



**NATASHA DANTAS LORENZO**

**DETECTION OF BLENDS AND PREDICTION OF NATURAL  
PIGMENTS IN VEGETABLE OILS USING A LOW-COST  
COLOR SENSOR**

**LAVRAS – MG**

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Agroquímica, concentração em Química/Bioquímica para obtenção do título de Doutor.

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**DETECÇÃO DE MISTURAS E PREDIÇÃO DE PIGMENTOS NATURAIS EM  
ÓLEOS VEGETAIS USANDO UM SENSOR DE COR DE BAIXO CUSTO**

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Agroquímica, concentração em Química/Bioquímica para obtenção do título de Doutor.

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## RESUMO

Neste trabalho foi avaliado o potencial de usar um sensor de cor para identificar diferentes misturas de óleos vegetais e prever níveis de pigmentos naturais em óleo de abacate. Os objetivos foram autenticar o óleo de abacate contra misturas contendo óleos de canola, girassol, milho, azeitona e soja, bem como explorar o uso do sensor de cor para prever o conteúdo total de carotenoides e clorofila em azeites de abacate e oliva, além de sua cor espectrofotométrica total (CET). Os estudos envolveram medições de parâmetros RGB sob dois tipos de iluminação: UV (395 nm) e luz branca. Métodos de classificação, como Análise Discriminante Linear (LDA) e Máquina de Vetores de Suporte por Mínimos Quadrados (LS-SVM), assim como Regressão Linear Múltipla (MLR) e LS-SVM foram empregados para detecção e quantificação das misturas. Para predição de níveis totais de pigmentos foi utilizado MLR e LS-SVM usando diferentes parâmetros de cor (RGB, HSV ou L\*a\*b\*). Em geral, os modelos LS-SVM superaram os de MLR e LDA, demonstrando boa capacidade de predição sem evidência de ajustes aleatórios, tanto para o estudo envolvendo misturas, quanto para o que envolveu pigmentos. No estudo com misturas, o modelo LS-SVM alcançou  $R^2$  superior a 0,9 em todos os casos, inclusive na validação externa. Além disso, usando luz branca, os modelos LS-SVM produziram valores de raiz quadrada do erro-médio (RMSE) variando entre 1,17 e 3,07%, indicando boa acurácia na quantificação das misturas. Tais resultados demonstraram eficácia do sensor de cor na identificação de misturas de outros óleos vegetais em óleo de abacate, sugerindo seu potencial como uma alternativa eficiente e de baixo custo para autenticação de óleo vegetal. No estudo envolvendo o teor de pigmentos, diferentes métodos foram usados para determinar os teores de pigmentos usados como referência para a construção dos modelos. A iluminação UV demonstrou melhorar o desempenho preditivo do conteúdo total de clorofilas quando a resposta é baseada em métodos livres de solvente (métodos IUPAC e AOCS). Nos outros casos, a iluminação branca se mostrou mais apropriada. Dados de cor no sistema HSV foi mais afetivo para prever clorofilas totais quando referenciadas em métodos livres de solvente ( $R^2 > 0,9$  para validação externa). Dados RGB forma mais adequados para prever CET e clorofilas totais referenciados em métodos que utilizam diluição com solvente ( $R^2 > 0,9$  para validação externa). Finalmente, o conteúdo de carotenoides totais foi mais bem predito usando valores L\*a\*b\* como descritores ( $R^2$  de 0,8 para validação externa). Portanto, métodos de baixo custo baseados em um sensor de cor se mostrou uma alternativa promissora para a predição de pigmentos e CET em óleos vegetais, sem a necessidade de pré-tratamento da amostra ou diluição em solventes tóxicos.

**Palavras chaves:** clorofilas; carotenoides; autenticação; quimiometria; colorimetria.

## ABSTRACT

In this study, the potential of using a color sensor to identify different mixtures of vegetable oils and predict levels of natural pigments in avocado oil was evaluated. The objectives were to authenticate avocado oil against blends containing canola, sunflower, corn, olive and soybean oils, as well as to explore the use of color sensing to predict total carotenoid and chlorophyll content in avocado and olive oils, in addition to their total spectrophotometric color (TSC). The study involved RGB interference under two types of lighting: UV (395 nm) and white light. Classification methods such as Linear Discriminant Analysis (LDA) and Least Squares Support Vector Machine (LS-SVM), as well as Multiple Linear Regression (MLR) and LS-SVM were used for detection and quantification of mixtures. To predict total pigment levels, MLR and LS-SVM were employed using different color parameters (RGB, HSV or L\*a\*b\*). In general, the LS-SVM models outperformed those of MLR and LDA, demonstrating good prediction capacity without evidence of random adjustments, both for the study involving mixtures and for those involving pigments. In the study with mixtures, the LS-SVM model achieved R<sup>2</sup> greater than 0.9 in all cases, including external validation. Furthermore, using white light, the LS-SVM models produced root mean square error (RMSE) values varying between 1.17 and 3.07%, indicating good accuracy in quantifying mixtures. Such results were demonstrated by the color sensor in identifying mixtures of other vegetable oils in avocado oil, showing its potential as an efficient and low-cost alternative for vegetable oil authentication. In the study involving pigment content, different methods were used to determine the pigment content used as a reference for building the models. UV illumination has been shown to improve the predictive performance of total chlorophyll content when the response is based on solvent-free methods (IUPAC and AOCS methods). In other cases, white lighting proved more effective. Color data in the HSV system were more effective in predicting total chlorophylls when referenced to solvent-free methods (R<sup>2</sup> > 0.9 for external validation). RGB data is the most appropriate way to predict TSC and total chlorophylls referenced in methods that use solvent dilution (R<sup>2</sup> > 0.9 for external validation). Finally, total carotenoid content was best predicted using L\*a\*b\* values as descriptors (R<sup>2</sup> of 0.8 for external validation). Therefore, low-cost methods based on a color sensor have proven to be a promising alternative for predicting pigments and TSC in vegetable oils, without the need for sample pretreatment or dilution in toxic solvents.

**Keywords:** chlorophylls; carotenoids; authentication; chemometric; colorimetry.

## **INDICADORES DE IMPACTO**

A regulamentação de misturas de azeite e óleos virgem é crucial para garantir a integridade e a qualidade do produto final. Conforme a instrução normativa nº49, de 22 de dezembro de 2006, óleo composto são produtos obtidos através da mistura de óleos e gorduras vegetais sendo que, também podem ter adição de outros ingredientes com finalidade de fornecer sabor, desde que não descaracterize o produto e estas adições devem estar claramente rotuladas na embalagem do óleo ou azeite. Por outro lado, devido ao seu alto valor, os azeites são um dos alimentos mais fraudados do mundo. Em alguns casos ocorrem até mesmo adulterações com adição de óleos impróprios para consumo, o que pode prejudicar a reputação do setor produtivo e a saúde do consumidor. Uma possível forma de detectar essas adulterações ou misturas é por meio da utilização de técnicas analíticas como espectroscopia, cromatografia gasosa e análise de fluorescência em conjunto com ferramentas quimiométricas para identificar possíveis frações indesejadas. No entanto, muitas dessas técnicas exigem alto conhecimento específico, alto custo de operação e manutenção, além de elevado tempo de operação. Além disso a análise no local é muitas vezes excluída devido à natureza estacionária da maioria dos equipamentos. Todos os aspectos mencionados estão de acordo com as perspectivas de inovação de produção responsável e bem-estar do consumidor. A viabilidade do uso de um sensor de cor de baixo custo para a detecção de blends de azeite de oliva e óleo de abacate foram satisfatórios. Além de o sensor ser de baixo custo (custo aproximado de 250,00 reais) em comparação a outros equipamentos de laboratórios, também foi possível utilizá-lo para determinar pigmentos como clorofila e carotenoides nos azeites, o que útil para avaliar a qualidade de produto. É possível utilizar o equipamento para fazer análises in loco, sendo uma alternativa inovadora para uso na fiscalização, contribuindo para a proteção do consumidor contra práticas fraudulentas envolvendo este tipo de produto.

## **IMPACT INDICATORS**

The regulation of olive oil and virgin oil blends is crucial to ensure the integrity and quality of the final product. According to normative instruction no. 49, of December 22, 2006, compound oils are products obtained through a mixture of vegetable oils and fats, which may also have the addition of other ingredients for the purpose of providing flavor, if it does not distort the product's characteristics, and these additions must be clearly labeled on the oil or olive oil packaging. On the other hand, due to their high value, olive oil is one of the most fraudulent foods in the world. In some cases, adulteration even occurs with the addition of oils unsuitable for consumption, which can harm the reputation of the production sector and the health of the consumer. A possible way to detect these adulterations or mixtures is using analytical techniques such as spectroscopy, gas chromatography and fluorescence analysis in conjunction with chemometric tools to identify possible unwanted fractions. However, many of these techniques require high specific knowledge, high operation, and maintenance costs, in addition to long operating times. Furthermore, on-site analysis is often excluded due to the stationary nature of most equipment. All aspects mentioned are in line with the innovation perspectives of responsible production and consumer well-being. The feasibility of using a low-cost color sensor for detecting blends of olive oil and avocado oil was satisfactory. In addition to the sensor being low cost (approximate cost of 250.00 reais) compared to other laboratory equipment, it was also possible to use it to determine pigments such as chlorophyll and carotenoids in olive oils, which is useful for evaluating product quality. It is possible to use the



equipment to carry out on-site analyses, being an innovative alternative for use in inspections, contributing to consumer protection against fraudulent practices involving this type of product.

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## **PRIMEIRA PARTE**

## 1 INTRODUÇÃO

Assim como o azeite de oliva, o óleo de abacate é reconhecido por sua composição saudável, especialmente uma elevada proporção de ácidos graxos monoinsaturados, como o ácido oleico, e outros ácidos graxos poli-insaturados, como o ácido linoleico (FLORES et al., 2019; PÉREZ-CALABUIG et al., 2023; TAN, 2019). Quanto aos compostos minoritários, que representam menos de 2% da composição, destacam-se os compostos fenólicos. Esses compostos têm demonstrado potencial como agentes antioxidantes, anti-inflamatórios e antimicrobianos (JIMENEZ-LOPEZ et al., 2020). O óleo de abacate também possui alta concentração de fitosteróis (FLORES et al., 2019; PÉREZ-CALABUIG et al., 2023; TAN, 2019). A presença de fitosteróis em alimentos está intrinsecamente associada à promoção da redução de doenças da pele e dos olhos, bem como à redução de problemas cardiovasculares, têm atividade antioxidante e antimutagênica e também atua na prevenção de doenças degenerativas (FERRUZZI; BLAKESLEE, 2007).

Devido à concentração de pigmentos presentes em óleos e azeites, a cor desempenha um papel importante nos padrões de qualidade comercial principalmente para azeites extra virgens, uma vez que eles possuem um padrão visual próprio baseado na cor verde/amarelo (DEKHILI; SIRIEIX; COHEN, 2011; GILA et al., 2023; ILAK PERŠURIĆ, 2020). Este atributo visual pode influenciar na qualidade dos azeites virgens, pois é resultado da quantidade e composição dos pigmentos naturais (JIMENEZ-LOPEZ et al., 2020). A clorofila e os carotenoides têm papel importante nos azeites como pigmentos naturais que promovem estabilidade oxidativa e qualidade funcional em óleos vegetais (DE CARVALHO; NUNES, 2021; TAN, 2019). As clorofilas são os principais pigmentos que afetam a cor dos óleos virgens e são responsáveis pela cor esverdeada dos óleos vegetais. Os carotenoides atuam como antioxidantes naturais para inibir as reações em cadeia dos radicais livres e são responsáveis pela pigmentação amarela, vermelha e laranja nos óleos (PÉREZ-GÁLVEZ; VIERA; ROCA, 2020; TAN, 2019; WONG et al., 2008).

A mistura de óleos vegetais é aceitável se for devidamente rotulada, garantindo a conformidade com a legislação e facilitando, ao mesmo tempo, o escrutínio para validar a exatidão das informações do rótulo. No entanto, apesar da confiabilidade analítica dos métodos utilizados para avaliar a composição de óleos vegetais, especialmente cromatografia e espectroscopia (BORELLO; RONCUCCI; DOMENICI, 2021; RODRIGUEZ-SAONA et al.,

2020), eles são caros, necessitam de analistas altamente treinados e equipamentos especializados. Além disso, alguns métodos são demorados, requerem etapas preparatórias e reagentes, além de gerarem resíduos químicos (HAKONEN; BEVES, 2018). Além disso, a análise no local é muitas vezes excluída devido à natureza estacionária da maioria dos equipamentos. Assim, alternativas para a procedimentos analíticos dispendiosos estão sendo cada vez mais estudadas. Entre as alternativas mais acessíveis estão as câmeras de smartphones e os sensores colorimétricos (DE CARVALHO; NUNES, 2021; HAKONEN; BEVES, 2018; SONG et al., 2020a).

Portanto, o objetivo deste estudo foi avaliar a aplicabilidade de um sensor de cor de baixo custo em combinação com ferramentas quimiométricas para analisar óleo de abacate visando detectar misturas de outros óleos vegetais e determinar pigmentos naturais. Na primeira parte experimental o sensor foi testado quando à capacidade de detectar e quantificar misturas de óleos de canola, milho, soja, oliva e girassol em óleo de abacate. Na segunda parte foi avaliada sua capacidade preditiva dos níveis de carotenoides e clorofila em óleos de abacate e suas misturas com outros óleos vegetais, além da cor espectrofotométrica total.

## **2 REFERENCIAL TEÓRICO**

### **2.1 Misturas e fraudes em óleos comestíveis**

A regulamentação das misturas em azeite virgem é crucial para garantir a integridade e a qualidade do produto final. Conforme a Instrução Normativa nº 49, de 22 de dezembro de 2006, óleo composto são produtos obtidos através da mistura de óleos e gorduras vegetais, sendo que, também podem ter adição de outros ingredientes com finalidade de fornecer sabor, desde que não descaracterize o produto como óleo ou gordura. As misturas legais podem envolver diferentes variedades de azeites virgens, desde que estejam claramente rotuladas e em conformidade com as regulamentações vigentes (BRASIL, 2006). No entanto, é importante ressaltar que a adulteração deliberada ou não autorizada de azeites virgens com outros óleos é proibida e considerada uma prática ilegal e antiética. Tais misturas ilícitas comprometem a qualidade e a autenticidade do azeite virgem, prejudicando a confiança do consumidor e a reputação do setor (BORELLO; RONCUCCI; DOMENICI, 2021; RODRIGUEZ-SAONA et al., 2020).

Há uma possibilidade de que essa tendência aumente nos próximos anos, especialmente devido à diminuição da produção de azeite em Andaluzia, na Espanha, responsável por 45% da oferta global de azeite (GRATSEA et al., 2022). Com a escassez de azeite decorrente de secas e ondas de calor, há um aumento monetário de 112% desde o ano passado. Diante desses desafios na produção e exportação de azeites, países como México, Brasil, Chile e Equador estão investindo ainda mais na produção de óleo de abacate (ARENAS-CASTRO et al., 2020; GRATSEA et al., 2022). Esse aumento de produção está relacionado principalmente aos benefícios nutricionais e de saúde semelhantes aos do azeite de oliva, bem como à possibilidade de rotacionar a produção durante o período de entressafra e manter um preço justo nos azeites (CANAL RURAL, 2024).

Com isso, há possibilidade de um problema futuro relacionado à mistura de óleo de abacate, como ocorre com o azeite de oliva, a mistura com outros óleos comestíveis, como a adição de óleo de soja. Uma possível forma de detectar essa adulteração ou mistura é por meio da verificação da rotulagem e da utilização de técnicas de autenticação, como espectroscopia, GC-MS e análise de fluorescência, em conjunto com ferramentas quimiométricas para identificar possíveis frações indesejadas. No entanto, muitas dessas técnicas exigem alto conhecimento específico, alto custo, manutenção e tempo de operação (NELIS et al., 2020; RODRIGUEZ-SAONA et al., 2020; ROHMAN et al., 2023; SONG et al., 2020a).

Um método de adulteração mais comumente utilizado é através de adição de algum outro óleo vegetal refinado, como exemplo, o óleo de soja, com isso, notícias sobre azeites fraudados não são incomuns, as quais relatam diferentes tipos de azeites comestíveis e não comestíveis numa matriz alimentar denominada “azeite extra virgem” acontecem periodicamente. A adulteração de alimentos pode levar a situações perigosas, não envolve apenas questões monetárias ou de credibilidade da marca e do mercado, mas pode afetar a saúde do consumidor e em algumas ocasiões a morte, como o escândalo de 2021 no Brasil, onde 24 marcas de azeites foram suspensas por adulteração com diversos tipos de óleos, alguns deles impróprios para consumo (G1, 2021).

É necessário enfatizar que a prevenção da adulteração de alimentos foi uma das sete peças fundamentais da Lei de Modernização da Segurança Alimentar de 2011. Para colocar isso em prática, são necessários testes autênticos de alimentos e detecção de adulterantes em vários produtos alimentícios para garantir a proteção do consumidor contra atividades fraudulentas

(PÉREZ-GÁLVEZ; VIERA; ROCA, 2020). A legislação, multas e penas de prisão são utilizadas para servir como dissuasores contra a adulteração de alimentos.

## 2.2 Pigmentos em óleos vegetais

A cor dos azeites virgens é a primeira característica percebida pelos consumidores e exerce forte influência em sua decisão e comportamento de compra (DE CARVALHO; NUNES, 2021; GILA et al., 2023). No entanto, esse atributo visual não impacta significativamente na qualidade dos azeites e em suas demais propriedades organolépticas. Na verdade, a cor dos azeites não é considerada um padrão de qualidade na legislação aplicável atualmente, como no Conselho Oleícola Internacional e nas normas da Comissão Europeia, uma vez que a simples percepção da cor dos azeites pode distorcer fortemente as avaliações de outras propriedades do azeite (GILA et al, 2023).

A cor em verde-amarelo em óleos virgem de origem vegetal é principalmente devida a dois tipos de pigmentos naturais: carotenoides e clorofilas. Os pigmentos carotenoides conferem o amarelo, enquanto os pigmentos das clorofilas proporcionam o verde (BORELLO; RONCUCCI; DOMENICI, 2021; JIMENEZ-LOPEZ et al., 2020; PÉREZ-GÁLVEZ; VIERA; ROCA, 2020).

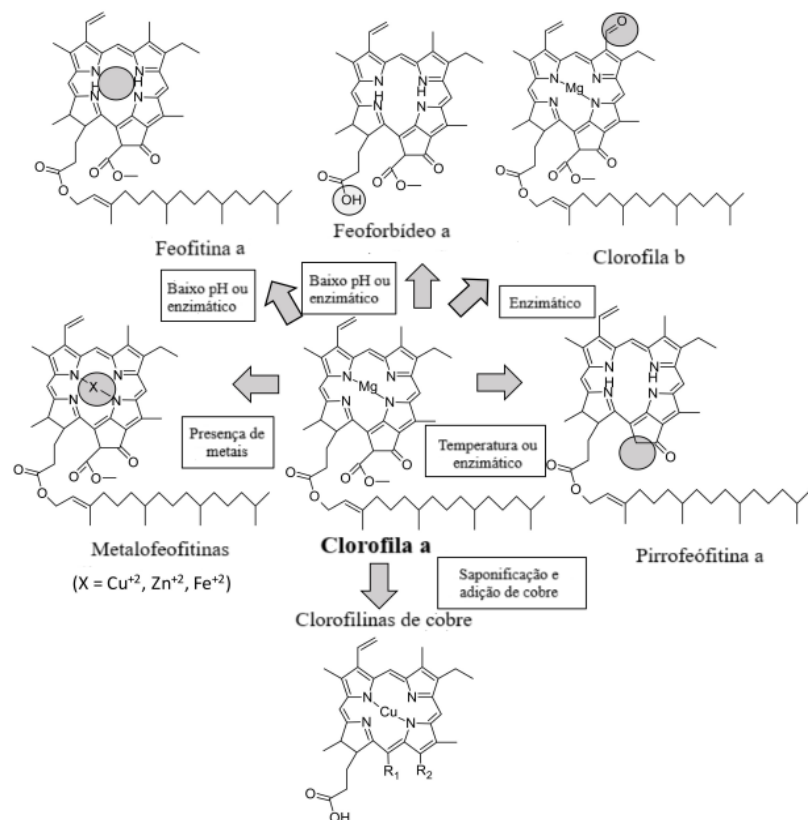
O papel dos pigmentos está principalmente relacionado ao fato de que eles determinam a cor distintiva do azeite, assim, como sua vida de prateleira. Sua composição química relativa varia durante a vida do azeite extravirgem e depende de muitos fatores, como o tipo de cultivar, as condições climáticas/ambientais, o estado de maturação do fruto no momento da colheita, o método de amostragem das azeitonas, o método de produção do azeite e as condições de armazenamento do produto final (JIMENEZ-LOPEZ et al., 2020; PÉREZ-GÁLVEZ; VIERA; ROCA, 2020).

Os pigmentos podem ser divididos em duas classes principais: carotenoides e clorofilas com seus derivados. Devido à sua natureza lipofílica, esses pigmentos são transferidos das membranas tilacoides das células da azeitona para a fase oleosa (BORELLO; RONCUCCI; DOMENICI, 2021). Os azeites contêm uma variedade relativamente rica de carotenoides (como  $\beta$ -caroteno, luteína, violaxantina, neo xantina e outras xantofilas em percentagens menores) e clorofilas com seus derivados (como clorofilas a e feofitinas a, clorofilas b e feofitinas b, e outros derivados menores) (BORELLO; RONCUCCI; DOMENICI, 2021; CERVANTES-

PAZ; YAHIA, 2021; JIMENEZ-LOPEZ et al., 2020; PÉREZ-GÁLVEZ; VIERA; ROCA, 2020).

A estrutura dos pigmentos da clorofila é responsável pela absorção das ondas eletromagnéticas na região visível do espectro dos azeites. A clorofila a tem uma cor azul esverdeada, enquanto a clorofila b é verde-amarelado. No entanto, as clorofilas são compostas muito instáveis no ambiente naturalmente ácido do óleo e, portanto, passam rapidamente por processos de transformação. Apenas em "óleos frescos", o teor de clorofila ainda é elevado (BORELLO; RONCUCCI; DOMENICI, 2021; PÉREZ GÁLVEZ; VIERA; ROCA, 2020). Na Figura 1, estão representadas a Clorofila a e outras estruturas formadas a partir de diferentes reações físico-químicas.

**Figura 1** - Fórmulas estruturais de clorofilas formada por diferentes reações físico-químicas.



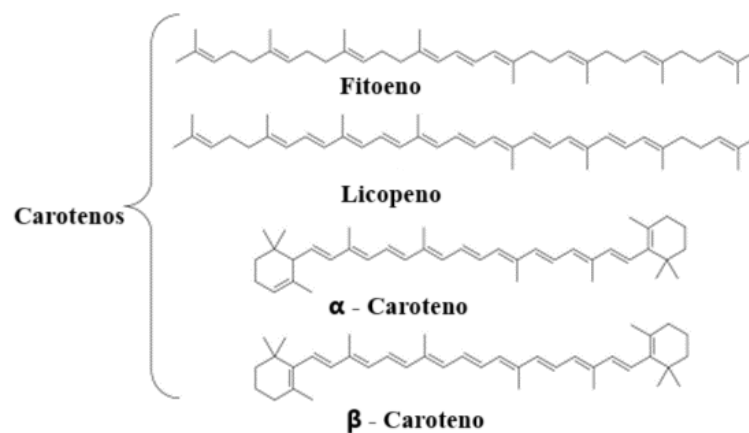
Fonte: Adaptado de Pérez-Gálvez, Roca (2020).

Os carotenoides são divididos em duas classes distintas de compostos: carotenos e xantofilas. Alguns deles são precursores da vitamina A e sua ingestão, por meio da nutrição,



promove a redução de doenças de pele e olhos, bem como a redução de problemas cardiovasculares, como a oxidação do colesterol de baixa densidade (LDL) (BORELLO; RONCUCCI; DOMENICI, 2021; PÉREZ-GÁLVEZ; VIERA; ROCA, 2020). Por esta razão, o interesse em desenvolver novos métodos para identificar e quantificar pigmentos é benéfico para a saúde humana. Na Figura 2 estão representadas as diferentes estruturas mais conhecidas do grupo de carotenoides.

**Figura 2** - Diferentes estruturas do grupo de carotenoides mais conhecidos.



Fonte: Adaptado de Pérez-Gálvez, Roca (2020).

A clorofila e os carotenoides têm papel importante nos azeites virgens como pigmentos naturais para promover estabilidade oxidativa e qualidade funcional em óleos vegetais (DE CARVALHO; NUNES, 2021; TAN, 2019). Enquanto as clorofilas afetam a cor o “frescor” de óleos e azeites, os carotenoides atuam como antioxidantes naturais para inibir reações em cadeia de radicais livres e são responsáveis pelos pigmentos amarelo, vermelho e laranja nos óleos (PÉREZ-GÁLVEZ; VIERA; ROCA, 2020; TAN, 2019; WONG et al., 2008). A presença de carotenoides está associada à promoção da redução de doenças da pele e dos olhos, bem como à redução de problemas cardiovasculares, como a oxidação do colesterol de baixa densidade (LDL). Por outro lado, as clorofilas têm atividade antioxidante e antimutagênica, também prevenindo doenças degenerativas (FERRUZZI; BLAKESLEE, 2007).

### 2.3 Sensores colorimétricos na análise de óleos e gorduras

Muitos métodos tradicionais, geralmente com alto custo, demandam mão de obra e tempo para determinação de pigmentos naturais em misturas de óleos vegetais, tais como espectroscopia e cromatografia (BORELLO; RONCUCCI; DOMENICI, 2021; JIMÉNEZ-SOTELO et al., 2016). Apesar da robustez e confiabilidade dos resultados gerados por espectroscopia e cromatografia (BORELLO; RONCUCCI; DOMENICI, 2021; RODRIGUEZ-SAONA et al., 2020), para se obter tal feito, muitas das vezes se é necessário utilizar métodos dispendiosos, além da necessidade de analistas altamente treinados para realização de análises específicas e na operação desses equipamentos, assim como, de manutenções periódicas para manter o seu bom funcionamento e a constância de resultados confiáveis. Além disso, alguns métodos são demorados, requerem etapas preparatórias e reagentes, além de gerarem resíduos químicos, consomem muita energia (HAKONEN; BEVES, 2018) e há inviabilidade em análises no local devido à natureza estacionária da maioria dos equipamentos. Portanto, a indústria agroalimentar exige equipamentos de fácil utilização, econômicos, portáteis, não destrutivos, potentes, rápidos e aplicáveis em linha para garantir a qualidade e segurança dos alimentos.

O desenvolvimento de potenciais equipamentos portáteis permite a execução de protocolos de química analítica demorados e complicados *in loco*, com resultados em pequeno intervalo de tempo, sem a necessidade de equipamentos caros ou altos níveis de especialização. A produção de novos sistemas microeletromecânicos, lasers de estado sólido, componentes ópticos, seletores de comprimento de onda e detectores que podem ser resfriados termo eletricamente ao ar permitiram a miniaturização de espectrômetros com desempenho espectral comparável aos seus equivalentes de laboratório de bancada. Essas novas capacidades posicionaram a espectroscopia vibracional (NIR, infravermelho médio e Raman) na vanguarda tecnológica para controle de qualidade no seu lugar de origem, autenticação e prevenção da segurança alimentar de produtos alimentícios (NELIS et al, 2020; RODRIGUEZ-SAONA et al, 2020).

Com este ponto de partida, o desenvolvimento de sensores de baixo custo que possam fornecer leituras semelhantes às de um espectrômetro começou a ser explorado em laboratórios de pesquisa. Uma das aplicações para comprovar a eficácia desses sensores está na sua utilização em autenticação e controle de adulteração de azeites e outras matrizes alimentícias.

A abundância de pesquisas utilizando material de azeite pode estar relacionada aos métodos exigidos pela Comissão da União Europeia, pelo Conselho Oleícola Internacional e pelo Comitê do Códex sobre Gorduras e Azeites, que estão lidando com a regulamentação e supervisão do azeite extravirgem (RODRIGUEZ- SAONA et al, 2020).

Além disso, a crescente atenção da sociedade para a segurança alimentar e proteção do meio ambiente tem levado ao desenvolvimento de novas formas de detectar adulteração em alimentos utilizando instrumentos tecnológicos diários para identificar ou verificar algumas propriedades de determinados tipos de alimentos, sem conhecimento profundo neste assunto (BI et al., 2019). Outro problema que poderia ser resolvido com esse tipo de método é que a indústria pode lançar seus produtos no mercado sem demora.

A demanda por ferramentas analíticas de ponta impulsionou o uso de instrumentos portáteis para espectroscopia vibracional, incluindo a espectroscopia infravermelha, para a vanguarda da avaliação de qualidade no local, autenticação de alimentos e garantia de segurança (NELIS et al., 2020; RODRIGUEZ-SAONA et al., 2020). Alternativas para evitar procedimentos caros estão sendo estudadas, e as mais acessíveis encontradas são a câmera de smartphone e sensores colorimétricos (DE CARVALHO; NUNES, 2021; HAKONEN; BEVES, 2018; SONG et al., 2020). O desenvolvimento de dispositivos portáteis facilitou análises no local sem a necessidade de equipamentos caros. Da mesma forma, dispositivos portáteis alternativos têm sido investigados para análise de alimentos, como câmeras (ANCONI; BRITO; NUNES, 2022; HAKONEN; BEVES, 2018; RESENDE; MAGALHÃES; NUNES, 2023; SONG et al., 2020a, 2020b) e sensores ópticos (ARSLAN et al., 2021; RODRIGUEZ-SAONA et al., 2020). Sensores ópticos ou colorimétricos têm sido empregados para avaliar a qualidade de óleos e gorduras comestíveis por meio da aplicação de abordagens quimiométricas ou de aprendizado de máquina.

Carvalho e Nunes (2021) propuseram um método baseado em smartphone para determinar os teores de clorofila e carotenoides em óleos de oliva e abacate, mas um método laborioso de transferência de calibração foi necessário para mitigar erros causados por efeitos de luz e diferenças nas câmeras de smartphones. Portanto, sensores portáteis parecem ser uma alternativa mais atraente do que smartphones, uma vez que estes últimos são muito dependentes das condições analíticas envolvidas no desenvolvimento do método e em seu uso (DE CARVALHO et al., 2023). Assim, a necessidade urgente de desenvolver um protótipo pequeno e portátil ou tecnologia pré-existente que possa utilizar um sistema RGB ou outro método que

forneça resultados rápidos é importante na área de autenticação de alimentos, especialmente a adulteração de azeites.

Bi et al (2019) desenvolveram um espectrômetro portátil de detecção de fluorescência LED ultravioleta em miniatura. O espectrômetro de fluorescência portátil era composto de quatro partes: uma fonte de luz de diodo emissor de luz UV (LED), um sistema dispersivo de luz, um sistema de detecção e um sistema de processamento de dados. Um LED de 365 nm foi escolhido como fonte de luz UV. Para testar o desempenho do espectrômetro, foram medidos os espectros de fluorescência de óleo comestível, plantas e papel.

Lastra-Mejias et al (2019) desenvolveram um equipamento para monitorar as condições de armazenamento e localizar adulterações de azeite extravirgem, que consiste em um sensor de fluorescência formado por uma estrutura física que contém a amostra, uma fonte de luz e um fotodetector de baixo custo. Além disso, dois tipos de fontes de luz foram testados de forma independente: diodos emissores de luz (LEDs) e diodos laser. O sistema foi projetado para utilizar o mesmo equipamento para ambas as fontes de luz (LASTRA-MEJIAS et al., 2019).

Sanaeifar et al (2018) propuseram uma nova abordagem baseada na fusão de espectroscopia dielétrica e visão computacional para a caracterização de índices de qualidade do azeite durante o armazenamento, visando reduzir o tempo de análise, consumo de reagentes, mão de obra e equipamentos de alto custo. Foram extraídas características de cor nos espaços RGB, HSV e  $L^*$ ,  $a^*$ ,  $b^*$ , bem como características dielétricas na faixa de frequência de 40 kHz a 20 MHz para cada amostra de azeite. Após o pré-processamento dos dados, foram desenvolvidos e comparados modelos de classificação e predição. Várias técnicas de aprendizado de máquina foram investigadas para classificação de tempo de armazenamento e previsão de índices de qualidade, incluindo rede neural artificial (RNA), máquina de vetores de suporte (SVM), rede bayesiana (BN) e regressão linear múltipla (MLR) (SANAEIFAR; JAFARI; GOLMAKANI, 2018).

Li et al (2018) utilizaram um espectrômetro Raman portátil para coletar os dados espectrais. O objetivo era detectar a adulteração do azeite com óleo alimentar residual (LI et al., 2018). Outro trabalho que utilizou dispositivo portátil disponível no mercado foi MacArthur et al (2020); eles usaram um espectrômetro NIR portátil em uma faixa de espectro de 740 nm a 1070 nm, com resolução de 1 nm, para gravação de espectros de dados. Este estudo teve como objetivo prever a adulteração do óleo de palma (MACARTHUR; TEYE; DARKWA, 2020).

Os métodos de sensores ópticos de cor portáteis têm se destacado como uma abordagem inovadora na análise de parâmetros de cor como resposta analítica. Esses sensores, como descrito por Liu et al. (2019), operam com base na detecção e interpretação das propriedades espectrais da luz refletida ou transmitida por uma amostra. A cor, um atributo visual perceptível, pode ser quantificada e relacionada a características específicas de materiais, substâncias ou ambientes (LIU et al., 2019). A capacidade dos sensores ópticos portáteis de capturar informações precisas sobre a cor tem aplicações significativas em uma variedade de setores, desde a avaliação da qualidade de alimentos e bebidas até a monitorização ambiental. Por exemplo, conforme evidenciado por Zhang et al. (2020), esses sensores são usados para identificar mudanças sutis na cor de óleos comestíveis, indicando sua frescura e estabilidade oxidativa. Além disso, eles são empregados na detecção de impurezas em produtos químicos e na caracterização de materiais em processos industriais. A portabilidade e a capacidade de fornecer resultados rápidos e precisos tornam os sensores ópticos de cor uma ferramenta valiosa para profissionais em diversas áreas, contribuindo para uma análise mais eficiente e acessível de parâmetros essenciais (ZHANG et al., 2018).

## **2.4 Operacionalização de métodos analíticos baseados em sensores colorimétricos**

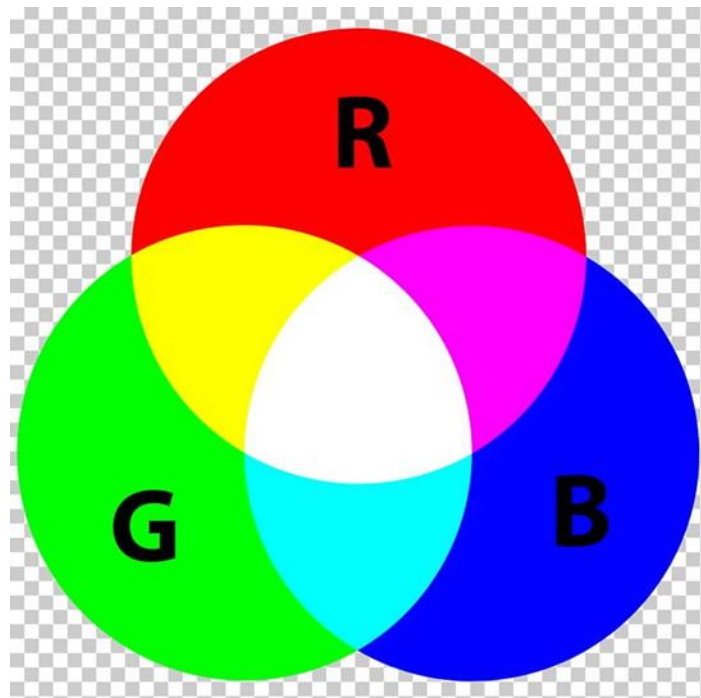
Em geral, os métodos analíticos baseados em sensores colorimétricos usam a resposta de saída do sensor para determinar ou prever alguma propriedade de interesse na amostra. A resposta de saída é comumente constituída das intensidades de parâmetros de algum sistema de cor, os quais são calibrados contra a propriedade a ser predita usando diferentes métodos lineares ou não lineares.

Os sistemas de cor desempenham um papel fundamental na análise de imagens e na interpretação de dados obtidos por sensores, influenciando diretamente o desempenho dos modelos preditivos. A escolha do sistema de cor a ser utilizado como descritor pode ter um impacto significativo na acurácia e na robustez dos modelos desenvolvidos. Como discutido por diversos estudos (ANCONI; BRITO; NUNES, 2022; DE CARVALHO; NUNES, 2021; RESENDE; MAGALHÃES; NUNES, 2023), diferentes sistemas de cor, como  $L^*a^*b^*$  e RGB, oferecem abordagens distintas para representar e quantificar a cor. Por exemplo, o espaço de cor  $L^*a^*b^*$  é conhecido por abranger toda a gama da percepção humana de cores, fornecendo informações detalhadas sobre luminosidade e componentes cromáticos. Por outro lado, o sistema RGB é baseado em um modelo aditivo que combina as cores primárias para criar outras

cores. Portanto, compreender as nuances e implicações de diferentes sistemas de cor é essencial para desenvolver modelos preditivos eficazes e robustos em uma variedade de contextos e aplicações.

As imagens digitais são geralmente baseadas no sistema de cores vermelho-verde-azul (RGB, do inglês red-green-blue), onde cada canal de cor possui uma escala de 8 bits. Portanto, cada pixel pode assumir uma das 256 possibilidades de valores de intensidade (0–255). A mistura entre essas três cores gera todas as outras cores, que podem ser percebidas por uma região do espectro visível. Histogramas de cores são frequentemente utilizados como fonte de informações em imagens RGB. Esse histograma descreve a distribuição estatística dos pixels em função do componente de cor. Um aspecto essencial dos histogramas de cores podem ser utilizados como dados de entrada para a análise multivariada, uma vez que possuem uma estrutura de dados unidimensional semelhante a um espectro (COSTA et al., 2020). Na Figura 3, está representado o diagrama do sistema RGB.

**Figura 3** - Diagrama representativo do sistema RGB.

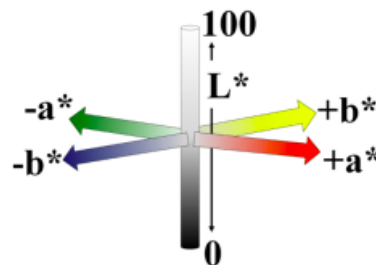


Fonte: Phuangsaikai et al, 2021.

O sistema  $L^*a^*b^*$  é um espaço de cores tridimensional composto por três eixos. Eixo  $L^*$  é uma escala de cinza que varia de 0 (preto) a 100 (branco), correlacionando-se com o nível de luminosidade. Eixo  $a^*$  representa o espectro de vermelho/verde, enquanto o eixo  $b^*$

representa o espectro de amarelo/azul (LY et al., 2020). Este é um sistema de cores que replica a percepção humana das cores na imagem (SHARIFZADEH et al., 2014), o qual descreve as cores usando três componentes. O primeiro componente é a luminosidade da cor ( $L^*$ ), enquanto os outros dois são os componentes cromáticos ( $a^*$  e  $b^*$ ). Este sistema de cores é semelhante ao HSV, onde a intensidade é separada da cromaticidade. Como as mudanças de tonalidade são lineares, as diferenças de cromaticidade podem ser calculadas usando a distância euclidiana. Portanto, o CIELAB é considerado um modelo de cores independente de dispositivo (PHUANGSAIJAI; JAKMUNEE; KITTIWACHANA, 2021). Na Figura 4, está representado o diagrama do sistema CIELAB.

**Figura 4** - Diagrama representativo do sistema CIELAB.

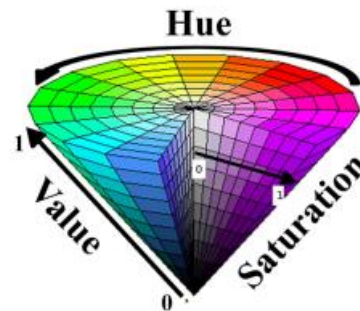


Fonte: Phuangsaikai et al, 2021.

Matiz (H), Saturação (S) e Valor (V) ou HSV formam um espaço de cores cilíndrico comumente utilizado em imagens digitais. No modelo HSV, o matiz (H) corresponde ao tom de cor "espectral", representado como valores angulares de 0 a 1 (parte circular do cilindro). A saturação (S) representa a diferença máxima entre os canais RGB (raio do cilindro), enquanto o valor (V, às vezes referido como brilho, B) é o valor máximo do canal (HAKONEN; BEVES, 2018).

O modelo de cores HSV descreve o espectro de cores como um modelo multidimensional composto por três fatores diferentes: matiz, saturação e valor. O maior diferencial neste sistema é a saturação ou croma, onde 100% de saturação representa a cor mais pura possível. Com menos saturação, a cor aparecerá mais acinzentada. Já o valor varia de 0, que indica preto absoluto, a 1, que representa 100% de brilho sem mistura com preto (PHUANGSAIJAI; JAKMUNEE; KITTIWACHANA, 2021). Na Figura 5, está representado o diagrama do sistema HSV.

**Figura 5** - Diagrama representativo do sistema HSV.



Fonte: Phuangsaikai et al, 2021.

Os sistemas de cor desempenham um papel fundamental na análise de imagens e na interpretação de dados obtidos por sensores, influenciando diretamente o desempenho dos modelos preditivos. Tendo isso em vista, é importante avaliar qual o melhor sistema de cor que se enquadra ao sensor colorimétrico em óleos vegetais. Alguns pesquisadores se utilizaram do sistema RGB, como o caso de Resende et al (2023), Song et al (2020) e Hakonen e Beves (2018); HSV em Hakonen e Beves (2018) e CIELAB como observado em Moyano et al (2008). Contudo, é preferível se utilizar de mais de um sistema de cor para avaliar qual melhor se adequa ao sensor estudado, como o caso de Sanaeifar et al (2018), Anconi et al (2023), de Carvalho et al (2021) que se utilizaram de pelo menos 3 sistemas de cores para validação do método.

Para obter dados confiáveis, é crucial considerar alguns parâmetros. De acordo com Chong et al. (2008), a iluminação desempenha um papel fundamental em aplicações onde o processamento visa operar com base em propriedades intrínsecas, em vez de intensidades observadas sob um iluminante específico (CHONG; GORTLER; ZICKLER, 2008). Além disso, ferramentas quimiométricas são de suma importância na escolha do sistema de cores mais adequado. Entre as diversas possibilidades métodos de calibração lineares e não lineares têm sido usados. Como exemplo, a Regressão Linear Múltipla (MLR) é um método que se apoia na relação linear entre variáveis dependentes e independentes (SIKORSKA et al., 2008). Por outro lado, o Máquina de Vetores de Suporte (LS-SVM) utiliza um algoritmo não linear, sendo mais intensivo computacionalmente, porém com maior capacidade de generalização (AMMARI et al., 2012). Alguns autores ressaltam que técnicas de aprendizado de máquina, como o SVM, complementam a implementação de sensores de baixo custo, superando limitações no design e



na fabricação (LLEÓ et al., 2016). Isso é viável porque o SVM opera como um modelo baseado em otimização, procurando o melhor hiperplano para maximizar margens em um espaço de alta dimensão. Ao contrário da regressão linear, onde o impacto de todos os pontos de dados é uniforme, no SVM, cada ponto de dados influencia a otimização final (SONG et al., 2020b).

### **3 CONSIDERAÇÕES GERAIS**

O uso de sensores ópticos é uma alternativa na área de segurança alimentar para reduzir os custos dos ensaios químicos em comparação com os métodos tradicionais, que geralmente são conduzidos em condições laboratoriais, tornando-os indisponíveis para uso no local ou em procedimentos de fácil operação.

Quando se trata de avaliar a qualidade dos óleos, os sensores podem desempenhar um papel crucial na determinação do frescor dos óleos vegetais. A partir da cor, é possível avaliar o teor de oxidação, identificar impurezas e contaminação, além de controlar processos industriais para monitorar continuamente a qualidade dos óleos utilizados em diversos contextos, como frituras em restaurantes.

Os sensores ópticos portáteis também têm um enorme potencial para revolucionar o atual sistema de segurança alimentar. Até mesmo fiscais sem conhecimento especializado podem utilizar esse tipo de ferramenta, auxiliando na vigilância da qualidade e na detecção de adulterações em alimentos.

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**SEGUNDA PARTE – ARTIGOS**

**ARTIGO 1**

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**FEASIBILITY OF USING A CHEAP COLOUR SENSOR TO DETECT BLENDS OF VEGETABLE OILS IN AVOCADO OIL.**

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**Abstract:** This proof-of-concept study explored the use of an RGB colour sensor to identify different blends of vegetable oils in avocado oil. The main aim of this work was to distinguish avocado oil from its blends with canola, sunflower, corn, olive and soybean oils. The study involved RGB measurements conducted using two different light sources: UV (395 nm) and white light. Classification methods, such as Linear Discriminant Analysis (LDA) and Least Squares Support Vector Machine (LS-SVM), were employed for detecting the blends. The LS-SVM model exhibited superior classification performance under white light with an accuracy exceeding 90%, demonstrating robust prediction capability without evidence of random adjustments. A quantitative approach was followed as well, employing Multiple Linear Regression (MLR) and LS-SVM for the quantification of each vegetable oil in the blends. The LS-SVM model consistently achieved good performance ( $R^2 > 0.9$ ) in all examined cases, both for internal and external validation. Additionally, under white light, LS-SVM models yielded root mean square errors (RMSE) between 1.17% - 3.07%, indicating a high level of accuracy in blend prediction. The method proved to be rapid and cost-effective, without a necessity of any sample pretreatment. These findings highlight the feasibility of a cost-effective colour sensor in identifying avocado oil blended with other oils, such as canola, sunflower, corn, olive, and soybean oils, suggesting its potential as a low-cost and efficient alternative for on-site oil analysis.

**Keywords:** colour; classification; RGB; authentication; chemometrics.



## 1. INTRODUCTION

The production of avocado oil has been steadily increasing due to its recognized health benefits, attributed to a high concentration of unsaturated fatty acids, specifically oleic acid. Recent comparative studies have been conducted to provide qualitative and quantitative evidence of the nutritional value of avocado oil in respect to olive oil (FLORES et al., 2019). Avocado oil has been associated with the potential lower risk of chronic degenerative illnesses (CERVANTES-PAZ; YAHIA, 2021). As a result, both avocado and olive oils are esteemed as premium delicacies due to their health benefits, categorizing them as high-quality oils of considerable value. These benefits justify their popularity in the culinary world.

Due to their premium pricing, extra virgin oils are susceptible to fraudulent practices. Notably, avocado oils have been a central point of various studies investigating adulteration, blend quantification, and geographical provenance (NELIS et al., 2020; ROHMAN et al., 2023; SONG et al., 2020a). On the other hand, mixing different oils is acceptable if appropriately labelled, ensuring compliance with legislation and facilitating inspection to validate the accuracy of the label information.

Despite the analytical performance of methods commonly used to assess oil quality, especially chromatographic and spectroscopic methods (ROHMAN et al., 2023), they are costly and necessitate trained analysts and specialized equipment. These analyses are time-intensive, involve many preparatory steps and the use of chemicals, generate chemical waste, and are energy-intensive (SONG et al., 2020a). Additionally, on-site analysis is often impeded due to the stationary nature of these equipments. Therefore, the agro-food industry urges user-friendly, cost-effective, portable, non-destructive, powerful, rapid, and in-line-applicable equipment to ensure food quality and safety.

The demand for cutting-edge analytical tools has propelled the use of portable instruments of vibrational spectroscopy, including infrared spectroscopy, to the forefront of on-site quality evaluation, food authentication, and safety assurance (NELIS et al., 2020; RODRIGUEZ-SAONA et al., 2020). The development of portable devices has facilitated the on-site analyses without requiring costly equipment. Similarly, alternative portable devices have been investigated for food analysis, such as mobile cameras (ANCONI; BRITO; NUNES, 2022; HAKONEN; BEVES, 2018; NELIS et al., 2020; RESENDE; MAGALHÃES; NUNES, 2023; SONG et al., 2020a) and optical sensors (ARSLAN et al., 2021; RODRIGUEZ-SAONA et al., 2020).

Optical or colourimetric sensors have been employed to assess the quality of edible oils and fats through the application of chemometric or machine-learning approaches. Sanjaya et al. (2018) used a device comprising various light-emitting diodes and a light sensor to distinguish palm oil, olive oil, sesame oil, soybean oil, and lard (SANJAYA et al., 2018). Furthermore, a low-cost fluorescence sensor discriminated between olive oils of different quality levels, namely extra virgin olive oil, virgin olive oil, and lampante olive oil (VENTURINI et al., 2021). In another study, a hyphenated photonics sensor detected fraud in extra virgin olive oil with refined and virgin olive oils, olive-pomace olive oils, and other common edible oils (WEESEPOEL et al., 2021). Similarly, a method based on a colourimetric sensor array was used to identify extra virgin olive oil mixed with soybean oil and corn oil (HUANG; WANG; LIU, 2022).

The advancements in technology, specifically in miniaturization and cost reduction, can be leveraged to explore alternatives with potential to be scaled up for quality assurance. In this context, a rapid and inexpensive analytical tool could be useful for assessing the quality of avocado oils, especially for detecting mixtures with cheap oils, even in a screening approach. Therefore, the main objective of this proof-of-concept study was to explore the feasibility of using a low-cost colour sensor to detect the presence of soybean, canola, corn, sunflower, and olive oils in avocado oil. This study focused on evaluating the responsiveness of the sensor output to the presence of blends in avocado oil and its potential to predict their proportion in the blend. Furthermore, the study explored the more suitable modelling approach (linear or nonlinear) and the type of illuminant (white or UV light).

## **2. MATERIAL AND METHODS**

### **2.1. Samples preparation**

Soybean, canola, corn, and sunflower refined oils were purchased from a local market, while virgin avocado oil and extra virgin olive oil were obtained from local producers. All oils were acquired within their expiration dates and stored at a temperature of 5°C until subjected to analysis.

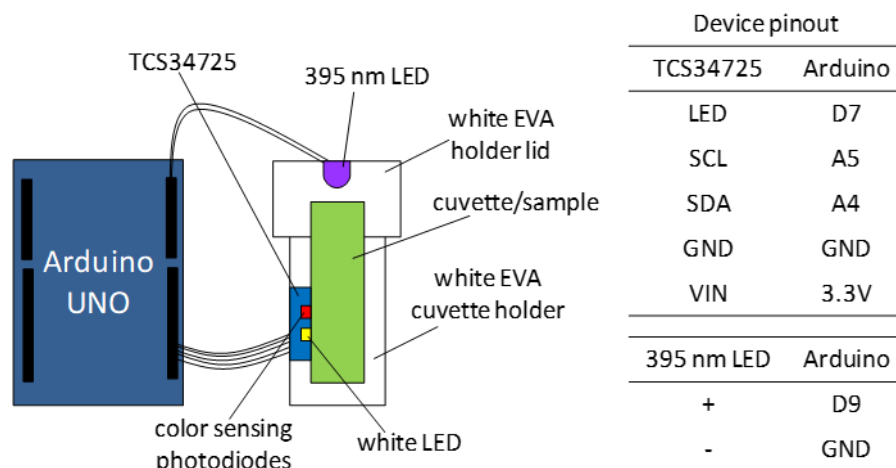
For classification purposes, the pure avocado oil was analyzed in 25 replicates. Additionally, blends of avocado oil combined with 5%, 10%, 20%, 35%, and 50% of each oil (namely soybean, canola, corn, sunflower, and olive oil) were analyzed in 5 replicates, resulting in total 125 blends (5 oils  $\times$  5 concentrations  $\times$  5 replicates).

For calibration purposes, both pure avocado oil and its blends with 5% to 50% in 5% intervals of these oils were analyzed in 5 replicates, resulting in 55 samples for each blend. The blending procedure involved weighing the respective oils in 4 mL glass vials and manually agitating them for approximately 30 seconds. The analyses were conducted at least 4 hours after sample preparation, with no visible bubbles in the oil medium.

## 2.2. Colour sensor Analysis.

A TCS34725 colour sensor (Texas Advanced Optoelectronic Solutions Inc.) was utilized to acquire digital readings of red, green, blue (RGB), and clear light (C) values. This sensor was interfaced with an Arduino Uno, employing an integration time of 24 milliseconds and a gain set at 1x. A sample holder was constructed using white ethylene-vinyl acetate (EVA) material. The samples, contained in 4 mL cuvettes, were analyzed without the use of any solvent. The analysis of the samples occurred under two distinct lighting conditions: ultraviolet light (3V, 395 nm LED) and white light from the sensor's light source (Figure 1). The closed sample holder ensured lighting standardization. Raw readings for red (R), green (G), blue (B), and clear light (C) outputs were acquired by averaging 10 readings. Subsequently, the raw RGB values were normalized by dividing them by the C value, resulting in the R, G, and B values used as descriptors in the models. The sensor's output was captured using Realterm software (version 2.0.0.70, i2cchip).

**Figure 1:** Device used to read colour parameters of oils based on a TCS34725 sensor interfaced with Arduino.



## **2.3. Statistical Analysis**

The data analysis was explored through two main tasks: a supervised classification to discriminate blends of different vegetable oils in avocado oil and a multivariate calibration to predict the proportion of a specific oil blended with avocado oil. Two classification methods were used: Linear Discriminant Analysis (LDA) and Least Squares Support Vector Machine (LS-SVM). LDA is a technique that finds a linear combination of features that characterizes or separates two or more classes, resulting in a linear classifier (IZENMAN, 2008). LS-SVM, widely used in classification and nonlinear function estimation, is a version of SVM that solves linear equations instead of a quadratic programming problem. This overcomes the major drawback of SVM, which is its higher computational burden for constrained optimization programming (HAIFENG WANG; DEJIN HU, 2005). In the same way, both linear and nonlinear methodologies were used for the multivariate calibrations: Multiple Linear Regression (MLR) and LS-SVM.

### **2.3.1. Supervised Classification**

The classification task was executed using two distinct approaches: (i) a binary classification contrasting pure avocado oil against blends of avocado oil mixed with soybean, canola, corn, sunflower, and olive oils; and (ii) a simultaneous six-class classification involving pure avocado oil and each of the five oil blends. Two classification methods were employed for this task: LDA and LS-SVM employing a radial basis function, implemented through the LS-SVMlab version 1.8 toolbox (SUYKENS et al., 2002). All computations were conducted using Octave version 5.2.0 (EATON; BATEMAN; HAUBERG, 2008).

The dataset was partitioned into a calibration set, encompassing 70% of the total samples, and a test set consisting of the remaining 30%, employing the Kennard-Stone algorithm (YUN, 2022).

The robustness of the models was assessed by the y-randomization test, which consists of fixing the X matrix (independent variables) and shuffling the y vector (dependent variables, classes) to obtain new models. It is expected that the predictive performance will decrease as the response is truly related to its predictor, thus validating the relationship between independent and dependent variables.

Model validation parameters encompassed Precision (PRE), Recall (REC), Accuracy (ACU), Error rate (ERR), F1-score (F1S), and Matthews Correlation Coefficient (MCC)

(GRANDINI; BAGLI; VISANI, 2020; SOKOLOVA; LAPALME, 2009) based on the total of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN):

$$PRE = \frac{TP}{TP+FP} \times 100 \quad (1)$$

$$REC = \frac{TP}{TP+FN} \times 100 \quad (2)$$

$$ACU = \frac{TP+TN}{TP+TN+FP+FN} \times 100 \quad (3)$$

$$ERR = \frac{FP+FN}{TP+TN+FP+FN} \times 100 \quad (4)$$

$$F1S = \frac{2TP}{2TP+FP+FN} \times 100 \quad (5)$$

$$MCC = \frac{TP \times TN - FN \times FP}{\sqrt{(TP+FN)(TP+FP)(TN+FN)(TN+FP)}} \quad (6)$$

The MCC is a reliable statistical parameter that produces a high score only if the prediction achieves good results in all four categories of the confusion matrix (true positives, false negatives, true negatives, and false positives), proportionally to both the number of positive and negative elements in the dataset (CHICCO; JURMAN, 2020). The MCC values can range from -1 to +1. A value of +1 indicates a perfect prediction, 0 represents a random prediction, and -1 indicates an inverse prediction.

### 2.3.2. Multivariate Calibration

Two modelling approaches were examined to predict the proportion of soybean, canola, corn, sunflower, and olive oils blended within avocado oil: MLR and LS-SVM. All computations were conducted using Octave version 5.2.0 (EATON; BATEMAN; HAUBERG, 2008).

The dataset was partitioned into a calibration set, encompassing 70% of the total samples, and a test set, comprising the remaining 30%, employing the Kennard-Stone algorithm. The adequacy of model fitting was evaluated using the determination coefficient ( $R^2$ ) and the root mean squared error (RMSE) for calibration, y-randomization, and external validation (test set).

The  $R_m^2$  value was computed (Equation 7) to ensure that the predicted values obtained through external validation not only exhibit a strong correlation with the observed values but also demonstrate congruency. A threshold of 0.5 was adopted as valid (MITRA; SAHA; ROY, 2010).

$$R_m^2 = R^2 \left( 1 - \sqrt{R^2 - R_0^2} \right) \quad (7)$$

Where  $R^2$  and  $R_0^2$  represent the quadratic correlation coefficients between the actual and predicted values, with and without the intercept, respectively.

Furthermore, the robustness of the models was examined via the y-randomization test. The  $cR_p^2$  value was computed, which accounts for the distinction between the y-randomization  $R^2$  ( $R_{rand}^2$ ) and calibration  $R^2$  ( $R_{cal}^2$ ) (Equation 8). A  $cR_p^2 > 0.5$  was established to attest to the absence of overfitting or random adjustment (MITRA; SAHA; ROY, 2010).

$$cR_p^2 = R_{cal}^2 \left( \sqrt{R_{cal}^2 - R_{rand}^2} \right) \quad (8)$$

### 3. RESULTS

#### 3.1. Discriminating between pure and blended avocado oil

The initial approach was a binary assessment for evaluating the discriminative potential between pure avocado oil and its blends with various vegetable oils. To achieve this, blends of 5%, 10%, 20%, 35%, and 50% vegetable oils were designated to constitute the blended class, whereas pure avocado oil was considered as the pure class. The effect of the light source, whether white or UV light, on the classification outcomes was analyzed. Detailed classification results are presented in Table 1.

**Table 1:** Performance parameters for LDA and LS-SVM two-class models to discriminate pure avocado oil from its blends with canola, sunflower, corn, olive, and soybean oils using the TCS34725 colour sensor under white and UV light.

		white light						UV light					
		LDA			LS-SVM			LDA			LS-SVM		
		overall	pure	blended*	overall	pure	blended*	overall	pure	blended*	overall	pure	blended*
calibration	PRE	42.0	0.0	84.0	100.0	100.0	100.0	92.9	100.0	85.9	95.3	92.9	97.7
	REC	50.0	0.0	100.0	100.0	100.0	100.0	53.3	6.7	100.0	92.7	86.7	98.8
	ACU	84.0	84.0	84.0	100.0	100.0	100.0	86.0	86.0	86.0	97.0	97.0	97.0
	ERR	16.0	16.0	16.0	0.0	0.0	0.0	14.0	14.0	14.0	3.0	3.0	3.0
	F1S	0.46	0.00	0.91	1.00	1.00	1.00	0.52	0.13	0.92	0.94	0.90	0.98
	MCC	0.00	0.00	0.00	1.00	1.00	1.00	0.24	0.24	0.24	0.88	0.88	0.88
y-rand.	PRE	42.0	0.0	84.0	51.5	18.2	84.9	52.5	20.0	85.1	59.0	30.0	88.1
	REC	50.0	0.0	100.0	53.0	6.3	99.8	50.2	1.3	99.1	60.3	20.7	100.0
	ACU	84.0	84.0	84.0	84.8	84.8	84.8	84.4	84.4	84.4	88.1	88.1	88.1
	ERR	16.0	16.0	16.0	15.2	15.2	15.2	15.6	15.6	15.6	11.9	11.9	11.9
	F1S	0.46	0.00	0.91	0.50	0.08	0.92	0.47	0.03	0.92	0.57	0.21	0.94
	MCC	0.00	0.00	0.00	0.09	0.09	0.09	0.01	0.01	0.01	0.22	0.22	0.22
test	PRE	41.0	0.0	82.0	100.0	100.0	100.0	40.0	0.0	80.0	82.7	75.0	90.5
	REC	50.0	0.0	100.0	100.0	100.0	100.0	50.0	0.0	100.0	77.5	60.0	95.0
	ACU	82.0	82.0	82.0	100.0	100.0	100.0	80.0	80.0	80.0	88.0	88.0	88.0
	ERR	18.0	18.0	18.0	0.0	0.0	0.0	20.0	20.0	20.0	12.0	12.0	12.0
	F1S	0.45	0.00	0.90	1.00	1.00	1.00	0.44	0.00	0.89	0.80	0.67	0.93
	MCC	0.00	0.00	0.00	1.00	1.00	1.00	0.00	0.00	0.00	0.60	0.60	0.60

PRE: Precision. REC: Recall. ACU: Accuracy. ERR: Error rate. F1S: F1-score. MCC: Matthews correlation coefficient. \*avocado oil blended with 5, 10, 20, 35, or 50 % canola, sunflower, corn, olive, or soybean oils.

The differentiation between the two classes, namely pure and blended avocado oil, was attempted through LDA or LS-SVM models. The LDA models, whether employing white or UV light, exhibited unsatisfactory performance in the classification of pure avocado oil versus blended oil. The results revealed a 100% recall for the blended avocado oil class but produced a recall of 0 for pure avocado oil, both for calibration and test sets. The practical interpretation of these findings indicates that this model exhibited excessive stringency, thereby categorizing all samples, including those of pure avocado oil, as blends.

Conversely, the LS-SVM model yielded praiseworthy results in the binary classification between pure and blended avocado oil (Table 1), achieving flawless classifications (with an accuracy of 100%) under white light. The MCC values of +1 for both calibration and test sets further corroborated these outcomes. Additionally, the low MCC values observed in the y-randomization test indicated the absence of overfitting or random adjustments in the model.

### 3.2. Discriminating between blend types in avocado oil

The next approach aimed to achieve multi-class discrimination between pure avocado oil and avocado oil blended with canola, sunflower, corn, olive, and soybean oils. Similar to the initial approach, discrimination among samples was attempted with LDA or LS-SVM models

under two distinct illuminants (white or UV light). The performance parameters of this approach are presented in Table 2.

The use of white light generally resulted in superior performance compared to the UV light condition across all classes, for both LDA and LS-SVM models. As in the binary classification, the LDA models exhibited inferior performance to the LS-SVM. Specifically, the LDA models had accuracies hovering around 80%; however, the low values of precision, recall, F1-score, and MCC indicated inadequate classification performance across all classes. Moreover, the relatively high accuracy observed in the y-randomization test suggested a potential tendency towards random adjustments. Thus, it can be inferred that the colourimetric data read by the sensor might not have been adequate to linearly describe the differentiation between pure avocado oil and its blends.

On the other hand, the LS-SVM models had superior performance, particularly under white light condition, with a low error rate across all classes. The models exhibited accuracy levels exceeding 90% for most classes in both the calibration and test sets. Furthermore, the elevated values of precision, recall, F1-score, and MCC confirmed the efficacy of this approach, while the poor performance in the y-randomization test, especially with low MCC values, suggested the absence of overfitting or random adjustments (MITRA; SAHA; ROY, 2010).

Lower MCC values were observed for the test set in the cases of canola (MCC = 0.56) and soybean (MCC = 0.78) blends. In these instances, a few samples assumed to be soybean blends were classified as canola oil blends (Table S2, in supplementary material in <https://doi.org/10.3390/foods13040572>). Nonetheless, the model indicated that these samples were not pure avocado oil; rather, they lacked the specific oil blended with the avocado oil.



**Table 2:** Performance parameters for LDA and LS-SVM six-class models to classify pure avocado oil and avocado oil blended with canola, sunflower, corn, olive, and soybean oils using the TCS34725 colour sensor under white and UV light.

		white light													
		LDA							LS-SVM						
		overall	pure	canola	sunflower	corn	olive	soybean	overall	pure	canola	sunflower	corn	olive	soybean
calibration	PRE	62.1	82.4	42.2	100.0	68.2	80.0	0.0	98.5	100.0	90.9	100.0	100.0	100.0	100.0
	REC	56.8	87.5	95.0	6.7	88.2	63.2	0.0	97.8	100.0	100.0	100.0	100.0	94.7	92.3
	ACU	87.0	95.0	73.0	86.0	91.0	90.0	87.0	99.3	100.0	98.0	100.0	100.0	99.0	99.0
	ERR	13.0	5.0	27.0	14.0	9.0	10.0	13.0	0.7	0.0	2.0	0.0	0.0	1.0	1.0
	F1S	0.51	0.85	0.58	0.13	0.77	0.71	0.00	0.98	1.00	0.95	1.00	1.00	0.97	0.96
	MCC	0.49	0.82	0.50	0.24	0.72	0.65	0.00	0.98	1.00	0.94	1.00	1.00	0.97	0.96
y-rand.	PRE	19.2	11.8	20.7	50.0	17.7	15.3	0.0	38.2	28.2	38.7	35.2	45.3	45.2	36.8
	REC	16.2	12.5	46.5	3.3	22.9	12.1	0.0	36.7	76.9	75.5	8.0	15.3	18.9	25.4
	ACU	72.7	71.0	53.6	85.0	68.8	70.6	87.0	79.4	63.7	69.7	86.0	84.5	83.3	89.3
	ERR	27.3	29.0	46.4	15.0	31.2	29.4	13.0	20.6	36.3	30.3	14.0	15.5	16.7	10.7
	F1S	0.1	0.1	0.3	0.1	0.2	0.1	0.0	0.30	0.41	0.51	0.12	0.22	0.24	0.29
	MCC	0.01	-0.05	0.02	0.10	0.01	-0.04	0.00	0.26	0.28	0.36	0.15	0.22	0.23	0.28
test	PRE	26.6	63.6	16.7	0.0	54.5	25.5	0.0	89.7	100.0	38.5	100.0	100.0	100.0	100.0
	REC	41.6	77.8	80.0	0.0	75.0	16.7	0.0	86.7	100.0	100.0	70.0	100.0	83.3	66.7
	ACU	78.7	88.0	58.0	80.0	86.0	84.0	76.0	94.7	100.0	84.0	94.0	100.0	98.0	92.0
	ERR	21.3	12.0	42.0	20.0	14.0	16.0	24.0	5.3	0.0	16.4	6.0	0.0	2.0	8.0
	F1S	0.30	0.70	0.28	0.00	0.63	0.20	0.00	0.85	1.00	0.56	0.82	1.00	0.91	0.80
	MCC	0.25	0.63	0.21	0.00	0.56	0.12	0.00	0.84	1.00	0.56	0.81	1.00	0.90	0.78
		UV light													
		LDA							LS-SVM						
		overall	pure	canola	sunflower	corn	olive	soybean	overall	pure	canola	sunflower	corn	olive	soybean
calibration	PRE	36.0	46.7	40.0	50.0	33.3	46.2	0.0	99.0	93.8	100.0	100.0	100.0	100.0	100.0
	REC	42.5	93.3	31.6	36.8	33.3	60.0	0.0	99.1	100.0	100.0	94.7	100.0	100.0	100.0
	ACU	81.3	83.0	78.0	81.0	80.0	78.0	88.0	99.7	99.0	100.0	99.0	100.0	100.0	100.0
	ERR	18.7	17.0	22.0	19.0	20.0	22.0	12.0	0.3	1.0	0.0	1.0	0.0	0.0	0.0
	F1S	0.38	0.62	0.35	0.42	0.33	0.52	0.00	0.99	0.97	1.00	0.97	1.00	1.00	1.00
	MCC	0.29	0.58	0.22	0.32	0.22	0.39	0.00	0.99	0.96	1.00	0.97	1.00	1.00	1.00
y-rand.	PRE	14.0	14.0	16.0	22.9	11.3	19.6	0.0	41.7	31.6	39.7	45.6	43.9	54.3	35.0
	REC	15.7	28.0	12.6	16.8	11.3	25.5	0.0	44.0	86.0	65.3	33.7	13.3	41.0	25.0
	ACU	72.2	63.4	70.8	73.4	73.4	64.2	88.0	81.7	67.6	77.1	83.3	86.1	86.0	90.3
	ERR	27.8	36.6	29.2	26.6	26.6	35.8	12.0	18.3	32.4	22.9	16.7	13.9	14.0	9.7
	F1S	0.14	0.19	0.14	0.19	0.11	0.22	0.00	0.36	0.46	0.48	0.33	0.19	0.43	0.26
	MCC	-0.01	-0.02	-0.03	0.04	-0.04	-0.01	0.00	0.32	0.37	0.39	0.31	0.20	0.42	0.26
test	PRE	34.9	45.0	14.3	28.6	100.0	21.4	0.0	52.1	63.6	25.0	62.5	66.7	33.3	61.5
	REC	36.7	90.0	16.7	33.3	20.0	60.0	0.0	51.4	70.0	33.3	83.3	40.0	20.0	61.5
	ACU	78.0	76.0	78.0	82.0	84.0	74.0	74.0	85.0	86.0	80.0	92.0	84.0	88.0	80.0
	ERR	22.0	24.0	22.0	18.0	16.0	26.0	26.0	15.0	14.0	20.0	8.0	16.0	12.0	20.0
	F1S	0.29	0.60	0.15	0.31	0.33	0.32	0.00	0.51	0.67	0.29	0.71	0.50	0.25	0.62
	MCC	0.23	0.51	0.03	0.21	0.41	0.24	0.00	0.42	0.58	0.17	0.68	0.43	0.20	0.48

PRE: Precision. REC: Recall. ACU: Accuracy. ERR: Error rate. F1S: F1-score. MCC: Matthews correlation coefficient. Canola, sunflower, corn, olive, and soybean oil percentages in avocado oil were 5, 10, 20, 35, or 50 %.

### 3.3. Predicting the blend level in avocado oil

In order to construct the calibration models, samples for each blend, ranging from 0% to 50% vegetable oil (canola, corn, soy, sunflower, and olive), were analyzed. The statistical parameters from the MLR and LS-SVM models for all blends under the two illuminants (white and UV light) are detailed in Table 3.

**Table 3:** Performance parameters for MLR and LS-SVM models to predict the proportion of canola, sunflower, corn, olive, or soybean oils blended with avocado oil using the TCS34725 colour sensor under white and UV light.

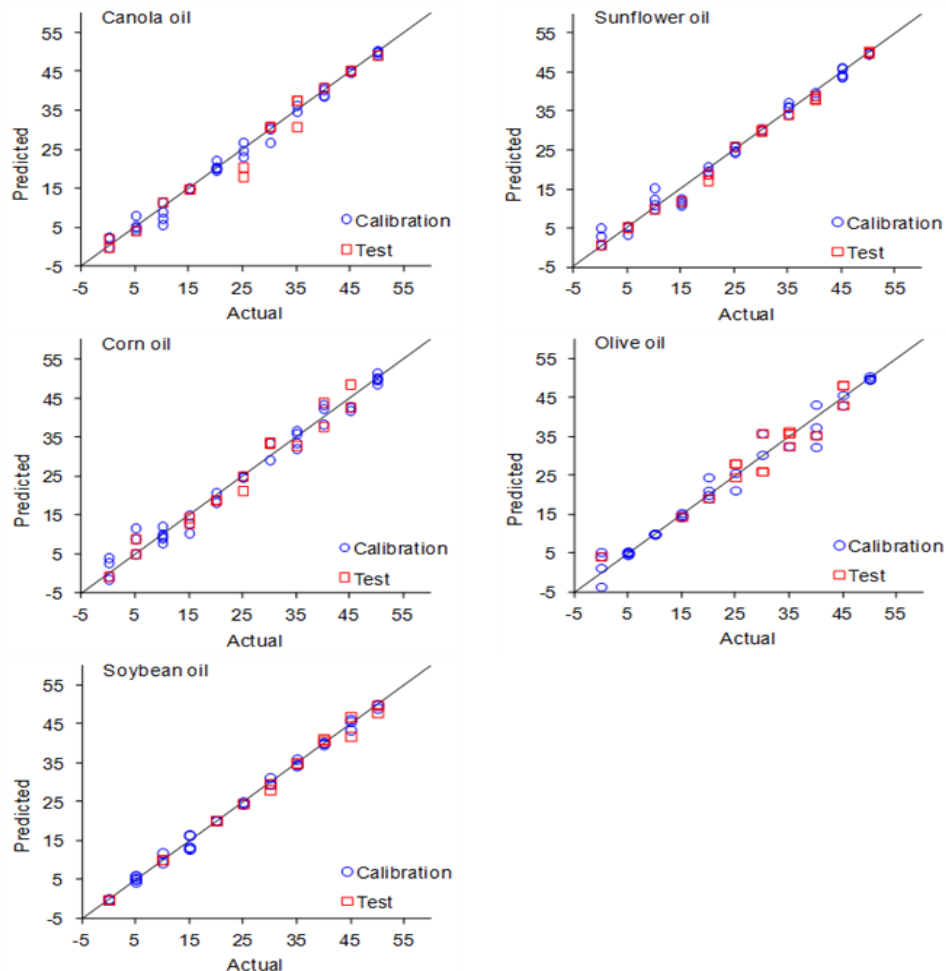
		white light		uv light	
		MLR	LS-SVM	MLR	LS-SVM
canola oil					
calibration	RMSE	6.02	1.44	7.60	5.37
	R <sup>2</sup>	0.86	0.99	0.78	0.90
y-randomization	RMSE	20.97	9.48	21.40	13.52
	R <sup>2</sup>	0.02	0.64	0.01	0.44
	cR <sup>2</sup> p	0.85	0.59	0.77	0.64
test	RMSE	6.36	2.55	7.12	7.35
	R <sup>2</sup>	0.83	0.97	0.77	0.77
	R <sup>2</sup> m	0.70	0.97	0.57	0.60
sunflower oil					
calibration	RMSE	3.08	1.89	2.89	2.91
	R <sup>2</sup>	0.96	0.99	0.97	0.97
y-randomization	RMSE	22.38	6.77	21.19	10.65
	R <sup>2</sup>	0.06	0.78	0.04	0.61
	cR <sup>2</sup> p	0.93	0.45	0.95	0.59
test	RMSE	2.63	1.37	3.86	3.81
	R <sup>2</sup>	0.97	0.99	0.94	0.94
	R <sup>2</sup> m	0.96	0.98	0.92	0.92
corn oil					
calibration	RMSE	4.38	2.29	8.95	4.61
	R <sup>2</sup>	0.93	0.98	0.66	0.91
y-randomization	RMSE	23.37	6.03	19.95	8.25
	R <sup>2</sup>	0.01	0.72	0.03	0.81
	cR <sup>2</sup> p	0.93	0.51	0.64	0.30
test	RMSE	4.31	2.62	7.53	7.91
	R <sup>2</sup>	0.91	0.97	0.76	0.77
	R <sup>2</sup> m	0.88	0.96	0.58	0.64
olive oil					
calibration	RMSE	7.14	2.53	6.88	4.08
	R <sup>2</sup>	0.82	0.98	0.82	0.94
y-randomization	RMSE	22.07	11.71	21.81	13.61
	R <sup>2</sup>	0.02	0.60	0.01	0.57
	cR <sup>2</sup> p	0.81	0.61	0.82	0.59
test	RMSE	6.39	3.07	5.74	4.92
	R <sup>2</sup>	0.75	0.93	0.87	0.90
	R <sup>2</sup> m	0.54	0.87	0.53	0.75
soybean oil					
calibration	RMSE	2.45	0.90	2.20	1.54
	R <sup>2</sup>	0.98	1.00	0.98	0.99
y-randomization	RMSE	22.26	12.79	22.37	8.07
	R <sup>2</sup>	0.03	0.40	0.01	0.63
	cR <sup>2</sup> p	0.96	0.77	0.97	0.60
test	RMSE	2.23	1.17	2.27	1.62
	R <sup>2</sup>	0.98	0.99	0.98	0.99
	R <sup>2</sup> m	0.96	0.99	0.98	0.99

The MLR models were able to predict the levels of canola, sunflower, corn, olive, and soybean oils blended with avocado oil, achieving calibration  $R^2 > 0.8$  for both illuminants (Table 3). However, the MLR models for canola and corn oil blends exhibited relatively lower calibration  $R^2$  values (0.78 and 0.66, respectively) under UV light. Also,  $R^2$  values ranging from

0.75 to 0.98 were obtained for the test set, in addition to  $R^2_m$  values  $> 0.5$ , indicating a congruence between actual and predicted values. Despite high RMSE values and low  $R^2$ , the  $cR^2_p > 0.5$  suggested no overfitting or random adjustments.

Conversely, the LS-SVM models produced superior outcomes compared to the MLR models, particularly under white light, with  $R^2$  values  $> 0.93$  for both calibration and test sets. The RMSE values from the LS-SVM models were notably lower than those obtained from the MLR models. Furthermore, the elevated  $R^2_m$  values (ranging from 0.87 to 0.99) indicated excellent congruence between actual and predicted values. The LS-SVM models exhibited poor performance in the y-randomization test, with  $cR^2_p$  values exceeding 0.5, suggesting the robustness of these models without overfitting or random adjustments. Comparisons between actual and predicted percentages of the vegetable oils in the blends with avocado oil by LS-SVM using white light for both calibration and test sets are presented in Figure 2.

**Figure 2:** Actual and predicted proportion of canola, sunflower, corn, olive, or soybean oils blended with avocado oil using the TCS34725 colour sensor under white light and LS-SVM models.



#### 4. DISCUSSION

Certainly, the outcomes reveal that the models generated under white-light conditions exhibited superior classification metrics compared to those under UV light for both calibration and test sets. A long-wavelength band at 350-420 nm in excitation and 660-700 nm in emission is attributed to the fluorescence of pigments of the chlorophyll group in olive oils (SIKORSKA et al., 2008), while excitation at 320-420 nm and emission at 400-500 nm is associated with oxidation products (AMMARI et al., 2012; LLEÓ et al., 2016). According to Hakonen and Beves (HAKONEN; BEVES, 2018), the fluorescence detected at a 90° angle from an excitation light might produce inner-filter effects due to the presence of particles in a liquid medium. They suggested that this effect, when using a 405 nm excitation light, could provide additional informative details for distinguishing pure oils based on colour parameters obtained via a smartphone camera. However, they highlighted a possible drawback of inhomogeneous sample images, which might have contributed to the unsatisfactory classification performance for the oil blends using the colour sensor under UV light. The evident difference in performance attributed to different light sources aligns with the known significance of illumination in colourimetric techniques (DE CARVALHO et al., 2023; LLEÓ et al., 2016; SONG et al., 2020b).

A noticeable disparity in classification performance and superior outcomes for LS-SVM models over LDA was evident. LDA stands out as a prevalent choice among supervised methods for tasks like food matrix authentication, characterization, and adulteration detection (ESTEKI; SHAHSAVARI; SIMAL-GANDARA, 2018). LDA is frequently used as a linear classifier (YE; JANARDAN; LI, 2004), similar to MLR, a regression method relying on the linear relationship between dependent and independent variables. In contrast, LS-SVM employs a non-linear algorithm, which, although more computationally intensive, exhibits greater generalization power (YU; WANG; CAO, 2009). Some authors emphasize that machine-learning techniques such as SVM complement the implementation of low-cost sensors, compensating for limitations in their design and manufacture (PAYETTE; VAUSSENAT; CLOUTIER, 2023). This is feasible because SVM operates as an optimization-based model, seeking the best hyperplane to maximize margins within a high-dimensional space. Unlike in linear regression, where the impact of all data points is uniform, in SVM, each data point influences the final optimization (OTHMAN et al., 2023). Therefore, the superiority of LS-SVM over LDA can be attributed to the mathematical qualities of each model in terms

of data processing and their ability to discriminate between classes, with LS-SVM classification performing better than LDA.

These outcomes confirm that the RGB sensor coupled with LS-SVM not only detected blends of vegetable oils in avocado oil but was also able to predict the blend proportion. Assessing oil authenticity typically involves quantifying triacylglycerol components using gas chromatography or high-performance liquid chromatography, especially when evaluating blends of virgin oils, such as avocado or olive, with refined vegetable oils (DE LA MATA-ESPINOSA et al., 2011). Another common approach involves spectroscopic methods coupled with chemometric tools (AROCA-SANTOS et al., 2019). However, both of these methods require the use of expensive equipment, reagents, and/or considerable time, in contrast to the colour sensor-based device used in the present work.

This study had similar or superior performance compared to previous studies that used colourimetric sensors or traditional analytical techniques to assess oil quality. Huang et al. (2022) achieved cross-validation with accuracy of 90.7% and 81.5% for distinguishing extra virgin olive oil from its mixtures with soybean and corn oil, respectively, using a colourimetric sensor array (SUYKENS et al., 2002). Naik et al. (2023) demonstrated that a paper-based colour sensor was able to detect the presence of palm oil and sunflower oil added to cow ghee at a concentration of 2.5% or more (NAIK et al., 2023). Amit et al. (2020) achieved an RMSE lower than 1% for a test set when using Fourier transform infrared spectroscopy to detect fried coconut oil mixed with pure coconut oil (AMIT et al., 2020). Tian et al. (2019) reported that gas chromatography can reliably identify peanut oil mixed with a minimum of 5% rapeseed oil based on fatty acid profiles (TIAN et al., 2019). Similar to the present study and others (DE LIMA; MUSSO; BERTOLDO MENEZES, 2020; JAMWAL et al., 2021; POPA et al., 2020; SU et al., 2021; WANG et al., 2019), all of these studies focused on demonstrating the feasibility of analytical techniques without examine mixtures of oils from different suppliers. Therefore, once the feasibility of an analytical technique has been demonstrated, further research can be conducted to validate and investigate the robustness of methods using larger and more diverse sample sets from different sources and suppliers.

## **5. CONCLUSION**

This proof-of-concept study presents evidence over a cost-effective colour sensor (RGB), which can be a feasible solution for detecting and distinguishing blends of vegetable

oils in avocado oil. This RGB sensor was able to quantify blends in avocado oil and their proportions.

The classification models effectively identified mixtures of various vegetable oils in avocado oil, with an accuracy exceeding 90%. Notably, non-linear LS-SVM models performed better under white light conditions, using the sensor's light source, thereby streamlining the analysis process. In addition, the method proved to be rapid and cost-effective, without the necessity of any additional sample pretreatment.

This work supporting evidence can be used in future research, for the further validation and robustness studies with even higher number of samples and other oils or mixtures, thus the further establishment of this portable device for detecting accurately the oil blends.

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# Feasibility of using a cheap colour sensor to detect blends of vegetable oils in avocado oil

Natasha Lorenzo, Roney A. da Rocha, Emmanouil H. Papaioannou, Yhan Mutz, Leticia Tessaro, and Cleiton Nunes

## Supplementary material

**Table S1.** Confusion matrix for the test set obtained by LDA two-class model to discriminate pure avocado oil from its blends with canola, sunflower, corn, olive, and soybean oils using the TCS34725 sensor under white light.

	pure	blended*
pure	0	9
blended*	0	41

\*avocado oil blended with 5, 10, 20, 35, or 50 % canola, sunflower, corn, olive or soybean oils.

**Table S2.** Confusion matrix for the test set obtained by LDA two-class model to discriminate pure avocado oil from its blends with canola, sunflower, corn, olive, and soybean oils using the TCS34725 sensor under UV light.

	pure	blended*
pure	0	10
blended*	0	40

\*avocado oil blended with 5, 10, 20, 35, or 50 % canola, sunflower, corn, olive or soybean oils.

**Table S3.** Confusion matrix for the test set obtained by LS-SVM two-class model to discriminate pure avocado oil from its blends with canola, sunflower, corn, olive, and soybean oils using the TCS34725 sensor under white light.

	pure	blended*
pure	9	0
blended*	0	41

\*avocado oil blended with 5, 10, 20, 35, or 50 % canola, sunflower, corn, olive or soybean oils.

**Table S4.** Confusion matrix for the test set obtained by LS-SVM two-class model to discriminate pure avocado oil from its blends with canola, sunflower, corn, olive, and soybean oils using the TCS34725 sensor under UV light.

	pure	blended*
pure	6	4
blended*	2	38

\*avocado oil blended with 5, 10, 20, 35, or 50 % canola, sunflower, corn, olive or soybean oils.

## ARTIGO 2

Artigo elaborado de acordo com a norma do Food Research International Journal

### EVALUATION OF NATURAL PIGMENTS CONTENT OF VEGETABLE OILS BY USING A CHEAP COLOR SENSOR.

Natasha Lorenzo, Roney A. da Rocha, Emmanouil Papaioannou, Cleiton Nunes.

**Abstract:** Natural pigments, such as chlorophyll and carotenoids, play an important role in virgin vegetable oils oxidative stability and organoleptic quality. Usually, labor and time demanding methods are commonly used to determine the natural pigments content in vegetable oils, such methods include spectroscopic and chromatographic protocols. Nowadays, there are intense research efforts on low-cost sensors application for these pigments' reliable analysis. This study assesses the feasibility of a low-cost color sensor to predict the total content of carotenoids and chlorophyll in avocado and olive oils, and their total spectrophotometric color (TSC). Different color parameters (RGB, HSV or L\*a\*b\*) under two lighting conditions (white or UV) were evaluated in order to predict the pigments levels in 43 oils samples. The Least-Square Support Vector Machine (LS-SVM) models used had better performance than the Multiple Linear Regression (MLR). UV improve the predictive performance for total chlorophylls content. The white lighting was more appropriated in general. HSV predicted better the total chlorophylls ( $R^2 > 0.9$  for external validation, without use of solvents)), whereas RGB was more adequate to predict TSC and total chlorophylls ( $R^2 > 0.9$  for external validation, diluted with hexane). Finally, total carotenoids content was better predicted using the L\*a\*b\* analysis tool ( $R^2$  of 0.8 for external validation). The proposed here color sensor-based method proved a valid alternative for a quick and cost-efficient prediction of pigments content in vegetable oils, without having any necessity of sample pretreatment.

**Keywords:** colorimetry; chemometrics; chlorophyll; carotenoids; olive; avocado

## 1. INTRODUCTION

Olive oil is globally recognized by its elevated proportion of monounsaturated fatty acid, such as oleic acid, that render to this oil its health benefits. There are also in it other minor compounds, < 2%, with phenolic compounds being the most important. These olive oil compounds have shown potential as antioxidant, anti-inflammatory and antimicrobial agents (JIMENEZ-LOPEZ et al., 2020). Avocado oil has a composition similar to olive oil, plus high concentration of phytosterols and antioxidants (FLORES et al., 2019; PÉREZ-CALABUIG et al., 2023; TAN, 2019).

In addition to health benefits of these oils and their composition their color plays an important role in consumers choice to purchase these extra virgin oils (DEKHILI et al., 2011; GILA et al., 2023; ILAK PERŠURIĆ, 2020). The quality of virgin oils are correlated with nutritional value, freshness and functional properties, all of them closely related to the natural pigments content (JIMENEZ-LOPEZ et al., 2020; LAZZERINI & DOMENICI, 2017; TENA et al., 2015). In addition, their main pigments, which are chlorophyll and carotenoids, have an important role in promoting the oxidative stability and organoleptic quality of these vegetable oils (DE CARVALHO & NUNES, 2021; TAN, 2019). Chlorophylls are the main green color pigments and carotenoids are responsible for the yellowish or reddish color of vegetable oils (PÉREZ-GÁLVEZ et al., 2020; TAN, 2019; WONG et al., 2008). Chlorophylls have been reported to possess antioxidant, antimutagenic activity, and preventing degenerative diseases (FERRUZZI & BLAKESLEE, 2007). Carotenoids are natural antioxidants to inhibit free radical chain reactions. The presence of carotenoids is linked to reduced risk of diseases of the skin, eyes, and cardiovascular problems, especially those based on oxidation of low-density cholesterol (LDL).

The traditional analytical methods are used for determining natural pigments in oils tend to be, labor intensive and tedious. These methods include various spectroscopic and chromatographic procedures (BORELLO et al., 2021; JIMÉNEZ-SOTELO et al., 2016). From the alternatives selected to avoid these procedures the most promising are considered the use of smartphone camera and the colorimetric sensors (DE CARVALHO; NUNES, 2021; HAKONEN & BEVES, 2018; SONG et al., 2020). Carvalho and Nunes (2021) have proposed a smartphone-based method for the determination of both chlorophyll and carotenoid content in oils (olive and avocado). However, this method proved laborious as a calibration transfer was required to mitigate errors from light effects and differences in smartphone cameras. Portable

sensors seem to be a more alternative attractive than smartphones, since the mobile cameras are very dependent on the analytical condition involved on the method developing and its use (DE CARVALHO et al., 2023).

According to Rodriguez-Saona et al, (2020), optical sensors are highly optimized towards ‘point-and-shoot’ capabilities with special attention to ruggedness. They are considered a promising and emerging approach, due to their significantly reduce size, thus the production cost of vibrational spectrometers and their integration of micro-electro-mechanical systems (RODRIGUEZ-SAONA et al., 2020). This new approach increases the development of new optical sensor with wide range of applications in food analysis, including vegetable oils. Bi et al. (2019) based on this have developed a handheld miniature ultraviolet-led fluorescence spectrometer to evaluate oil mixtures. Lastra-Mejias et al (2019) developed a fluorescence sensor formed by a light source and a photodetector to monitor the storage conditions and locate adulterations of extra virgin olive oil.

Thus, these advancements in sensors technology, particularly involving miniaturization and cost reduction, can be utilized to explore alternatives that have the potential to be widely applied in the rapid and reliable quality assurance of food. The objective of this proof-of-concept study is to evaluate the feasibility of a cheap color sensor to predict at the same time carotenoids and chlorophyll levels, using different spectrophotometric methods as reference.

## **2. MATERIAL AND METHODS**

### **2.1. Samples**

Four virgin avocado oils were obtained from a local producer (Breda, Margarida, Fortuna, and Quintal avocado cultivars). One commercial avocado oil was obtained from Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG, Hass cultivar). Four commercial extra virgin olive oils (Andorinha, Larambia, Herdade, and Irarema) and one refined soybean oil (Liza) were obtained from local market. Two extra virgin olive oils (olive A and olive B) were obtained from a local producer. The oils were filtered in paper filter before storage and analysis. All oils were within the expiration date and were stored in a fridge at 5° C until their further analysis and not longer than 3 months.

In order to increase the diversity of samples, some random blends were prepared by mixing the oil samples with soy, avocado and olive oil, resulting in 43 distinct samples

according to Table 1. The blending was performed by weighing the respective oils in 10 ml glass vials and then manually vigorously shaking them for about 30 seconds before analysis. The analyses were conducted within 4 hours after the sample preparation. The oil mixture during the analysis was bubble free. The samples were analyzed in triplicate, totaling 129 observations.

**Table 1.** Samples of avocado and olive oils with their respective blends.

Oil	% m/m
Breda (avocado)	100
Margarida (avocado)	100
Fortuna (avocado)	100
Quintal (avocado)	100
Hass (avocado)	100
Olive A	100
Olive B	100
Andorinha (olive)	100
Larambia (olive)	100
Herdade (olive)	100
Irarema (olive)	100
Margarida + Andorinha	25:75
Margarida +Larambia	50:50
Margarida + Herdade	75:25
Margarida +Larambia	80:20
Olive A + Irarema	25:75
Olive A +Herdade	50:50
Olive A + Andorinha	75:25
Olive A + Soybean	50:50
Olive A +Herdade	80:20
Olive B + Larambia	24:75
Olive B + Soybean	50:50
Olive B + Herdade	50:50
Olive B + Soybean	85:15
Fortuna + Irarema	50:50
Fortuna + Larambia	75:25
Fortuna + Andorinha	75:25
Fortuna + Soybean	50:50
Fortuna + Herdade	80:20
Fortuna + Irarema	80:20
Quintal + Herdade	25:75
Quintal + Andorinha	50:50
Quintal + Soybean	50:50
Quintal + Larambia	50:50
Quintal + Larambia	85:15
Breda + Herdade	50:50
Breda + Irarema	25:75
Breda + Soybean	50:50
Breda + Andorinha	50:50
Breda + Andorinha	85:15
Breda + Soybean	85:15
Hass + Soybean	50:50
Hass + Herdade	75:25

## 2.2. Determination of total chlorophylls by spectrophotometry

Three different spectrophotometric methods were used to determine the total chlorophyll content (Tchl) in the oil samples. The determinations were carried out in a UV/Vis spectrophotometer (Drawell® DV-8200, UV-Pro software ver. 1.0.01) using 1 cm glass cuvette.

### 2.2.1 IUPAC method

Chlorophyll pigments were determined by direct measuring the absorbance of the sample (without solvent) at 670 nm, 630 nm, and 710 nm (POKORNY; KALINOVA; DYSSSELER, 1995). Tchl was calculated as in equation 2.1.

$$Tchl(mg.Kg^{-1}) = \frac{345.3x(A_{670}-0.5x A_{630}-0.5x A_{710})}{L} \quad (2.1)$$

Where:

Tchl: content of chlorophyll pigments in mg of pheophytin-a in 1 Kg of oil,

A: absorbance at the respective wavelength (nm),

L: optical path length (1 cm).

### 2.2.2 AOCS method

The standard analytical method of AOCS Cc 13d-55, as referenced in (SABAH, 2007) is based on the determination of the total chlorophyll content using absorbance of the sample (without solvent) at 630 nm, 670 nm, and 710 nm. Tchl was calculated as in equation 2.2.

$$Tchl(mg.kg^{-1}) = \frac{A_{670}-0.5(A_{630}+A_{710})}{0.0964 L} \quad (2.2)$$

Where:

Tchl: content of chlorophyll pigments in mg of pheophytin-a in 1 Kg of oil,

A: absorbance at the respective wavelength (nm),

L: optical path length (1 cm).

### 2.2.3 Minguez-Mosquera method

In this method (MINGUEZ-MOSQUERA et al., 1991), the absorbance at 670 nm provides a quantitative evaluation of the total content of chlorophyll and their derivatives, since the absorbance at 670 nm is due exclusively to the fraction of pigments, as example pheophytin

a. The samples were prepared by mixing 0.6 g of oil and 2 ml of n-hexane as solvent and blank. Tch1 was calculated as in equation 2.3.

$$Tchl (mg.Kg^{-1}) = \frac{A_{670} \times 10^6}{613 \times 100 \times L} \quad (2.3)$$

Where:

Tchl: content of chlorophyll pigments in mg of pheophytin-a in 1 Kg of oil,

A: absorbance at the respective wavelength (nm),

L: optical path length (1 cm).

### 2.3 Determination of total carotenoids by spectrophotometry

The total carotenoid content (Tcar) was determined by two different spectrophotometric methods. The determinations were carried out in a UV/Vis spectrophotometer (Drawell® DV-8200, UV-Pro software ver. 1.0.01) using 1 cm glass cuvette.

#### 2.3.1 Goodwin method

The samples were prepared by mixing 0.6 g of oil and 2 mL of n-hexane as solvent and blank. As described by Goodwin (1951) and adapted by Mba et al. (2017), the total carotenoids content was measured at 445 nm. Tcar was calculated using the equation 2.4.

$$Tcar (mg.kg^{-1}) = \frac{A_{445} \times v \times 10^6}{2500 \times W \times 1000} \quad (2.4)$$

Where:

Tcar: Total carotenoids content in mg in 1 Kg of oil,

A: absorbance at the respective wavelength (nm),

v: volume of n-hexane in mL,

W: weight of sample in g.

#### 2.3.2. Minguéz-Mosquera Method

According to Minguéz-Mosquera et al. (1991), the absorbance of 470 nm can be used to assess the total content of carotenoids in virgin oils, since absorbance at 470 nm comes largely from the absorption of carotenoids. The samples were prepared by mixing 0.6 g of oil and 2 mL of n-hexane as solvent and blank. Tcar was calculated using equation 2.5.



$$T_{car}(mg.Kg^{-1}) = \frac{A_{470} \times 10^6}{2000 \times 100 \times L} \quad (2.5)$$

Where:

T<sub>car</sub>: Total carotenoids content in mg in 1 Kg of oil,

A: absorbance at the respective wavelength (nm),

L: optical path length (1 cm).

#### 2.4 Determination of total spectrophotometric color by spectrophotometry

The total spectrophotometric color was determined according to the Polish Standard PN-A-86934:1995 (RÓŻAŃSKA; NAMIEŚNIK, 2017). The samples were prepared by mixing 0.2 g of oil and 2 mL of n-hexane for reading at 442 nm and mixing 1.0 g of oil and 1 ml of n-hexane for reading at 668 nm. The determinations were carried out in a UV/Vis spectrophotometer (Drawell® DV-8200, UV-Pro software ver. 1.0.01) using 1 cm glass cuvette. TSC was calculated using equation 2.6.

$$TSC = 1000 \times (A_{442} + A_{668}) \quad (2.6)$$

Where:

TSC: Total spectrophotometric color

A<sub>442</sub>: absorbance at the respective wavelength (nm),

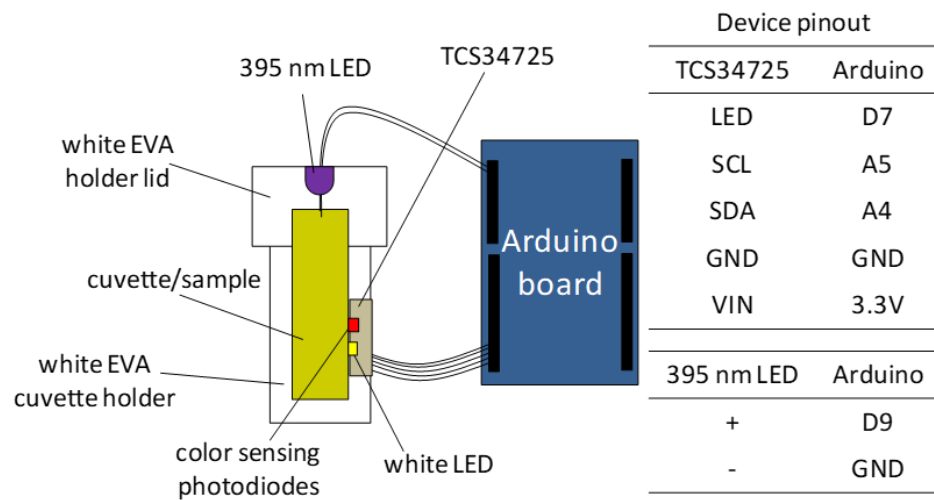
A<sub>668</sub>: absorbance at the respective wavelength (nm).

#### 2.5 Analysis using the color sensor

A TCS34725 color sensor (Texas Advanced Optoelectronic Solutions Inc.) was used to acquire digital readings of red, green, blue (RGB), and clear light (C) values as previously described (LORENZO et al., 2024). This sensor was interfaced with an Arduino Uno, employing an integration time of 24 milliseconds and a gain of 1x. A sample holder was built using white ethylene-vinyl acetate (EVA) material. The samples, contained in 4 ml cuvettes, were analyzed without the use of any solvent. The samples were analyzed under two distinct lighting conditions: ultraviolet light (3V, 395 nm LED) and white light from the color sensor's light source. The closed sample holder ensured the lighting standardization. Raw readings for

red (R), green (G), blue (B), and clear light (C) outputs were acquired by averaging 10 readings using Realterm software (version 2.0.0.70, i2cchip). The raw RGB values were normalized by dividing them by the C value, resulting in the R, G, and B values used as descriptors in the models. Further, the RGB values were converted to HSV and CIE L\*a\*b using the functions “rgb2hsv” and “rgb2lab” from Image Processing Octave package.

**Figure 1.** Device based on a TCS34725 sensor interfaced with Arduino used to read the color parameters of the oils.



## 2.6 Multivariate calibration

In order to predict the total chlorophylls and carotenoids in the oils, as well as the TSC, two modeling approaches were examined: Multiple Linear Regression (MLR) and Least-Square Support Vector Machine LS-SVM. MLR is a method that finds a linear combination of features that describe a dependent variable, resulting in a linear predictor (SLINKER & GLANTZ, 2008). LS-SVM, widely used in nonlinear function estimation, is a version of SVM that solves linear equations instead of a quadratic programming problem. This overcomes the major drawback of SVM, which is its higher computational burden for constrained optimization programming (HAIFENG WANG & DEJIN HU, 2005). All computations were conducted utilizing Octave version 5.2.0 (Eaton et al., 2019). LS-SVM models were implemented through the LS-SVMlab toolbox version 1.8 (SUYKENS et al., 2002) using a radial basis function.

The color parameters (R, G, and B) of the oil samples obtained using the color sensor (independent variables) were calibrated against the total chlorophylls, total carotenoids and TCS values determined spectrophotometrically (dependent variables). The entire dataset is

available as supplementary material. In order to find the better descriptors, different color systems (RGB, HSV, or L\*a\*b\*) and lighting condition (white or UV light) were used.

The dataset was divided into a calibration set, encompassing 75% (97 samples) of the total samples, and a test set (for external validation) comprising the remaining 25% (32 samples), employing the Kennard-Stone algorithm (Yun, 2022). The adequacy of model fitting was evaluated using the determination coefficient ( $R^2$ ) and the root mean squared error (RMSE) for calibration, y-randomization and external validation (test set).

The  $R_m^2$  value was computed (Equation 2.7) to ensure that the predicted values obtained through external validation not only exhibit a strong correlation with the observed values but also demonstrate congruency. A threshold of 0.5 was adopted as valid (MITRA; SAHA; ROY, 2010).

$$R_m^2 = R^2 \left(1 - \sqrt{R^2 - R_0^2}\right) \quad (2.7)$$

Where  $R^2$  and  $R_0^2$  represent the quadratic correlation coefficients between the actual and predicted values, with and without the intercept, respectively.

Furthermore, the robustness of the models was assessed by y-randomization test, which consists of fixing the X matrix (independent variables) and shuffling the y vector (dependent variable) to obtain new models. It is expected that the predictive performance will decrease as the response is truly related to its predictor, thus validating the relationship between independent and dependent variables. From this test, the  $cR_p^2$  value was computed, which accounts for the distinction between the y-randomization  $R^2$  ( $R_{rand}^2$ ) and calibration  $R^2$  ( $R_{cal}^2$ ) (Equation 2.8). A  $cR_p^2 > 0.5$  was established to attest the absence of overfitting or random adjustment (MITRA; SAHA; ROY, 2010).

$$cR_p^2 = R_{cal}^2 \left(\sqrt{R_{cal}^2 - R_{rand}^2}\right) \quad (2.8)$$

### 3. RESULTS

#### 3.1. Total Chlorophyll

The color parameters (RGB, HSV or L\*a\*b\*) from the color sensor were calibrated against the total chlorophyll (Tchl) content in the oils determined by spectrometry using three

different methods. The statistical parameters resulting from MLR and LS-SVM models under two distinct lighting conditions (white or UV) are detailed in Table 2.

**Table 2.** Performance parameters for MLR and LS-SVM models to predict the total chlorophylls (Tchl) in olive and avocado oils based on different methods using the TCS34725 color sensor.

		Tchl – IUPAC (mg kg <sup>-1</sup> )											
		MLR						LS-SVM					
		white light			uv light			white light			uv light		
		RGB	HSV	Lab	RGB	HSV	Lab	RGB	HSV	Lab	RGB	HSV	Lab
Calibration	RMSE	6.68	6.46	6.47	6.08	5.63	5.96	5.30	5.00	4.82	2.03	2.10	1.94
	R <sup>2</sup>	0.77	0.78	0.79	0.82	0.85	0.82	0.86	0.87	0.88	0.98	0.98	0.98
y-rand	RMSE	18.75	18.59	19.03	18.95	19.87	19.13	12.35	11.57	12.80	13.03	13.53	12.09
	R <sup>2</sup>	0.01	0.01	0.01	0.02	0.01	0.02	0.34	0.46	0.25	0.30	0.19	0.45
	cR <sup>2</sup> p	0.77	0.78	0.79	0.81	0.84	0.81	0.67	0.60	0.75	0.81	0.88	0.73
Test	RMSE	5.32	7.01	7.06	7.25	9.24	7.26	5.03	4.85	4.90	4.21	4.13	4.49
	R <sup>2</sup>	0.89	0.85	0.83	0.77	0.64	0.77	0.90	0.91	0.91	0.93	0.94	0.91
	R <sup>2</sup> m	0.86	0.81	0.78	0.62	0.57	0.68	0.85	0.88	0.90	0.76	0.90	0.81
		Tchl – AOCS (mg kg <sup>-1</sup> )											
		MLR						LS-SVM					
		white light			uv light			white light			uv light		
		RGB	HSV	Lab	RGB	HSV	Lab	RGB	HSV	Lab	RGB	HSV	Lab
Calibration	RMSE	2.01	1.94	1.94	1.83	1.69	1.79	1.59	1.50	1.45	0.61	0.64	0.59
	R <sup>2</sup>	0.77	0.78	0.79	0.82	0.85	0.82	0.86	0.87	0.88	0.98	0.98	0.98
y-rand	RMSE	5.64	5.51	5.76	5.74	5.89	5.67	3.52	3.83	3.52	3.72	4.16	3.67
	R <sup>2</sup>	0.01	0.01	0.01	0.00	0.02	0.01	0.39	0.37	0.39	0.43	0.23	0.41
	cR <sup>2</sup> p	0.76	0.78	0.79	0.81	0.84	0.82	0.64	0.66	0.66	0.73	0.89	0.75
Test	RMSE	1.60	2.11	2.12	2.18	2.77	2.18	1.51	1.46	1.47	1.27	1.23	1.34
	R <sup>2</sup>	0.89	0.85	0.83	0.77	0.64	0.77	0.90	0.91	0.91	0.93	0.94	0.91
	R <sup>2</sup> m	0.86	0.81	0.78	0.62	0.57	0.68	0.86	0.88	0.90	0.75	0.90	0.81
		Tchl – MM (mg kg <sup>-1</sup> )											
		MLR						LS-SVM					
		white light			uv light			white light			uv light		
		RGB	HSV	Lab	RGB	HSV	Lab	RGB	HSV	Lab	RGB	HSV	Lab
Calibration	RMSE	1.63	1.40	1.46	1.78	1.63	1.69	1.31	1.06	1.33	0.82	0.79	1.06
	R <sup>2</sup>	0.81	0.85	0.84	0.77	0.81	0.78	0.88	0.91	0.87	0.95	0.96	0.91
y-rand	RMSE	4.95	4.79	4.98	4.93	5.01	4.82	3.62	3.06	3.18	3.51	3.60	3.16
	R <sup>2</sup>	0.00	0.01	0.01	0.01	0.02	0.01	0.19	0.51	0.31	0.24	0.14	0.32
	cR <sup>2</sup> p	0.81	0.84	0.84	0.77	0.80	0.77	0.77	0.60	0.70	0.82	0.88	0.74
Test	RMSE	1.54	1.89	2.05	2.10	2.36	2.33	0.99	1.40	1.56	1.44	1.40	1.16
	R <sup>2</sup>	0.88	0.86	0.83	0.72	0.66	0.72	0.94	0.91	0.88	0.89	0.88	0.94
	R <sup>2</sup> m	0.82	0.79	0.74	0.55	0.56	0.49	0.91	0.89	0.83	0.85	0.87	0.81

y-rand: y-randomization test. IUPAC: based on Pokorny et al. (1995). AOCS: based on AOCS Cc 13i-96 method. MM: based on Mínguez-Mosquera et al. (1991).

MLR models demonstrated a better capability to predict the Tchl in olive and avocado oils based in both IUPAC, AOCS and Mínguez-Mosquera (MM) methods using while lighting and RGB values as descriptors, achieving R<sup>2</sup> ~0.8. R<sup>2</sup> values ~ 0.9 were obtained for the test set, in addition to R<sup>2</sup>m > 0.5, indicating substantial congruence between actual and predicted values. The high RMSE values and low R<sup>2</sup> for y-randomization test, besides cR<sup>2</sup>p > 0.5, suggested no overfitting or random adjustments.

On the other hand, the LS-SVM models outperformed the MLR models, in special the models based on IUPAC and AOCS methods using HSV values and UV lighting, and the model

based on MM method under white lighting with RGB values, with  $R^2$  values  $> 0.90$  for both calibration and test sets. The RMSE values resulting from the LS-SVM models were notably lower than those obtained from the MLR models. Furthermore, the elevated  $R^2_m$  values ( $R^2_m > 0.90$ ) indicated excellent congruence between actual and predicted values, as verified in Figure 1. The LS-SVM models exhibited poor performance in the y-randomization test, with  $cR^2_p$  values exceeding 0.5, suggesting the robustness of these models without overfitting or random adjustments.

### 3.2. Total carotenoids and total spectrophotometric color

As for total chlorophylls, the color parameters (RGB, HSV or  $L^*a^*b^*$ ) from the color sensor were calibrated against the total carotenoids ( $T_{car}$ ) content in the oils determined by spectrometric methods, as well as the total spectrophotometric color (TSC). The statistical parameters resulting from MLR and LS-SVM models under the two lighting conditions (white and UV) are presented in Table 3.

In general, the MLR models exhibited a poor performance to predict  $T_{car}$ , with  $R^2$  values for calibration ranging from 0.59 to 0.67 for white lighting, and between 0.47 and 0.52 for UV lighting. Despite relatively low  $R^2$  values for the test set (from 0.62 to 0.72),  $R^2_m > 0.5$  suggested some congruence between actual and predicted values when using white light. On the other hand, a considerable poor performance was obtained when using UV light, with  $R^2_m < 0.5$  for practically all the models. In addition, the  $cR^2_p$  values lower or close to 0.5 in the y-randomization test suggested overfitting or random adjustments when using UV-lighting.

The LS-SVM models produced superior outcomes in comparison to the MLR models to predict  $T_{car}$  based on both GW and MM methods, especially when using  $L^*a^*b^*$  under white lighting, with  $R^2$  values close to 0.8 for test set. The RMSE values resulting from the LS-SVM models were lower than those obtained from the MLR models. Furthermore,  $R^2_m > 0.5$  indicated a valid congruence between actual and predicted values, as verified in Figure 1. In addition, the LS-SVM models exhibited elevated RMSE and low  $R^2$  in the y-randomization test, particularly when using  $L^*a^*b^*$  and white light, with  $cR^2_p$  values exceeding 0.5, suggesting no overfitting or random adjustments.

The MLR models were able to predict TSC with a regular performance when using white lighting (Table 3), with calibration  $R^2$  close or superior to 0.8 for both calibration and test

sets.  $R^2_m > 0.5$  indicating a good congruence between actual and predicted values. Also, the high RMSE and low  $R^2$  values, with  $cR^2_p > 0.5$ , suggested no overfitting or random adjustments.

**Table 3.** Performance parameters for MLR and LS-SVM models to predict the total spectrophotometric color (TSC) and total carotenoids (Tcar) in olive and avocado oils based on different methods using the TCS34725 color sensor.

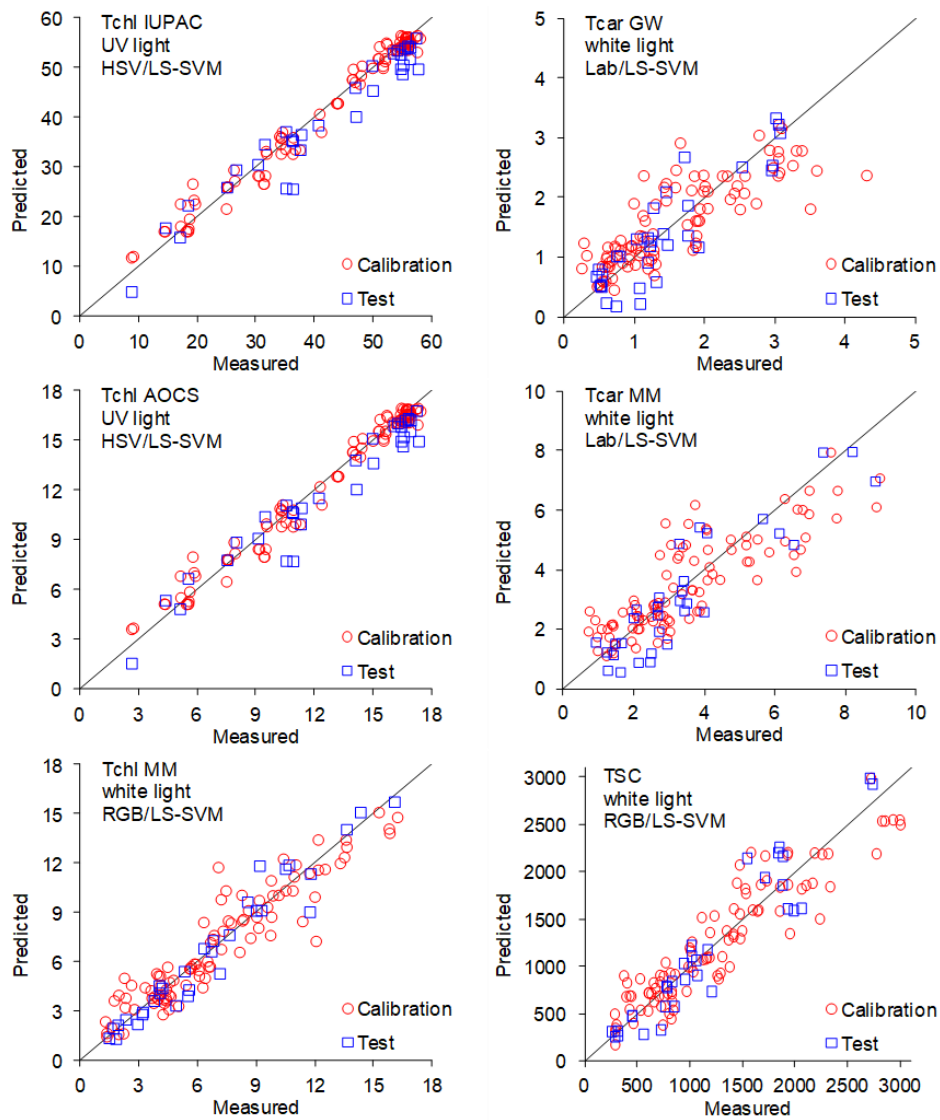
		Tcar – GW (1951) (mg kg <sup>-1</sup> )											
		MLR						LS-SVM					
		white light			uv light			white light			uv light		
		RGB	HSV	Lab	RGB	HSV	Lab	RGB	HSV	Lab	RGB	HSV	Lab
calibration	RMSE	0.56	0.57	0.57	0.64	0.66	0.66	0.48	0.50	0.53	0.41	0.51	0.44
	R <sup>2</sup>	0.59	0.61	0.61	0.50	0.48	0.47	0.70	0.70	0.67	0.80	0.69	0.76
y-rand	RMSE	1.11	1.16	1.17	1.11	1.11	1.06	0.76	0.75	0.87	0.85	0.84	0.77
	R <sup>2</sup>	0.00	0.01	0.01	0.01	0.01	0.02	0.44	0.46	0.24	0.24	0.31	0.48
	cR <sup>2</sup> <sub>p</sub>	0.59	0.60	0.61	0.50	0.47	0.47	0.43	0.41	0.54	0.67	0.51	0.46
Test	RMSE	0.62	0.59	0.62	0.70	0.66	0.64	0.61	0.44	0.42	0.72	0.57	0.49
	R <sup>2</sup>	0.62	0.67	0.64	0.39	0.48	0.50	0.63	0.77	0.79	0.50	0.62	0.73
	R <sup>2</sup> <sub>m</sub>	0.49	0.62	0.62	0.12	0.28	0.24	0.50	0.64	0.79	0.40	0.50	0.53
		Tcar – MM (mg kg <sup>-1</sup> )											
		MLR						LS-SVM					
		white light			uv light			white light			uv light		
		RGB	HSV	Lab	RGB	HSV	Lab	RGB	HSV	Lab	RGB	HSV	Lab
calibration	RMSE	1.21	1.09	1.15	1.31	1.36	1.29	1.03	0.92	1.06	0.76	0.93	1.01
	R <sup>2</sup>	0.63	0.67	0.65	0.52	0.52	0.52	0.73	0.77	0.70	0.85	0.77	0.71
y-rand	RMSE	2.40	2.47	2.59	2.32	2.42	2.25	1.70	1.72	1.73	1.81	1.87	1.70
	R <sup>2</sup>	0.01	0.01	0.01	0.01	0.01	0.00	0.38	0.34	0.35	0.29	0.18	0.25
	cR <sup>2</sup> <sub>p</sub>	0.62	0.67	0.65	0.52	0.51	0.51	0.50	0.58	0.50	0.69	0.68	0.57
Test	RMSE	1.12	1.33	1.29	1.62	1.50	1.65	1.02	1.09	0.92	1.28	1.13	1.11
	R <sup>2</sup>	0.72	0.67	0.70	0.44	0.48	0.48	0.76	0.76	0.82	0.65	0.71	0.78
	R <sup>2</sup> <sub>m</sub>	0.68	0.61	0.68	0.13	0.28	0.17	0.73	0.62	0.81	0.41	0.63	0.51
		TSC											
		MLR						LS-SVM					
		white light			uv light			white light			uv light		
		RGB	HSV	Lab	RGB	HSV	Lab	RGB	HSV	Lab	RGB	HSV	Lab
calibration	RMSE	326.6	285.9	298.6	366.8	369.7	365.6	268.5	258.8	267.8	206.5	206.4	233.3
	R <sup>2</sup>	0.78	0.82	0.81	0.70	0.71	0.70	0.85	0.85	0.85	0.91	0.91	0.88
y-rand	RMSE	922.9	936.2	912.6	862.3	884.7	867.5	632.4	629.8	603.5	623.9	627.5	625.1
	R <sup>2</sup>	0.01	0.01	0.02	0.01	0.01	0.00	0.28	0.19	0.36	0.36	0.27	0.21
	cR <sup>2</sup> <sub>p</sub>	0.78	0.82	0.80	0.70	0.71	0.69	0.70	0.75	0.64	0.70	0.77	0.76
Test	RMSE	300.0	366.3	391.3	464.1	456.2	458.6	251.2	263.9	294.4	363.1	296.1	263.7
	R <sup>2</sup>	0.86	0.83	0.82	0.63	0.64	0.67	0.90	0.90	0.88	0.79	0.85	0.89
	R <sup>2</sup> <sub>m</sub>	0.79	0.77	0.70	0.39	0.51	0.44	0.85	0.89	0.80	0.75	0.81	0.77

y-rand: y-randomization test. GW: based on T.W. Goodwin (1951). MM: based on Minguéz-Mosquera et al. (1991).

As for Tchl and Tcar, the LSSVM models produced better performance to predict TSC compared to the MLR models, particularly under white lighting using RGB values as descriptors, with  $R^2$  of 0.85 for calibration and 0.90 for test set. RMSE values resulting from the LS-SVM models were considerably lower than those obtained from the MLR models. Furthermore, the elevated  $R^2_m$  values (close to 0.80) indicated considerable congruence between actual and predicted values, as verified in Figure 1. In addition, the LSSVM models

exhibited poor performance in the y-randomization test, with  $cR^2_p$  values exceeding 0.5, suggesting the robustness of these models without overfitting or random adjustments.

**Figure 2.** Measured and predicted total spectrophotometric color (TSC), total chlorophylls (Tchl), and total carotenoids (Tcar) in olive and avocado oils based on different methods using the TCS34725 color sensor and the best tested models.



#### 4. DISCUSSION

The outcomes revealed that the models for the prediction of Tchl based on solvent free methods (IUPAC and AOCS) had a better performance when using UV light. On the other hand, the predictive models for Tcar or TSC (that include carotenoids absorption) had better performance when using white light. As reported to Hakonen and Beves (2018), fluorescence

might cause inner-filter effects when detected at a 90° angle from the excitation light, due to the existence of particles in a liquid medium. This effect would be even more pronounced in oils rich in chlorophylls (fluorescent species), as olive and avocado oils are. They suggested that this effect, when using a 405 nm excitation light, could provide additional information for distinguishing vegetable oils based on color parameters. This could explain the better performance when predicting Tchl based on solvent free methods. This is based on the more concentrated medium, that could be more susceptible to inner-filter effects, thus contributing to a better correlation between the color parameters and the chlorophyll content.

The prediction of Tchl based on solvent free methods also had superior outcomes, when using HSV values as descriptors that can be attributed to fluorescence effects. According to Hakonen and Beves (2018), HSV (Hue, Saturation, Value) is a color space commonly used in digital imaging, and the hue parameter was introduced as a quantitative tool for optical chemical sensors, including fluorescence measurements. They reported that oils rich in chlorophylls, such as olive oils, presented hue values considerably higher than that with low chlorophylls content when illuminated by a 405 nm LED. This suggests that the hue could have a strong correlation with the content of fluorescent species, such as chlorophylls.

The color space used as descriptor influences considerably the model performance (ANCONI et al., 2022; DE CARVALHO & NUNES, 2021; RESENDE et al., 2023). This effect occurs due how the color space describes the observed color. For example, L\*a\*b\* is three-dimensional color space that covers the entire range of human color perception. The L\* value (lightness) defines a scale from black to white, while a\* and b\* are chromatic components, being a\* representing green-red opponent colors, and b\* the blue-yellow opponents (IBRAHEEM et al., 2012). RGB system describes a color by an additive model, in which the linear combination of the three primary colors, red (R), green (G), and blue (B). Therefore, these different ways to describe a color impact its correlation with the interested response, such as the content of pigments in oils. This effect results in differences in modeling performance depending on the color system used as descriptor, which is consistent with conclusions of other studies. Carvalho and Nunes (2021) presented some good models, when predicting Tchl in olive and avocado oils using RGB or HSV values obtained with smartphone camera. Similarly, Moyano et al. (2008) found that L\*a\*b\* had a good correlation with carotenoid content in olive oils, when using complex regression models, with  $R^2 > 0.86$ .



The superiority of LS-SVM over MLR was observed for all the predictive models, including TchI, Tcar, and TSC. In fact, some authors emphasize that machine learning techniques, such as SVM, can complement the implementation of cheap sensors, compensating some limitations in their design and manufacture (PAYETTE et al., 2023; LORENZO et al., 2024). While MLR is a regression method that relying on the linear relationship between dependent and independent variables (SLINKER & GLANTZ, 2008), LS-SVM employs a non-linear and computationally intensive algorithm that exhibits greater generalization power (YU et al., 2009). SVM operates as an optimization-based model that seeks the best hyperplane to maximize margins within a high-dimensional space. Consequently, in SVM, each data point influences the final optimization (OTHMAN et al., 2023), unlike linear regression, in which the impact of all data points is uniform.

Even though not predicting the exact natural pigments content, the use of optic or colorimetric sensors has been used to assess properties depended on the chlorophylls or carotenoids content in oils. The main applications involve the authentication of oils, especially blends of extra virgin oils (high natural pigment content) with other vegetable oils (commonly refined and with low or no pigment content). LORENZO et al. (2024) demonstrated that the same color sensor used in this study was able to detect blends of vegetable oils in avocado oil. In other study, a colorimetric sensor array associated to LDA was used for distinguishing extra virgin olive oil from its mixtures with soybean and corn oil (HUANG et al., 2022). Also, a hyphenated photonics sensor was used to discriminate extra virgin olive oil adulterated with other edible oils using and one-class classification modelling (WEESEPOEL et al., 2021). All these works based on the color sensor-based methods achieved considerable performances, corroborating the findings of the present study.

## **5. CONCLUSION**

In this proof-of-concept study, a device based on a color sensor and chemometrics models proven to be able to predict the levels of carotenoids and chlorophylls in olive and avocado oils, in addition to their TSC. The superiority of LS-SVM over MLR observed for all the predictive models can be attributed to mathematical skills of each method regarding data processing capabilities and their ability to describe the predicted response. The use of a UV lighting demonstrated to improve the predictive performance for total chlorophylls content when the response is based on solventless methods (IUPAC and AOCS methods). Otherwise, a white lighting was more appropriated. The color space used as descriptor also influenced the

predictive accuracy depending on the type of variable and reference method. HSV predicted better total chlorophylls referenced on solventless methods. RGB was more adequate to predict TSC and total chlorophylls referenced on methods that use solvent dilution. Finally, total carotenoids content was better predicted using  $L^*a^*b^*$  values as descriptors. Thus, the color sensor-based method demonstrated to be feasible as an alternative for rapid and cost-effective prediction of pigments content in vegetable oils without the necessity of sample pretreatment.

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