July, p. 121931, 2019.

MACFIE, H. J. H. Assessment of the Sensory Properties of Food. **Nutrition Reviews**, v. 48, n. 2, p. 87–93, 27 abr. 2009.

MAUL, A. A.; WASICKY, R.; BACCHI, E. M. Extração por fluido supercrítico. **Revista Brasileira de Farmacognosia**, v. 5, n. 2, p. 185–200, 1996.

MEDEIROS, L. C. S.; SPERIDIÃO, P. G. L.; SDEPANIAN, V. L.; FAGUNDES-NETO, U.; MORAIS, M. B. Nutrient intake and nutritional status of children following a diet free from cow's milk and cow's milk by-products. **Jornal de Pediatria**, v. 80, n. 5, p. 363–370, 2004.

MEILGAARD, M. R.; CIVILLE, G. V.; CAR, B. T. **Sensory evaluation techniques**. 4. ed. Boca Raton: CRC Press, 2007.

MOSKOWIT, H. R. **Product testing and sensory evalution of foods**. Westport: Food & Nutrition Press, 1983.

MUKHOPADHYAY, M. **Natural extracts using supercritical carbon dioxide**. 1. ed. Boca Raton: CRC Press, 2000.

MUSSATTO, S. I.; ROBERTO, I. C. Xilitol: edulcorante com efeitos benéficos para a saúde humana. **Revista Brasileira de Ciências Farmacêuticas**, v. 38, n. 4, p. 401–413, 2002.

NAGAGATA, B. A.; CARVALHO, C. F. DE; SANTOS, L. P.; SANTANA, I.; FREITAS, S. M. DE L.; GUIMARÃES, R. R. Desenvolvimento de burgueres veganos: estudo com consumidores e pesquisa de mercado. **Research, Society and Development**, v. 9, n. 7, p. e622974542, 28 may 2020.

NRC [NATIONAL RESEARCH COUNCIL] - USA. **Dietary Reference Intakes: The Essential Guide to Nutrient Requirements**. Washington: The National Academies Press, 2006.

ROCHE, H. M. Olive oil , high-oleic acid sunflower oil and CHD. **British Journal of Nutrition**, v. 85, p. 3–4, 2001.

ROSS, B. Ten tips to winning at consumer centricity: for retailers and manufacturers. **Journal of Consumer Marketing**, v. 26, n. 6, p. 450–454, 11 sept. 2009.

SCHULZE, M. B.; MANSON, J. A. E.; LUDWIG, D. S.; COLDITZ, G. A.; STAMPFER, M. J.; WILLETT, W. C.; HU, F. B. Sugar-sweetened beverages, weight

gain, and incidence of type 2 diabetes in young and middle-aged women. **Journal of the American Medical Association**, v. 292, n. 8, p. 927–934, 2004.

TURECK, C.; GESSER CORREA, V. G.; PERALTA, R. M.; KOEHNLEIN, E. A. Intakes of antioxidant vitamins and minerals in the Brazilian diet. **Nutricion Clinica y Dietetica Hospitalaria**, v. 33, n. 3, p. 30–38, 2013.

UQUICHE, E.; DEL VALLE, J. M.; ORTIZ, J. Supercritical carbon dioxide extraction of red pepper (Capsicum annuum L.) oleoresin. **Journal of Food Engineering**, v. 65, n. 1, p. 55–66, nov. 2004.

VARGAS, M.; CHÁFER, M.; ALBORS, A.; CHIRALT, A.; GONZÁLEZ-MARTÍNEZ, C. Physicochemical and sensory characteristics of yoghurt produced from mixtures of cows' and goats' milk. **International Dairy Journal**, v. 18, n. 12, p. 1146–1152, 2008.

VEIGA, A. S.; REDIN, E. A pesquisa de mercado nas microempresas do setor de viandas e marmitarias de Santa Maria-RS. **Saber Humano: Revista Científica da Faculdade Antonio Meneghetti**, v. 7, n. 10, p. 155, 9 ago. 2017.

VERRUMA-BERNARDI, M. R.; DAMÁSIO, M. H. Análise descritiva de perfil livre em queijo mozarela de leite de búfala. **Ciência e Tecnologia de Alimentos**, v. 24, n. 4, p. 536–542, 2004.

VIDAL, L.; ARES, G.; HEDDERLEY, D. I.; MEYNERS, M.; JAEGER, S. R. Comparison of rate-all-that-apply (RATA) and check-all-that-apply (CATA) questions across seven consumer studies. **Food Quality and Preference**, v. 67, p. 49–58, 2018.

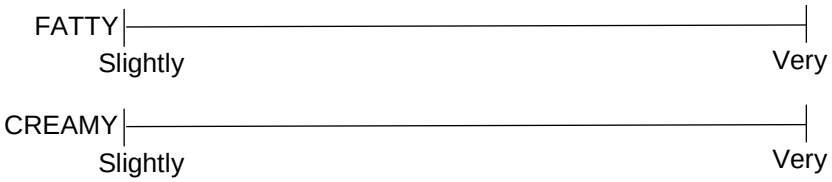
WILLIAMS, A. A.; LANGRON, S. P. The use of free-choice for the evaluation of commercial ports. **Journal of the Science of Food and Agriculture**, v. 35, p. 558–568, 1984.

APPENDIX I

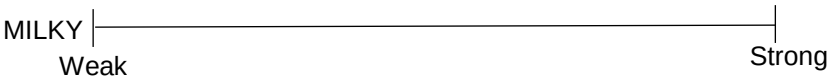
Free-choice profile files by evaluator

Evaluator 1

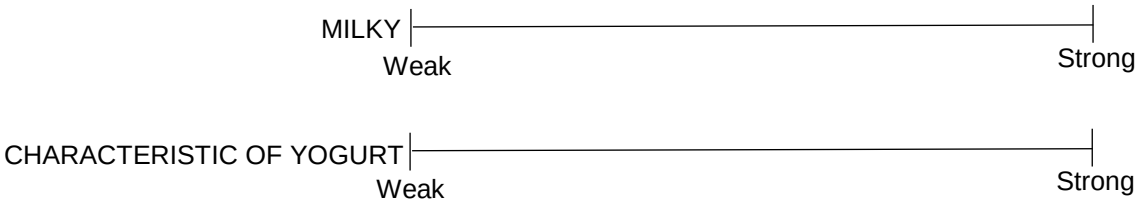
Appearance



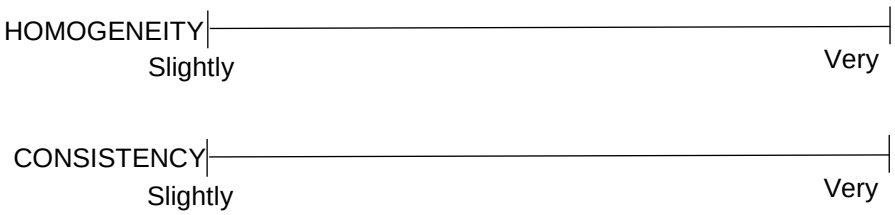
Aroma



Flavor

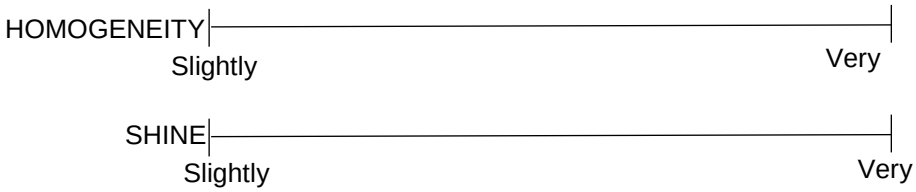


Texture

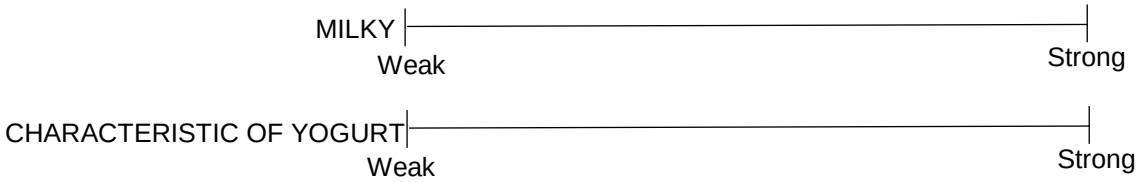


Evaluator 2

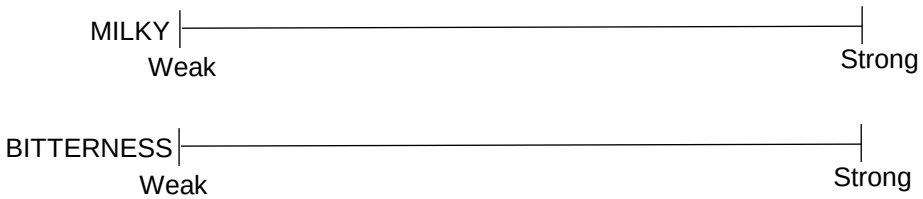
Appearance



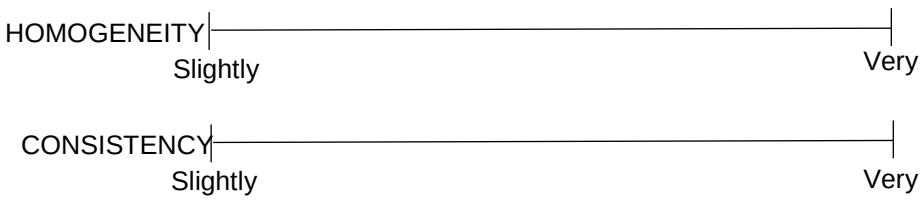
Aroma



Flavor

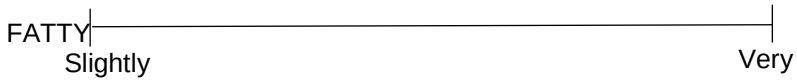


Texture

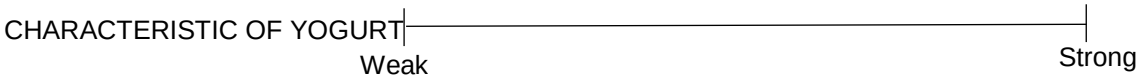


Evaluator 3

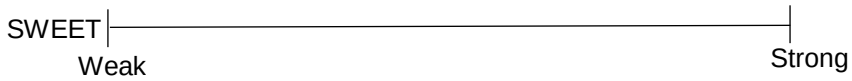
Appearance

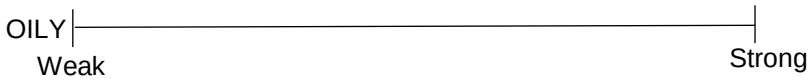


Aroma

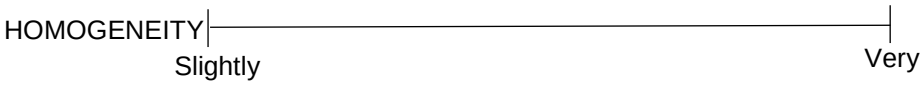


Flavor



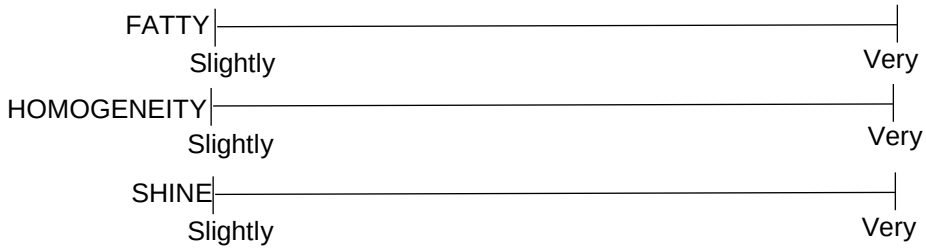


Texture

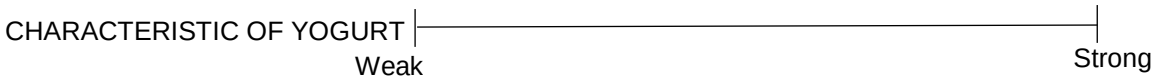


Evaluator 4

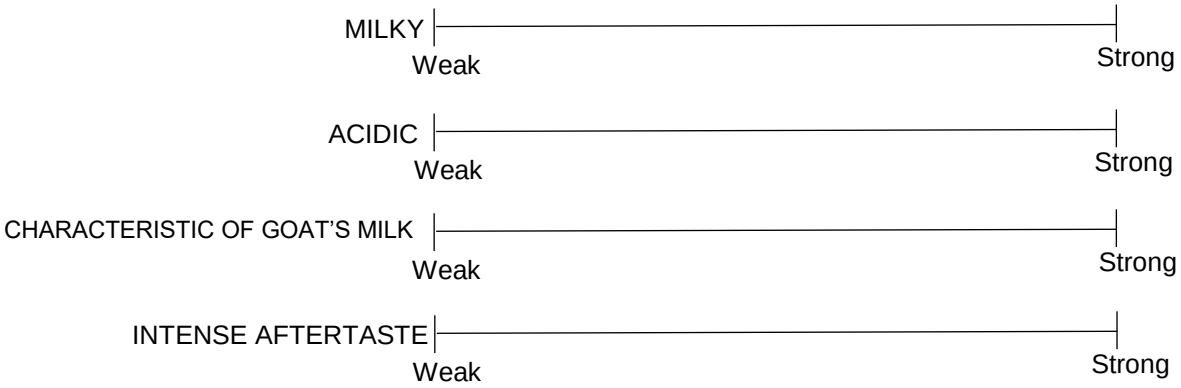
Appearance



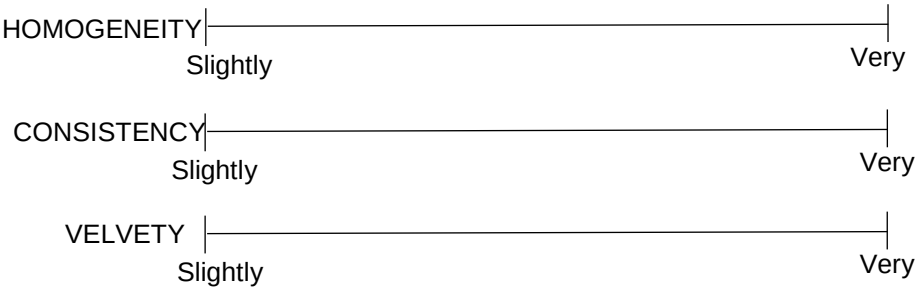
Aroma



Flavor

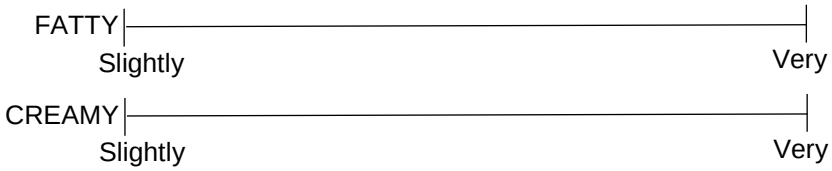


Texture

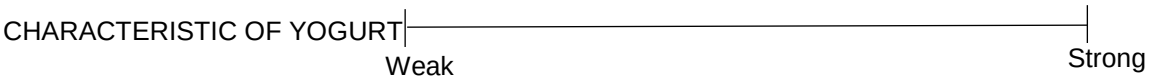


Evaluator 5

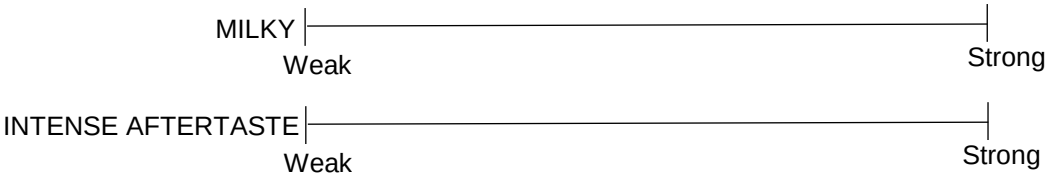
Appearance



Aroma



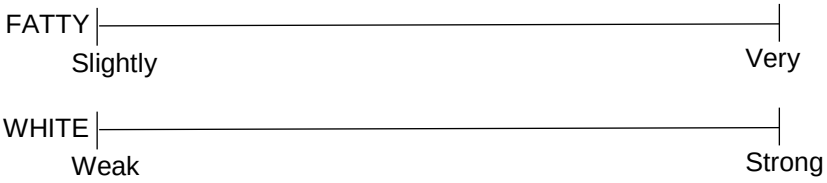
Flavor



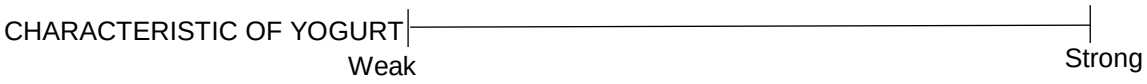
Texture

Evaluator 6

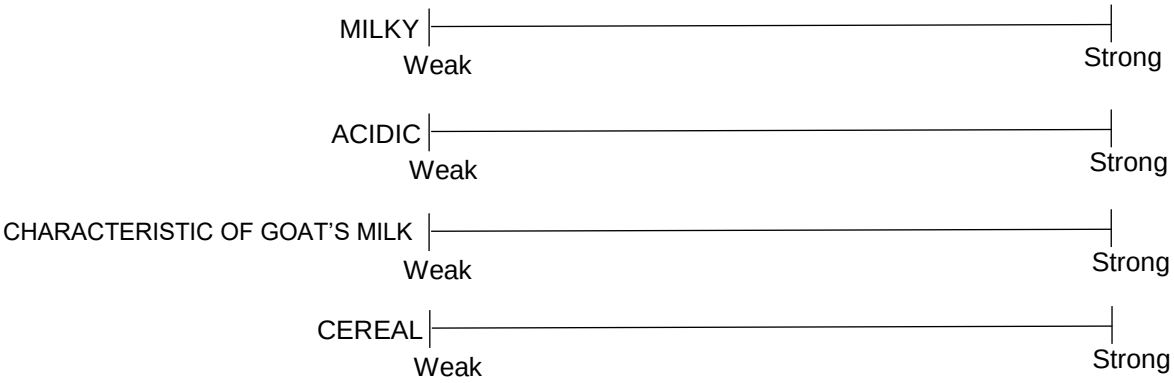
Appearance



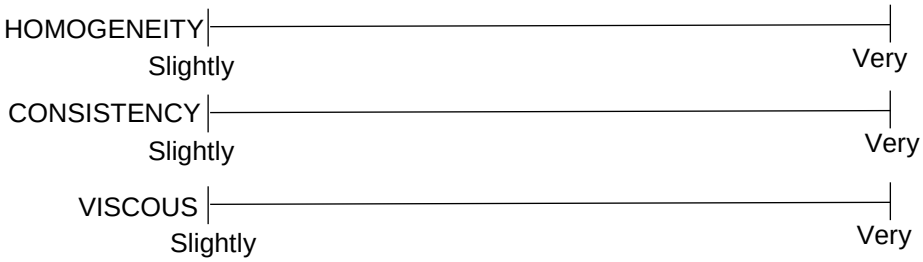
Aroma



Flavor

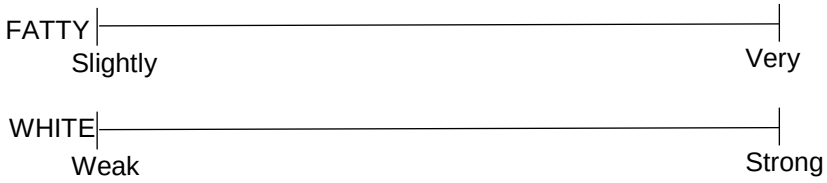


Texture

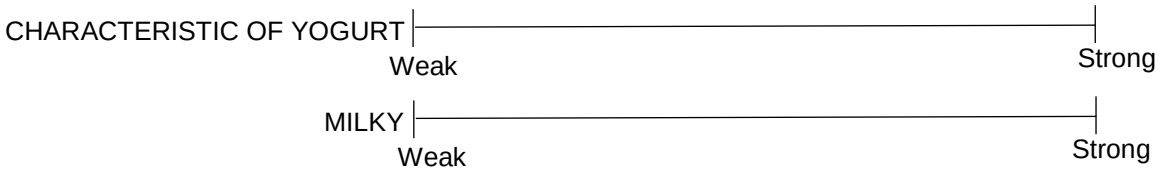


Evaluator 7

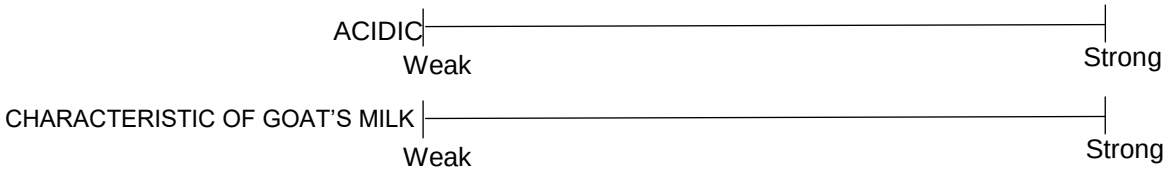
Appearance



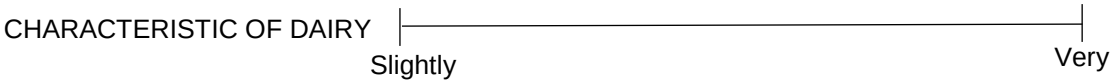
Aroma



Flavor

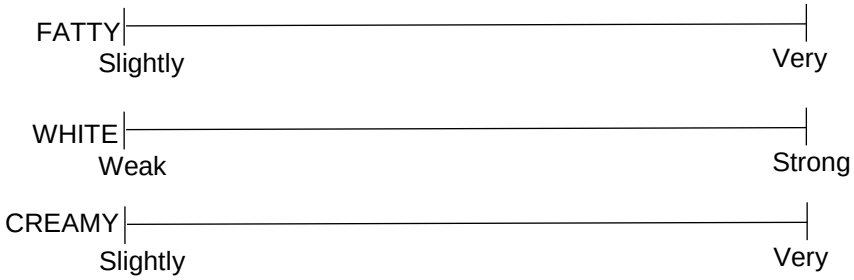


Texture

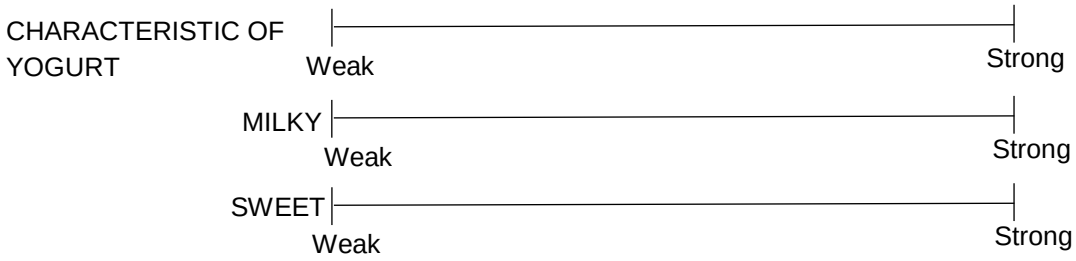


Evaluator 8

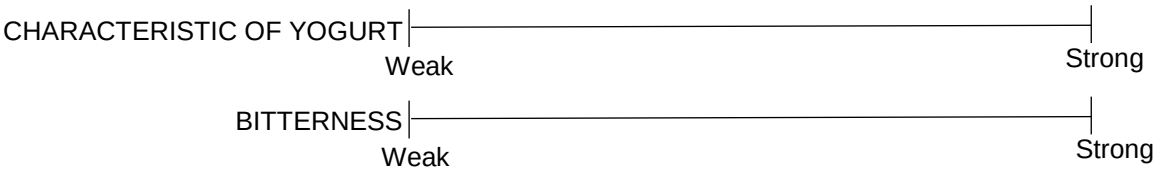
Appearance



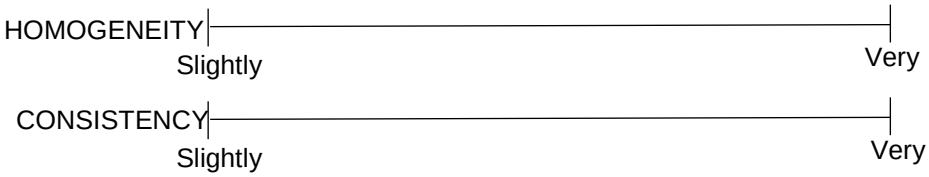
Aroma



Flavor

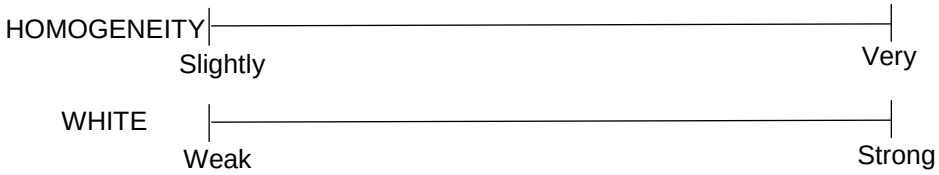


Texture

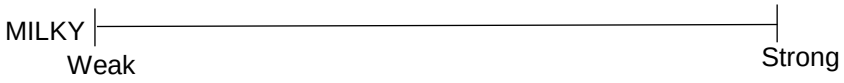


Evaluator 9

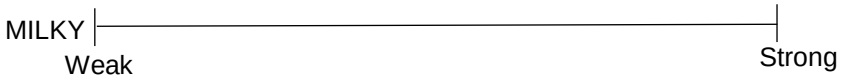
Appearance



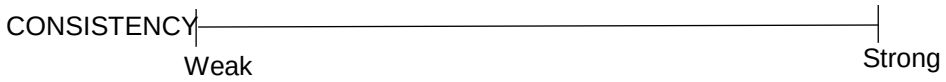
Aroma



Flavor



Texture

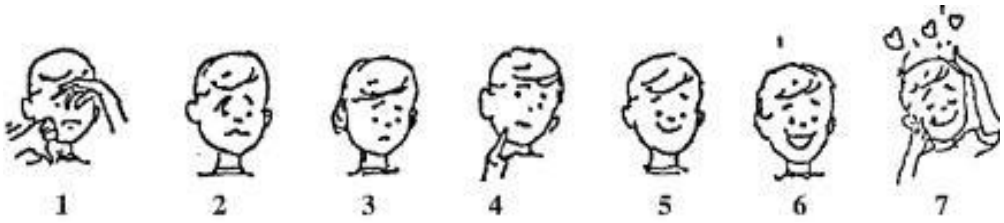


APPENDIX II

Name: _____ Sample: _____ Date: _____

Acceptance

Please circle the image that best represents your feeling regarding the overall impression of this sample:



CHAPTER VII: GENERAL CONCLUSIONS

This study resulted in important data regarding the use of green solvents, both in terms of the yield of the extractions, and in the valorization of the products obtained by them. Both the supercritical extraction (SFE) with carbon dioxide and extraction with pressurized liquid (PLE) using ethanol as a solvent are emerging technologies by using green and renewable solvents. In addition, especially with PLE, there has been a large reduction in extraction time, allowing extractions that would have taken several hours in the conventional process to be completed in a few minutes. When observing the behavior of sunflower oil extraction by PLE, using both rolled and ground seeds in continuous and intermittent processes, some interesting data were obtained. This study presents relevant results for the extraction of sunflower oil by two green techniques: carbon dioxide supercritical extraction (SFE) and pressurized liquid extraction (PLE) with ethanol as solvent. The emergence of these technologies is expected to replace traditional hexane extraction due to the use of non-toxic solvents. Ethanol and carbon dioxide are renewable solvents and therefore environmentally friendly extraction processes. Both technologies were expected to use green solvents to obtain sunflower oil with high levels of tocopherols with high biological value.

The yields of sunflower oil extracted with $scCO_2$ varied widely over the temperature and pressure range studied, from 19.48% to 87.58 % (w/w) oil recovery in 4 h without the need of further solvent separation, promoting a clean, high-quality oil.

PLE offers simplified technology, reduced execution time and lower solvent consumption. In approximately 30 min, the process was able to recover 75.37 to 93.93 % (w/w) of the oil in the seeds. Considering the discontinuous process, the solvent consumption is significantly lower than the conventional process. According to the probable composition of the TAG, the products obtained by SFE and PLE proved to be comparable to commercial products that have been integrated in the Brazilian market. This is considered a good guide because neither the use green technology nor harmless solvents will affect the lipid composition of the oil, maintaining its properties.

Also, the PLE kinetics showed that the yield varied for each process, as did the solvent consumption. In the continuous process, the yield was about 60 %, not differentiating concerning the size of the particles (rolled or grounded). In the intermittent process, the extraction occurred in the decreasing phase, not reaching the diffusion phase.

Thus, the yield was lower. However, the solvent consumption was also considerably lower. In both processes, convection predominates, which indicates that the solvent solubilizes the components on the surface of the particles.

As for the acidity content of the oils, it was observed that under optimized extraction conditions, sunflower oils obtained by SFE-scCO_2 and PLE showed a total titratable acidity of 3.59 and 3.17 g oleic acid/100 g oil, respectively, while the soybean oils presented an acidity of 2.10 ± 0.001 and 2.11 ± 0.008 g/100 g oleic acid in the PLE using ethanol and hexane as solvents, respectively.

As for the valorization of the final products, this study showed the possibility of enriching sunflower oil with tocopherols, especially α-tocopherol, one of the main components of Vitamin E, so indispensable to human health. Both technologies (SFE and PLE) proved capable of obtaining high levels of α-tocopherol in the extracted oil-but not always correlated to high total yields.

There were differences in tocopherol content: in oils obtained by SFE, temperature affected α-tocopherol (14.92 to 88.00 mg /100 g) content, while pressure affected γ-tocopherol (0.30 to 1.20 mg / 100 g) and δ-tocopherol (<0.02 to 0.43 mg / 100 g). Therefore, the total tocopherol content (22.89 to 91.17 mg / 100 g) was affected by these two variables.

Although both the linear and quadratic models have been shown to be quite predictive for the extraction yield by PLE (R^2 = 0.99 and adjusted R^2 = 0.97), the models are not predictive for tocopherols. In oils obtained by PLE, no variables affected tocopherol content and the total tocopherol content ranged from 40.30 to 83.16 mg / 100 g), being the α-tocopherol responsible for 36.32 to 76.99 mg / 100 g.

Subsequent to the publication of these data (Chapter V), the acidity of commercial sunflower and soybean oils and those obtained via soxhlet (petroleum ether) were determined: sunflower oil showed an acidity of 0.36 ± 0.02 and 2.52 ± 0.05 g oleic acid/100 g sunflower oil, respectively, while soybean oil showed 0.32 ± 0.03 1.97 ± 0.04 g oleic acid/100 g soybean oil. These results indicate that the pressurized extractions resulted in more acidic oils than the non-pressurized (soxhlet) using both matrices.

The total fatty acid profile was similar between the optimized samples and commercial sunflower oil, suggesting that the lipid composition of the product was not altered. This information was reinforced by the triacylglycerol analysis, which also proved to be similar. These results suggest that, in lipidic composition, the crude oil

obtained is similar to the refined oil obtained by conventional process, already well established in the Brazilian market.

However, there was little information in the literature regarding the composition of free fatty acids in oils, which are responsible for the acidity in oils. Usually, only the contents per total by titrimetric method are presented. In this study, an adapted methodology for the qualitative determination of free fatty acids present in sunflower oil and soybean oil obtained by green processes was described in detail. As expected, there was no variation in the major fatty acids, such as linoleic, oleic, palmitic, and stearic acid. However, the process influenced the composition of minority free fatty acids, such as eicosanoic and Arachidonic. The used methodology rendered satisfactory results for the determination of free fatty acids in crude sunflower and soybean oil extracted using green technologies.

After analyzing the oils, an online consumer survey was carried out to obtain relevant information on public perception of a goat milk yogurt enriched with sunflower oil. 204 volunteers participated. The consumer survey showed that the consumer public associates natural products with health and especially nutrient content. However, while the vast majority (70%) of participants stated that they were familiar with the benefits of vitamin E, few (38%) stated the same about goat milk, suggesting misinformation about this product. To formulate the yogurt, it was chosen the oil obtained by CO_2 that presented both good yield and good tocopherol content, without causing a significant increase in the acidity of the product in its raw form. The use of the oil did not influence the oxidation induction time of the yogurt, maintaining the quality of the natural product. As for possible changes in the characteristics of the enriched yogurt, it was noted that the oil influenced the brightness of the yogurt, but there was no influence on the color or hue. Even so, the evaluators visually perceived the presence of fat droplets mixed in the drink, also related to the perception of homogeneity.

Despite this visual perception, the main attributes mentioned for aroma and flavor were related to the dairy characteristics of the product, not to the oil, and no comments were made about the sweetener, xylitol. The use of this sweetener allows the product to be consumed by people with restrictions on the consumption of regular sugar, such as diabetics. As for product acceptance, both the natural yogurt (without oil) and the enriched one (with oil) were well accepted by the evaluators.

Thus, sunflower oil obtained by SFE is a viable alternative for dairy product enrichment. The goat milk yogurt enriched with sunflower oil obtained by supercritical

technology can be presented as an alternative to the consumption of traditional yogurts with cow milk, with good sensory characteristics and nutritional appeal.