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USO DE GOJI BERRY COMO CONSERVANTE E ESTABILIZANTE NATURAL EM HAMBÚRGUERES DE CARNE BOVINA

MELINA APARECIDA PLASTINA CARDOSO

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Tese apresentada ao programa de Pós Graduação em Ciência de Alimentos da Universidade Estadual de Maringá, como parte dos requisitos para obtenção do título de doutora em Ciência de Alimentos.

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BIOGRAFIA

Melina Aparecida Plastina Cardoso nasceu em 18 de fevereiro de 1989, na cidade de Arapongas, Paraná. Possui graduação em Engenharia de Alimentos pela Universidade Estadual de Ponta Grossa (UEPG) e mestrado em Ciências de Alimentos pela Universidade Estadual de Londrina (UEL). Atualmente, é doutoranda em Ciência de Alimentos pela Universidade Estadual de Maringá (UEM). Atuou como Agente Local de Inovação (ALI) pelo SEBRAE, de 2014 a 2016, e como professora adjunta nível B na Universidade Pitágoras UNOPAR e Universidade Anhanguera (UNIDERP) de 2017 a 2019. Tem experiência na área de Engenharia de Alimentos, com ênfase em Ciência e Tecnologia de Alimentos, atuando principalmente nos seguintes temas: ciência tecnologia de alimentos, engenharia de produção, gestão da inovação, empreendedorismo, gestão de projetos e gestão de produtos, desenvolvimento de novos produtos e gerenciamento e controle de qualidade.

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SUMÁRIO

APRESENTAÇÃO

Esta tese de doutorado está apresentada na forma de três artigos científicos publicados:

1. Melina Aparecida Plastina Cardoso, Charles Windson Isidoro Haminiuk, Alessandra Cristina Pedro, Isabela de Andrade Arruda Fernandes Fernandes, Maira Akemi Casagrande Yamato, Giselle Maria Maciel & Ivanor Nunes Do Prado (2023) **Biological Effects of Goji Berry and the Association with New Industrial Applications: A Review**, Food Reviews International, 39:5, 2990-3007.

2. Melina Aparecida Plastina Cardoso, Ana Carolina Pelaes Vital, Aylle Medeiros, Bianka Rocha Saraiva, Ivanor Nunes do Prado (2023) **Goji Berry effects on hamburger quality during refrigerated display time,** Food Sci. Technol, Campinas, 43, e68322.

3. Melina Aparecida Plastina Cardoso, Camila Fogaça de Oliveira, Rodolfo Lopes Coppo, Maira Akemi Casagrande Yamato, Alessandra Cristina Pedro, Pietro Martins de Oliveira, Venicio Macedo Carvalho, Ivanor Nunes do Prado (2022) **Computer vision as the golden tool: mathematical models for evaluating color and storage time of hamburgers with Goji Berry natural additive**. Food Sci. Technol, Campinas, 42, e35822

GENERAL ABSTRACT

Introduction

The Goji Berry or wolfberry (Lycium barbarum L.) belongs to the Lycium genus, originating from Asia and can be cultivated in different regions of the world. Among its different species, 35 are commonly used for consumption and/or medicinal purposes. Among its characteristics, its fruits stand out for being rich in phenolic antioxidants and have five classes of polyphenols: benzoic acids, catechins, cinnamic acids, flavonoids and tannins, as well as terpenes, organic acids and vitamin C. This source of phytochemicals can be related to important biological functions, such as increasing the activity of antioxidant enzymes, namely superoxide dismutase (SOD) and glutathione peroxidase (GPX).

Currently, there is an increase in demand for natural, healthier and safer foods, resulting in the replacement of synthetic antioxidants with natural products. In this search, the food industry has explored the antioxidant capacity of products, such as Goji Berry.

The phenols found in the fruit are associated with maintaining the integrity of food and its natural appearance. Therefore, this product can be considered an interesting additive, as it is safe for food products and improves their nutritional, physical-chemical and visual characteristics during storage time. Therefore, this work aimed to improve studies on the biological activities present in Goji Berry, evaluating its role in the quality of conditioning in the life of a hamburger grill, in addition to analyzing a computational model for its visual results.

Aim

This work aimed to verify the effect of Goji Berry on burger quality (pH, color, water loss, shear force, antioxidant activity and lipid oxidation) during 10 days under refrigeration. Furthermore, we sought to propose a mathematical model based on computer vision to investigate the performance of Goji Berry (in powder or aqueous extract) as a natural preservative to maintain the color of hamburger-type products.

Material and methods

Goji Berry or *Lycium barbarum L.*, black pepper (*Piper nigrum L.*) and commercial salt (Ajisal®) were purchased at the local market in Maringá, Paraná, Brazil. To verify the effect of Goji Berry (GB) on the quality of the burger, the GB fruit was dried in an oven at 55° C for 48 hours and crushed in a grain crusher (Hamilton Beach®). Different treatments were established: control (CONT) – hamburger without GB extract or powder, GBEX – hamburger with GB extract, GBPW – hamburger with GB powder and GBEC – hamburger with edible coating containing GB. Samples were analyzed on days 1, 3, 7 and 10 of exposure.

For GBEX, 30 g of GB was suspended with 1 L of water (70 °C) for 30 min. and the mixture was cooled to room temperature, added to the minced meat and homogenized. For GBEC, 20 g of alginate was dissolved in 1 L of the GB extract thus obtained (at 70 °C). The burgers were then submerged in alginate solution for 1 min, left to drain (to remove excess coating) for 1 min, submerged in calcium chloride solution (2% w/v) used as a crosslinker for 30 s to result in GBEC

samples with ~3% GB m/m. Each burger was packaged (polystyrene tray), covered with a shrink film and stored in an illuminated display (2° C) (fluorescent lamp, 1200 lux, 12 h day-1).

pH analysis was performed on days 1, 3, 7 and 10 of storage, using a digital pH meter (Tradelab, Contagem, MG, Brazil) with a penetration electrode. For water loss analysis, the individual weights of the burgers were recorded on each day of analysis. Shear force (N) was analyzed using the TA.XT Plus with a Warner-Bratzler blade. Color was assessed using a Minolta CR-400 colorimeter (10° viewing angle and a D65 illuminant). Four measurements were recorded on each burger: brightness (L*), red tone (a*) and yellow (b*) and the chroma and hue values were calculated.

For lipid oxidation, the malonaldehyde (MDA) content was quantified using the TBARS assay, the total phenolic compounds (TPC) content was quantified by the Folin-Ciocalteu assay. The radical scavenging test was carried out using the Gallic acid, 2, 20-azinobis-3-ethylbenzotiazoline-6-sulfonic acid (ABTS) and 2, 2-diphenyl-1-picrylhydrazyl (DDPH) test.

For statistical analysis, the general linear model (GLM) (SPSS, v.20.0) was used. Means and standard deviation were calculated for each variable. Exposure time and type of goji application were considered fixed factors (factorial design) with four replicates per treatment. The experiment was repeated twice. When the differences were significant, the Tukey test was applied ($P < 0.05$). All samples were analyzed in triplicate.

To propose the mathematical model based on computer vision, the GB fruit was dried in an oven at 55°C for 72 hours and finely crushed for 2 minutes. The aqueous extract was prepared by dissolving the powder in distilled water and then added in different concentrations to the burgers.

Ten burgers (~50 g, ~2 cm high) were prepared with a burger making machine with different proportions (% w/w) that varied up to 6% addition of GB powder and 6% GB aqueous extract.

All samples were placed in white Styrofoam trays, sealed with flexible polyvinyl chloride (PVC) film and stored randomly under refrigeration (2-4°C), free from contamination, under conditions comparable to commercial/domestic conditions.

The photographs were taken at intervals of 1, 3, 7 and 10 days through a black box where a cell phone camera was attached and operated without the use of flash and zoom. The analysis of the photographs was carried out using the CIE (International Electrotechnical Commission) laboratory system, transforming the RGB components (red, green and blue) into the XYZ model.

Results and discussion

Fresh burgers are susceptible to rapid deterioration, so the best combination is sought to maintain high moisture, protein and lipid content for optimal conservation. The use of Goji Berry in fresh burgers showed positive effects on quality during the exposure time. When compared to the different groups, samples with edible coating showed the best results, with lower losses of water ($P < 0.016$) and color (luminosity $P > 0.005$), lower pH values ($P < 0.05$) and greater softness. Another aspect observed through its use was the increase in the product's antioxidant activity and inhibition of lipid oxidation. These results demonstrated that the association of the edible coating on burgers results in the prevention of water loss, in addition to less oxidation.

The use of the mathematical model for the computational evaluation demonstrated that the addition of Goji Berry to burgers, introduced in a pioneering way in this study, presented advantageous characteristics both in terms of storage time and possible positive effect on human health, due to the polyphenols in the additive. Natural.

Among the different models evaluated, samples five (meat + commercial salt + pepper + 3% aqueous goji extract) and samples 7 to 10 (meat + Goji Berry only, in powder or aqueous extract) demonstrated different luminosity results, and colors perceptible to the human eye. The meat colors from these trials remained visually acceptable during a 10-day evaluation, proving the role of the fruit's antioxidants as a good preservative.

When it comes specifically to color, this study also provided a new insight into the predictive mathematical model, being able to present a quick, low-cost and viable homemade system to evaluate the color of meat and stipulate a realistic storage time, in which the visual characteristics remain. However, to expand the use of this computer vision model, some adjustments are necessary to obtain results with greater precision, such as using software with environmental control, automating the process of obtaining images and using Machine Learning before extrapolating the results.

These results demonstrated that GB has real potential for application in the meat industry to maintain/improve the characteristics of the final product during the conservation period. Both natural compounds isolated or incorporated with GB were able to improve the quality and shelf life of meat products, preventing their lipid oxidation.

Conclusion

The use of Goji Berry can provide positive effects on the quality of the visual characteristics and conservation of burgers. Its use was able to present positive results in relation to weight and color losses, pH values and greater softness, in addition to increasing the antioxidant activity of the product and inhibiting its lipid oxidation. As for the computational model, the results of the analysis demonstrated that it is a viable method. However, adjustments are necessary, such as the use of software with environmental control, automation of the image acquisition process and use of techniques such as Machine Learning before extrapolating the results.

Keywords: Food industry, Lycium barbarum, meat products, natural products, shelf life, mathematical modeling for foods.

RESUMO GERAL

INTRODUÇÃO

A Goji Berry ou wolfberry (*Lycium barbarum L.*) pertence ao gênero Lycium, originária da Ásia e pode ser cultivada em diferentes regiões do mundo. Entre suas diferentes espécies, 35 são comumente usadas para consumo e/ou com propósito medicinal. Entre suas características, seus frutos se destacam por serem ricos em antioxidantes fenólicos e apresentam cinco classes de polifenóis: ácidos benzóicos, catequinas, ácidos cinâmicos, flavonoides e taninos, além de terpenos, ácidos orgânicos e vitamina C. Essa fonte de fitoquímicos pode ser ser relacionada com importantes funções biológicas, como o aumento da atividade das enzimas antioxidantes, nomeadamente a superóxido dismutase (SOD) e a glutationa peroxidase (GPX).

Atualmente, vive-se o aumento da procura de alimentos naturais, mais saudáveis e seguros, consequentemente a substituição de antioxidantes sintéticos por produtos naturais. Nessa busca, a indústria de alimentos tem explorado a capacidade antioxidante de produtos, como a Goji Berry.

Os fenóis, encontrados no fruto, são associados a manutenção da integridade dos alimentos e sua aparência natural. Dessa forma, esse produto pode ser considerado um aditivo interessante, uma vez que é segura para os produtos alimentares e melhora as suas características nutricionais, físico-químicas e visuais durante o tempo de armazenamento. Portanto, este trabalho se propôs a aprimorar os estudos sobre as atividades biológicas presentes na Goji Berry, avaliando sua atuação na qualidade de condicionamento na vida de prateleira de hambúrgueres, além da análise de um modelo computacional para os seus resultados visuais.

OBJETIVOS

Os objetivos desse trabalho foram: verificar o efeito da Goji Berry na qualidade do hambúrguer (pH, cor, perdas de água, força de cisalhamento, atividade antioxidante e oxidação lipídica) durante 10 dias sob refrigeração. Além disso, buscou-se propor um modelo matemático baseado na visão computacional para investigar o desempenho da Goji Berry (em pó ou em extrato aquoso) como conservante natural para manter a cor de produtos do tipo hambúrguer.

MATERIAL E MÉTODOS

Goji Berry ou *Lycium barbarum L.*, pimenta-do-reino (*Piper nigrum L.*) e o sal comercial (Ajisal®) foram adquiridos no mercado local de Maringá, Paraná, Brasil. Para verificar o efeito da Goji Berry (GB) na qualidade do hambúrguer, a fruta da GB foi seca em estufa a 55° C por 48 horas e triturada em um triturador de grãos (Hamilton Beach®). Foram estabelecidos diferentes tratamentos: controle (CONT) – hambúrguer sem extrato ou pó de GB, GBEX – hambúrguer com extrato de GB, GBPW – hambúrguer com GB em pó e GBEC – hambúrguer com revestimento comestível contendo GB. As amostras foram analisadas nos dias 1, 3, 7 e 10 de exposição.

Para o GBEX, 30 g de GB foram suspensos com 1 L de água (70 °C) durante 30 min. e a mistura foi resfriada à temperatura ambiente, adicionada à carne picada e homogeneizada. Para o GBEC, 20 g de alginato foram dissolvidos em 1 L do extrato de GB assim obtido (a 70 ºC). Os hambúrgueres foram então submersos em solução de alginato durante 1 min, deixados a escorrer (para remover o excesso de revestimento) durante 1 min, submersos em solução de cloreto de cálcio (2% p/v) utilizada como reticulante durante 30 s para resultar nas amostras de GBEC com ~3% GB m/m. Cada hambúrguer foi embalado (tabuleiro de poliestireno), coberto com uma película retrátil e armazenado num expositor iluminado (2° C) (lâmpada fluorescente, 1200 lux, 12 h dia^{-1}).

A análise do pH foi realizada nos dias 1, 3, 7 e 10 de armazenamento, utilizando um medidor de pH digital (Tradelab, Contagem, MG, Brasil) com um eletrodo de penetração. Para análise de perda de água, os pesos individuais dos hambúrgueres foram registados em cada dia de análise. A força de cisalhamento (N) foi analisada utilizando o TA.XT Plus com uma lâmina Warner-Bratzler. A cor foi avaliada utilizando um colorímetro Minolta CR-400 (ângulo de visão de 10° e um iluminante D65). Foram registadas quatro medições em cada hambúrguer: luminosidade (L*), tom vermelho (a*) e amarelo (b*) e calculados os valores de croma e matiz.

Para a oxidação lipídica, o teor de malonaldeído (MDA) foi quantificado pelo ensaio TBARS, o teor de compostos fenólicos totais (TPC) foi quantificado pelo ensaio de Folin-Ciocalteu. O teste de eliminação de radicais foi realizado utilizando o teste do ácido gálico, ácido 2,20-azinobis-3-etilbenzotiazolina-6-sulfônico (ABTS) e 2,2-difenil-1-picrilhidrazil (DDPH).

Para análise estatística foi utilizado o modelo linear geral (GLM) (SPSS, v.20.0). As médias e o desvio padrão foram calculados para cada variável. O tempo de exposição e o tipo de aplicação da goji foram considerados fatores fixos (design fatorial) com quatro réplicas por tratamento. A experiência foi repetida duas vezes. Quando as diferenças foram significativas, foi aplicado o teste de Tukey ($P < 0,05$). Todas as amostras foram analisadas em triplicata.

Para a proposta do modelo matemático baseado na visão computacional a fruta da GB foi seca em estufa a 55°C por 72 horas e finamente triturada por 2 minutos. O extrato aquoso de foi preparado dissolvendo o pó em água destilada e depois adicionado em diferentes concentrações aos hambúrgueres.

Foram preparados dez hambúrgueres (~50 g, ~2 cm de altura) com uma máquina de fazer hambúrgueres com diferentes proporções (% w/w) que variavam até 6% de adição de GB em pó e 6% de extrato aquoso de GB.

Todas as amostras foram colocadas em tabuleiros de esferovite brancos, selados com película flexível de cloreto de polivinilo (PVC) e armazenadas aleatoriamente sob refrigeração (2-4°C), isentas de contaminação, em condições comparáveis às comerciais/domésticas.

As fotografias foram obtidas nos intervalos de 1, 3, 7 e 10 dias através uma caixa preta onde uma câmera de um celular foi acoplado e operado sem a utilização de flash e zoom. A análise das fotografias foi realizada utilizando o sistema de laboratório da CIE (Comissão Eletrotécnica Internacional) transformando os componentes de RGB (vermelho, verde e azul) para o modelo XYZ.

RESULTADOS E DISCUSSÃO

Hambúrgueres frescos são suscetíveis a uma rápida deteriorização, dessa forma busca-se a melhor combinação para manter elevado o teor de umidade, proteínas, lípidios para uma conservação ideal. A utilização da Goji Berry em hambúrgueres frescos apresentou efeitos positivos na qualidade durante o tempo de exposição. Quando comparados aos diferentes grupos, as amostras com revestimento comestível apresentaram os melhores resultados, com menores perdas de água ($P < 0,016$) e cor (luminosidade $P > 0,005$), menores valores de pH ($P < 0,05$) e maior maciez. Outro aspecto observado através da sua utilização foi o aumento da atividade antioxidante do produto e inibição da oxidação lipídica. Esses resultados demonstraram que a associação do revestimento comestível nos hambúrgueres resulta no impedimento da perda de água, além de uma menor oxidação.

A utilização do modelo matemático para a avaliação computacional demonstrou que a adição de Goji Berry nos hambúrgueres, introduzida de forma pioneira neste estudo, apresentou características vantajosas tanto em termos de tempo de armazenamento como de possível efeito positivo na saúde humana, devido aos polifenóis do aditivo natural.

Entre os diferentes modelos avaliados, as amostras cinco (carne + sal comercial + pimenta + 3% de extrato aquoso de goji) e amostras 7 a 10 (carne + Goji Berry somente, em pó ou em extrato aquoso) demonstraram resultados distintos de luminosidade, e cores perceptíveis ao olho humano. As cores da carne destes ensaios permaneceram visualmente aceitáveis durante uma avaliação de 10 dias, provando o papel dos antioxidantes da fruta como um bom conservante.

Em se tratando especificamente da cor, este estudo proporcionou ainda uma nova visão sobre o modelo matemático preditivo, sendo capaz de apresentar um sistema caseiro rápido, de baixo custo e viável para avaliar a cor da carne e estipular um tempo de armazenamento realista, em que as características visuais se mantêm. Todavia, para ampliação da utilização desse modelo de visão computacional, são necessários alguns ajustes para obter os resultados com maior precisão, como utilizar softwares com controle de ambiente, automatizar o processo de obtenção de imagens e utilizar *Machine Learning* antes de extrapolar os resultados.

Estes resultados demonstraram que a GB apresenta potencial real na aplicação na indústria da carne para manter/melhorar as características do produto final durante o período de conservação. Tanto os compostos naturais isolados ou incorporados com a GB foram capazes de melhorar a qualidade e o prazo de validade dos produtos cárneos, evitando a sua oxidação lipídica.

CONCLUSÕES

A uilização de Goji Berry pode proporcionar efeitos positivos na qualidade das características visuais e de conservação dos hambúrgueres. A sua utilização foi capaz de apresentar resultados positivos em relação a perdas de peso e cor, valores de pH e maior maciez, além de aumentar a atividade antioxidante do produto e inibir a sua oxidação lipídica. Quanto ao modelo computacional, os resultados da análise demonstraram ser um método viável. Entretanto, ajustes são necessários, como o uso de softwares com controle de ambiente, automatização do processo de obtenção de imagens e utilização de técnicas como *Machine Learning* antes de extrapolar os resultados.

Palavras chaves: Indústria alimentícia, *Lycium barbarum*, produtos cárneos, produtos naturais, vida de prateleira, modelagem matemática para alimentos.

REVISÃO BIBLIOGRÁFICA

1. Características, composição e principais utilizações do Goji Berry

A Goji Berry (GB) ou *wolfberries*, conhecida cientificamente como *Lycium barbarum*, tem recebido destaque devido às suas propriedades nutricionais e benefícios à saúde (Ma et al., 2023). É uma fruta vermelha originária da região asiática e tem sido cultivada há séculos por suas propriedades medicinais e nutricionais (Yao et al., 2018). Sua composição inclui uma variedade de nutrientes essenciais, como vitaminas (C, A, B2), minerais (ferro, zinco, selênio), aminoácidos, polissacarídeos e antioxidantes, como os carotenoides e flavonoides (Silva et al., 2022). Essa combinação única confere a Goji Berry propriedades antioxidantes, anti-inflamatórias (sendo associado à redução de processos inflamatórios no corpo) e imunomoduladoras, pois tem demonstrado em estudos a prevenção de doenças crônicas e ao fortalecimento do sistema imunológico (Donno et al., 2015).

A presença de polissacarídeos, especialmente beta-glucanos, é o que confere propriedades imunomoduladoras à fruta. O que se sabe é que estes polissacarídeos estimulam a produção de citocinas e ativam células do sistema imunológico, promovendo uma resposta equilibrada (Yao et al., 2018).

Os antioxidantes presentes na GB combatem os radicais livres, ajudando a prevenir o estresse oxidativo e contribuindo para a saúde celular. A capacidade antioxidante é atribuída principalmente aos carotenoides, como a zeaxantina e a beta-caroteno. Estes compostos são capazes de neutralizar os radicais livres, protegendo as células contra danos oxidativos (Silva et al., 2022).

Componentes bioativos, como quercetina e ácido ascórbico, contribuem para as propriedades anti-inflamatórias (interferindo em vias de sinalização celular que levam à produção de mediadores inflamatórios). Além disso, atuam também como antioxidantes, na inibição de enzimas oxidativas, modulação da expressão gênica, apresentam proteção cardiovascular e atividade anticancerígena (Soares et al., 2019).

Alguns estudos indicam que a Goji Berry pode, ainda, influenciar positivamente o metabolismo, auxiliando na regulação do peso e na melhoria dos níveis de glicose no sangue. Na medicina tradicional chinesa, a Goji Berry é utilizada para fortalecer o sistema imunológico, melhorar a visão e promover a longevidade. Pesquisas estão em andamento para avaliar seu potencial em tratamentos complementares, como em casos de diabetes e doenças neurodegenerativas (Ma et al., 2023).

A indústria farmacêutica também vem buscando sua utilização e inclusão, principalmente, no que diz respeito a formulações de suplementos nutricionais e multivitamínicos devido à sua rica composição (Lee & Choi, 2023; Manganaris et al., 2014; Püssa et al., 2008; Yu et al., 2023).

Na área da cosmética, têm-se estudado a inclusão da GB em produtos de cuidados com a pele devido às propriedades antioxidantes que podem ajudar na proteção contra danos causados pelos radicais livres. Novas pesquisas têm explorado seu uso em produtos anti-envelhecimento e de proteção solar e apontam resultados satisfatórios porém, que necessitam da avaliação das concentrações utilizadas e das formulações (Cheng et al., 2023).

Especificamente na indústria de alimentos, a GB tem sido utilizada na adição de barras de cereais, sucos, iogurtes, cereais matinais e produtos cárneos para enriquecer o seu valor nutricional, melhorar suas características sensoriais e buscar aumentar sua vida de prateleira (Bulambaeva et al., 2014; Donno et al., 2015; Manganaris et al., 2014). Tem sido amplamente utilizada, também, em chás e infusões para aproveitar as suas propriedades antioxidantes. Além disso, tem-se notado a sua inclusão em produtos de panificação, como pães e bolos, para agregar valor nutricional e funcional (Törrönen et al., 2013).

2. Influência da Goji Berry na qualidade e vida de prateleira de produtos alimentícios e de hambúrgueres

A inclusão da GB em produtos alimentícios tem sido uma estratégia adotada pela indústria para conferir benefícios à saúde e agregar valor aos produtos. Os antioxidantes presentes na GB contribuem para a estabilidade dos alimentos, impedindo a oxidação de lipídios e proteínas, o que pode resultar em uma maior vida de prateleira dos produtos (Püssa et al., 2008).

Além disso, a presença de vitaminas e minerais nesta fruta podem melhorar o perfil nutricional de alimentos processados, atendendo as demandas dos consumidores por opções mais saudáveis (Silva et al., 2022). A sua capacidade de modular o sabor e a cor dos alimentos também oferece oportunidades para inovações na indústria alimentícia (Castrica et al., 2020).

No contexto específico dos hambúrgueres, a adição de Goji Berry pode ser uma estratégia interessante para elevar o valor nutricional do produto (Lorenzo et al., 2018). A presença de aminoácidos essenciais e proteínas do fruto pode complementar a composição proteica do hambúrguer, tornando-o uma opção mais saudável (Tudor et al., 2022).

Os antioxidantes da GB podem proteger os lipídios da carne do hambúrguer contra a oxidação durante o armazenamento, contribuindo para a qualidade sensorial e garantindo uma vida de prateleira prolongada (Lorenzo et al., 2018; Manganaris et al., 2014). Além disso, a cor vibrante da GB pode conferir uma aparência visualmente atraente ao hambúrguer, o que é crucial na decisão de compra dos consumidores (Agradi et al., 2022).

3. A visão computacional como uma ferramenta de ouro na análise de cor de hambúrgueres

A oxidação lipídica em hambúrgueres é um processo em que os lipídios presentes na carne reagem com o oxigênio do ar, resultando na formação de compostos indesejados que afetam o sabor, aroma e cor (Amaral et al., 2018; Domínguez et al., 2019; Pateiro et al., 2018). Os antioxidantes presentes na GB podem ser capazes de neutralizar os radicais livres gerados durante a oxidação lipídica. Eles doam prótons aos radicais livres, interrompendo a reação em cadeia que leva à formação de compostos oxidados (Li et al., 2009; Lorenzo et al., 2018). Ao neutralizar os radicais livres, os antioxidantes da Goji Berry preservam a estabilidade dos lipídios na carne do hambúrguer, impedindo a formação de produtos de degradação que contribuem para a deterioração da qualidade do produto (Bulambaeva et al., 2014).

Com isso, o que se tem, dentre as inúmeras reações, é a perda pouco significativa do hambúrguer adicionado de GB quando comparado ao hambúrguer "*in natura*", que pode estar associado à capacidade dos antioxidantes em manter a integridade dos pigmentos naturais presentes na carne como, por exemplo, a mioglobina, responsável pela cor vermelha característica da carne fresca (Soares et al., 2019).

A visão computacional (VC) pode envolver o uso de análise espectral para avaliar as mudanças na cor (Girolami et al., 2013; Tarlak et al., 2016; Tomasević et al., 2019). Espectrogramas podem mostrar a distribuição de cores ao longo do tempo, permitindo identificar variações sutis (Jackman et al., 2011; O'Sullivan et al., 2003). Neste contexto, algoritmos de visão computacional podem comparar imagens de hambúrgueres antes e depois da adição de Goji Berry, tanto em pó, como na forma de extrato ou adicionados a formulações de coberturas comestíveis. Mudanças na intensidade e distribuição de cores podem ser detectadas e quantificadas.

Pode-se, então, extrair características específicas das imagens, como valores de cor média, saturação e brilho, para avaliar alterações na aparência do hambúrguer ao longo do tempo. Assim, considera-se também, as técnicas de aprendizado de máquina, que podem ser empregadas para treinar algoritmos a reconhecer padrões de mudanças na cor relacionadas à oxidação lipídica. Isso pode facilitar a detecção automática de pequenas variações na cor (Girolami et al., 2013; O'Sullivan et al., 2003; Tomasević et al., 2019).

A visão computacional tem sido explorada como uma ferramente rápida, barata e não invasiva como uma alternativa para análise de carne e seus co-produtos. Imagens digitais são capazes de captar informações gerais e permite estocar os dados para transforma-los sistemas demultivariadas cores para estudos futuros (Lima et al., 2022; O'Sullivan et al., 2003; Passetti et al., 2017, 2019)

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ARTIGO 1 - Biological Effects of Goji Berry and the Association with New Industrial Applications: A Revie

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Biological Effects of Goji Berry and the Association with New Industrial Applications: A Review

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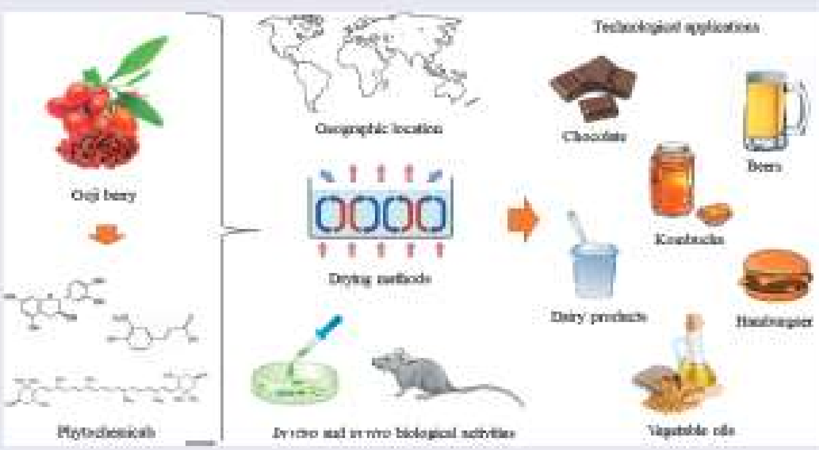
ABSTRACT

Several studies have shown that the consumption of goji berries, which belong to the *Lycium* genus, can reduce the risk of developing carcinogenic, neurodegenerative, ocular, nephrological and liver diseases. This fruit's functional characteristics have attracted the attention of researchers and industries from different areas. In this review, we present a critical and detailed analysis of the studies published for the last five years that are related to the techniques of geographic characterisation, biological activities *in vitro* and *in vivo*, processing methods and technological applications of goji berry. The studies demonstrate that the bioactive compounds of goji berry and their beneficial effects on the human organism are important for geographical classification, processing methods and particularly the development of new products for specific purposes. The current study will allow researchers to investigate new benefits of goji fruits and hence develop innovative products in different industrial segments.



KEYWORDS

Solanacea; wolfberry; bagas; biological activities; *Lycium barbarum*

Introduction

Goji berries or wolfberries belong to the Lycium genus and are grown in different regions of the world.[1] In East Asia, the *L. barbarum*, L. (Ningxiagouqi) and *L. chinense* Miller (Gouqi) are the main goji berries used for consumption and medicinal purposes. However, there are several varieties and species distributed worldwide, such as *L. barbarum* var. *aurantiocarpum* (Huangguogouqi), *L. chinense* var. *potaninii* (Beifanggouqi), *L. ruthenicum* (Heiguogouki) and *L. truncatum* (Jieegouki).[2] Every year, new species are found, which increases the worldwide consumption of goji berries and attracts the attention of the scientific academy.

The knowledge of geographic location is important for differentiating goji fruits. Characteristics, such as climate, soil type and cultivation methods, lead to differences in the composition of these fruits and directly influence their biological properties and modes of application.[3] Researchers have developed highly efficient methods for the geographic differentiation of goji fruits; spectrophotometric, chromatographic and statistical and chemometric analyses are employed to determine their morphological, botanical and phytochemical characteristics.[4–9]

Goji berries are sources of phytochemicals with important biological functions and are designated as superfruits. Polysaccharides are among the main bioactives found in goji fruits, followed by carotenoids, especially zeaxanthin, phenolic and flavonoid compounds, catechins and *p*-cumárico, as well as caffeic and chlorogenic acids. Finally, there are steroids and alkaloids in goji fruits.[10]

These phytochemicals have been related to the decrease in the risk of disease development in *in vitro* and *in vivo* studies: anti-hyperglycemic and anti-aging[11]; anti-coagulants and anti-platelets[12]; anti-inflammatory drugs[13]; antitumor[14] and neuroprotective.[15,16] In addition, they protect the liver and kidney,[17] as well as the ocular,[18,19] gastrointestinal[20] and skeletal muscle.[21] These properties are mainly associated with the increased activity of antioxidant enzymes, namely superoxide dismutase (SOD) and glutathione peroxidase (GPX).[16]

Commercially, goji fruits are dehydrated by different drying methods to increase the shelf life and improve logistics.[22] However, due to the high temperature and long drying periods, dehydration can cause nutritional, sensory and phytochemical losses. Thus, studies have been developed to reduce the drying time and maintain the qualitative properties of goji berry by applying chemical and physical pre-treatments.[23–25]

The reduction in the drying time of the goji fruit also determines the purpose of its application in product development. Goji fruits have been applied in the formulation of beers, milk and yogurts, chocolates, kombucha, muffins, hamburgers, and edible oils for functional and antioxidant purposes. The characteristics of goji berry will enable the establishment of future studies on various products.[26,27]

Due to the considerable interest in goji berries and the lack of detailed studies related to this fruit, the objectives of this review were to: (1) present the main botanical and morphological characteristics in addition to analytical techniques for their geographic characterisation; (2) discuss *in vitro* and *in vivo* studies that evaluate the biological activity of phytochemicals present in *Lycium* fruits; and (3) demonstrate the main processing methods and technological applications of goji berry.

Goji berry

Botanical and morphological characteristics

The goji berry belongs to the kingdom Plantae, family Solanaceae and genus *Lycium*. More than 90 species are known and distributed across different regions of the world. However, only 35 species are used for consumption and medicinal purpose.[1] The most commonly found names are 'goji berry,' 'wolfberry,' 'gou-qi-zi' and 'fructus Lycii'. The term 'goji' is an adaptation of a series of native words and was coined by Tanaduk Botanical Research Institute in 1973.[28]

In general, plants of the *Lycium* genus are thorny shrubs and known as 'boxthorn', with a height of up to 3 m. They are found in plateau regions (700–2700 m altitude) of arid and semi-arid climate. The shrub has overlapping, thorny and leafy branches, and the leaves are fleshy and narrowly linear. The flowers are small, can occur alone or in groups and have a purple or blue-violet corolla.[1]

The fruits are of great commercial importance and are up to 2 cm long. Besides, they are succulent, with a bitter sweet taste and a red-orange to black colour. The fruit's pericarp wall is composed of the exocarp (skin), mesocarp (pulp) and lignified endocarp around the seeds. The exocarp presents thin, amorphous, waxy epicuticular films with finely thickened collenchyma cells with periclinal walls, which makes the fruit soft and highly sensitive to mechanical damage. Exocarp and mesocarp cells have a high concentration of chromoplast which are heterogeneous organelles responsible for pigment storage and synthesis.[22] The structure of *Lycium* plants is shown in Figure 1.

The species *L. barbarum, L. chinense, L. ruthenicum, L. intricatum* and *L. europeaum* are the most frequently reported in the literature regarding *in vitro* and *in vivo* biological studies, food, cosmetic and pharmaceutical applications, in addition to nutritional and phytochemical composition studies. According to Yao et al.,[1] the species *L. barbarum, L. chinense* and *L. ruthenicum* are widely produced in China, Africa and Europe, while *L. intricatum* and *L. europeaum* have higher production in the Mediterranean and Middle East.

Among the species mentioned, the *L. ruthenicum* ('black goji') provides fruits with a black-purple colour and brownish seeds.[29] The *L. barbarum* fruits are red-orange, sweet, 2 cm long and have few yellow seeds. *L. chinense* fruits are orange, approximately 1 cm long, have many seeds and a bitter taste.[1] The *L. intricatum* species ('Awsadj') and *L. europeaum*, whose leaves, seeds and fruits are used for different purposes, are highly produced in Tunisia.[30]

The *L. barbarum* species is the most cultivated worldwide, and, although all the mentioned species are commercialised, the Pharmacopoeia of the People's Republic of China and the European Union's New Food Catalog recognise only *L. barbarum* as a food or food ingredient.[29] In recent years, the species *L. ferocissimum* and *L. amarum* have attracted the attention of the scientific community.

The *L. ferocissimum* (African boxthorn) is native to the Western Cape, Eastern Cape and Free State provinces of South Africa and has become naturalised in Australia and New Zealand.[31] In Australia, it is designated as a weed from coastal and semi-arid regions of southern Australia due to its relation to the formation of dense forests, which reduces pasture areas. However, it produces fruits with desirable characteristics for different applications.[32]

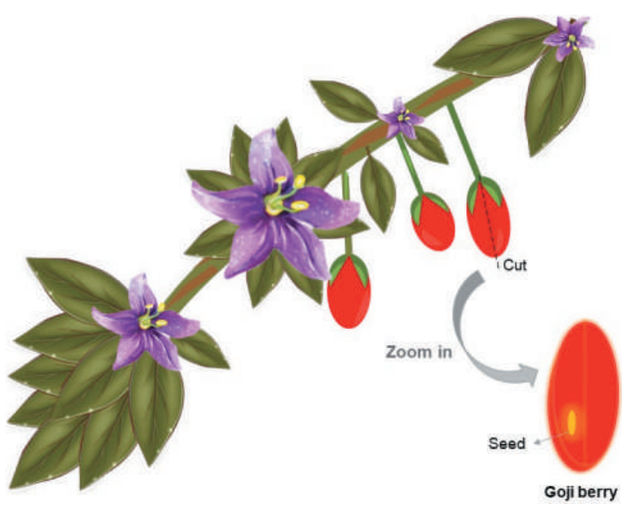


Figure 1. The basic structure of Lycium plants.

The species *L. amarum* originates from the Xizang region, in southwest China, semi-arid and with a temperate climate. This species develops on rocks and along the roadside. According to Xie et al.,[33] *amarum* refers to bitter fruits compared to other species of the *Lycium* genus that produce sweet fruits. The study showed morphological characteristics closer to those of the *L. chinense* species, presenting linear lanceolate leaves, three-lobed goblets, villous ring above the base of the filament and adjacent corolla tube.

The different cultivars, environment (land, climate, humidity, and attitude) and some other factors are responsible for varying the content of nutritional components. The nutritional components found in food are essential to human health. They are responsible for providing energy and substances needed to regulate chemical processes.[34] Nutritional components (Table 1) of goji berry include

Table 1. Nutritional composition of goji fruits.

Analysis Minerals	Composition (g/100 g)	References
Potassium (K)	2200.00 ± 21.02	[35]
Sodium (Na)	370.00 ± 5.82	
Phosphor (P)	183.75 ± 4.4	
Calcium (Ca)	149.50 ± 6.3	
Magnesium (Mg)	67.50 ± 3.54	
Iron (Fe)	7.42 ± 0.59	
Zinc (Zn)	1.96 ± 0.20	
Copper (Cu)	1.54 ± 0.20	
Manganese (Mn)	0.98 ± 0.20	
Total minerals	**21173.92**	
Fibers		
Soluble fibers	2.08 ± 0.13	
Insoluble fibers	7.81 ± 0.1	
Total fibers	**9.88 ± 0.2**	
Sugar		
Fructose	5.45–44.81	[34,35]
Glucose	4.15–30.79	
Sucrose	0.33–1.42	
Maltose	0.38–0.57	[34]
Organic acids		
Citric acid	0.90 ± 0.02	[35]
Oxalic acid	0.66 ± 0.01	
Tartaric acid	0.21 ± 0.01	
Fumaric acid	0.18 ± 0.01	
Quinic acid	0.04 ± 0.01	
Malic acid	0.07 ± 0.00	
Total organic acids	**2.06**	
Fatty acids		
Linoleic acid (C18:2n6cc)	54.68 ± 0.16	
Oleic acid (C18:1n9c)	21.01 ± 0.02	
Palmitic acid (C16:0)	10.68 ± 0.04	
Stearic acid (C18:0)	2.99 ± 0.01	
Linolenic acid n-6 (C18:3n6ccc)	2.57 ± 0.01	
Linolenic acid n-3 (C18:3n3ccc)	2.31 ± 0.01	
Docosadienoic acid cis-13,16 (C22:2)	1.25 ± 0.07	
Arachidonic acid (C20:4n6cccc)	1.01 ± 0.04	
Lignoceric acid (C24:0)	0.96 ± 0.07	
Behenic acid (C22:0)	0.75 ± 0.0	
Arachidic acid (C20:0)	0.68 ± 0.02	
Lauric acid (C12:0)	0.61 ± 0.01	
Palmitoleic acid (C16:1)	0.38 ± 0.18	
Myristic acid (C14:0)	0.12 ± 0.01	
Satured fatty acids	16.79 ± 0.0	
Monounsatured fatty acids	21.39 ± 0.05	
Polyunsatured fatty acids	61.82 ± 0.10	
Total fatty acids		
Moisture	**15.12 ± 0.10**	
Proteins	**4.12–9.57**	[34,35]

carbohydrates, fatty acids, proteins, organic acids, and fiber.[34,35] The presence of essential minerals such as magnesium, iron, and manganese in goji berry, contribute positively to the human diet. Beneficial compounds such as citric acid (organic acid) and linoleic acid (fatty acid) also were identified in higher concentration in this fruit. In this context, goji fruits can be considered a potential source of nutrients for the human diet and as an ingredient for the food industry.[35]

Techniques of geographic characterisation

The earliest record of goji fruits describes the discovery of the species *Lycium chinense* in 100 B.C. in the southern region of Hebei, China. Later, around 600 A.C., the species *L. barbarum* was found in sem-iarid regions of China, such as Ganzhou, Yecheng, Lanzhou, Jiuyuan and Lingzhou. In 1960, *Lycium* fruit production extended to other regions of China that were formed by plateaus and had different climatic conditions, such as Qinghai, Xinjiang, Ningxia, Tibet, and Mongolia.[36]

The high worldwide consumption of goji fruits has stimulated an increase in the total cultivated area in China, which exceeds 1,500 km^2, emphasising the regions of Ningxia, Hebei, Mongolia, Tibet, and the province of Qinghai.[36] Other countries also have large areas of fruit cultivation of the *Lycium*, such as North and South America, Africa, Australia and Europe. According to Knowles[37] Italy's production reached 50 tons in 2016, making it the European country with the largest production of goji, mainly in the areas of Calabria, Veneto, Puglia, Lazio and Tuscany.

Due to the considerable worldwide production of goji fruits, the geographical location, and climatic conditions (temperature, humidity, sunlight, and precipitation) must be known since they strongly influence the characteristics of the fruits. Such characteristics are related to appearance (size and colour), metabolite profile, traceability, commercial value and even the ways of using goji fruits.[38,39] Therefore, knowing the origin of the product is important, which is often not provided.

Hence, several methods of differentiating the geographic location of goji fruits have been developed (Table 2), such as near infrared spectroscopy (NIR), electronic nose and tongue, statistical analysis of principal components (PCA), multivariate and linear discriminant (LDA), gas chromatography

Table 2. Analytical methods for differentiating the geographic location of goji fruits.

Analysis method	Techniques[a]	References
Specific aromatic groups of flavonoids	NIR	[4]
Pixel-wise e object-wise	hyperspectral image of NIR and statistical analysis of PCA	[5]
Complex odors and volatile components	electronic nose, multivariate statistical analysis and CG-MS	[40]
Chemical characteristics (flavonoids and antioxidant activity), metabolomics and morphology (length, width and color intensity)	HPTLC fingerprint and ^{1}H NMR	[37]
Composition of fatty acids and phytosterols	CG-FID	[6]
Composition of stable isotopes, mineral and carotenoids profile, and statistical techniques	IR-MS, ICP-MS, HPLC-DAD-MS and statistical techniques	[39]
Anthocyanins composition and quantification	HPLC-ESI-MS and HPLC-DAD combined with statistical analysis of PCA and LDA	[7]
Isotopic composition of stable carbon (δ13 C) of volatile compounds (limonene, tetramethylpyrazine, safranal, geranylacetone and β-ionone)	CG-IR-MS associated with HP-SPME	[8]
Sense of human taste and quantification of components	Voltammetric electronic language and convolutional neural network algorithm	[9]
Average polysaccharide yield	HPLC-PAD associated with HPSEC-MALLS-RID	[41]

[a]NIR: near infrared spectroscopy; PCA: statistical analysis of principal components; CG-MS: gas chromatography coupled to mass spectroscopy; HPTLC: high performance liquid chromatography coupled to high performance thin layer;[1]H NMR: nuclear proton magnetic resonance; CG-FID: gas chromatography coupled to flame ionization; IR-MS: mass spectroscopy of stable isotopic ratio; ICP-MS: inductively coupled plasma; HPLC-DAD-MS: high performance liquid chromatography coupled to mass spectroscopy; LDA: statistical analysis of linear discriminant; CG-IR-MS: mass spectroscopy of stable isotopic ratio coupled to gas chromatography; HP-SPME: solid phase microextraction; HPLC-PAD: high performance liquid chromatography with pulsed amperometric detection; HPSEC-MALLS-RID: high performance liquid chromatography with laser light mirroring and refractive index detector.

coupled with mass spectroscopy (GC-MS) and flame ionisation (GC-FID), mass spectroscopy of stable isotopic ratio (IR-MS), inductively coupled plasma (ICP-MS) and stable isotopic ratio coupled with gas chromatography (GC-IR-MS), high performance liquid chromatography coupled with mass spectroscopy (HPLC-DAD-MS), electrospray ionisation (HPLC-ESI-MS), pulsed amperometric detection (HPLC-PAD), solid phase microextraction (HP-SPME), nuclear proton magnetic resonance[1H NMR], as well as with diode array detector (HPLC-DAD), high performance thin layer (HPTLC) and laser light mirroring and refractive index detector (HPSEC-MALLS-RID).

NIR is one of the most used and effective techniques for tracking the geographic origin of goji fruits. Through the identification of specific aromatic groups of flavonoids from goji berry, studied by Tingting et al.,[4] NIR enabled the differentiation and classification of the fruits based on their geographical origin. Using the same technique, Yin et al.[5] associated the hyperspectral image of NIR with the statistical analysis of PCA to differentiate samples of goji from different regions of China. Two approaches, namely pixel-wise and object-wise, were investigated to discriminate the origin of the samples. The classification in terms of pixels assigned a class to each individual fruit pixel, and, with this approach, the differences were reflected intuitively.

The use of an electronic nose, multivariate statistical analysis and a GC-MS by Li et al.[18] also proved to be efficient (91–100%) for determining the geographic origin of goji berries produced in different areas of China. The electronic nose can detect complex odours and volatile components, which differentiate production sites. Furthermore, it is portable, easy to operate and convenient for use in routine environments and it can be applied to different samples.

Yao et al.[1] associated goji fruits from different regions of China based on the chemical (HPTLC flavonoids fingerprint and antioxidant activity (AA)) and morphological (length, width and colour intensity) characteristics and metabolomics[1H NMR] of each fruit. The plateau and arid regions (Qinghai and Xinjiang) showed elongated oval fruits with less intense red colour, low AA, high sugar content and *L. barbarum* species, while in monsoon regions (Hebei), the fruits are short ovals with bright red colour, high AA and *L. chinense* species. Larger fruits tend to be more expensive, and consumers prefer those that are bright red in colour. The study suggests that elongated fruits are suitable for commercialisation in dehydrated form, those with a high level of sugar are useful as preserved foods, and those with high AA associated with bitterness are proper for medicinal use.

The analyses of fatty acid and phytosterols composition (GC-FID) of *L. barbarum* L. were used by Cossignani et al.[6] as geographic markers. The objective was to classify and distinguish samples from different production areas such as Italy, Mongolia and China. Fruits from regions in Italy had about 48% of fatty acids, while in Mongolia and China, the level was above 61%. Samples from Italy showed a predominance of the compound β-sitoesterol, and fruits of the regions of Mongolia and China showed higher content of Δ-5-avenaesterol and Δ-5, 23-stigmastadienol.

The study conducted by Bertoldi et al.[39] demonstrated that methods of stable isotope composition (IR-MS), minerals (ICP-MS) and carotenoids profile (HPLC/DAD-MS), as well as statistical techniques were effective in distinguishing the geographic origin of goji fruits from Italy and China. Stable isotopes of hydrogen and oxygen from plants are strongly linked to climatic conditions and geographical characteristics of cultivated areas. The results showed that goji berries produced in Italy had a higher content of carotenoids and minerals than those produced in China.

Wang et al.[7] used HPLC-ESI-MS and HPLC-DAD techniques combined with PCA and LDA analyses to develop models for discriminating the anthocyanin concentrations of fruits from different locations in China. *Lycium ruthenicum* Murray samples were effectively separated by geographic regions according to the type and concentration of anthocyanins.

Meng et al.[8] combined the GC-IR-MS with HP-SPME technique to discriminate the geographical origin of *L. barbarum* L. fruits from different provinces in China. These techniques are based on the isotopic composition of the stable carbon (δ13 C) of volatile compounds from goji fruits, such as limonene, tetramethylpyrazine, safranal, geranylacetone and β-ionone, reflecting their metabolic pathways C3, C4 or CAM. The δ13 C composition is subject to variations in genetic and environmental factors. Such variations will interfere with the morphological and nutritional characteristics of the fruits and will allow geographic differentiation among them. The study showed that the techniques used are 89% accurate and fast and can determine the geographical origin of different plant sources.

Yang et al.[9] developed a system integrated by a voltammetric electronic language (EL) and a deep learning algorithm – convolutional neural network (CNN) – for the classification of goji fruits from different geographical regions. EL is an intelligent electronic device that aims to imitate the theory of the sense of human taste. It is capable of employing qualitative and quantitative analyses for different products, with high detection speed, convenience and good repeatability. The CNN can be used for component quantification and differentiation between samples. The devices used in the study enabled the recognition, identification and discrimination of goji fruits from four geographic regions. They also showed 98.2% accuracy and the possibility of being used for the traceability of other agricultural products.

Polysaccharides are the main active ingredients in goji fruits. The study performed by Wang et al.[41] evaluated the yield, composition, molecular weight, conformation and biological activities of polysaccharides extracted from goji fruits from different regions of China. The HPLC-PAD technique associated with HPSEC-MALLS-RID was used and the average yield of polysaccharides was the best indicator of sample differentiation. The authors suggest that fruits with higher polysaccharides yields, such as Ningxia and Xinjiang, are more suitable as functional ingredients.

Phytochemicals of importance in goji fruits

Goji berries are considered as superfoods, that is, foods that are sources of phytochemicals with important biological functions in the human body. These compounds, also called bioactive, are found in the seeds and layers of pericarp and endocarp of goji fruits.[10] Polysaccharides, carotenoids, terpenoids (essential oils, steroids, sesquiterpenes and oleoresins) and phenolic compounds (phenolic acids, flavonoids, and tannins) are the main bioactive compounds found in goji berries. Seeds are rich in triterpenes, steroids, and alkaloids, while leaves have a high concentration of steroids,[22] as shown in Figure 2.

Polysaccharides are among the main and most important constituents of goji berry and correspond to 2–8% of the dried fruit's content. These compounds are basically made up of galactose, arabinose, glucose, rhamnose, xylose and mannose.[12,42,43] Studies show that the concentration of polysaccharides is mainly influenced by the degree of maturation and extraction method employed.[44] Bao et al.[45] reported a yield of 3.3% of crude polysaccharides precipitated with ethanol after extraction with boiling water from the dehydrated goji berry. Chien et al.[19] determined a high content of polysaccharides (843.50 mg/g) in the aqueous extract of goji fruits. The polysaccharides extracted by infusion at 100°C of black coloured goji fruits, by Liu et al.[46] showed a content of 150 mg/mL of tea.

Carotenoids are fat-soluble compounds (isoprenoids) precursors of vitamin A and are responsible for the yellow, orange, and red colouration of goji fruits.[47] Studies demonstrate different concentrations of carotenoids in goji fruits. Islam et al.[38] determined about 1.5–3.0 and 212–233 mg/100 g of carotenoids in black and red extracts (ethanolic butylated hydroxil toluene) respectively. Pedro et al.[48] determined a content of 31.20–9.97 mg/100 g of dried fruit in alcoholic extracts of goji berry. Bertoldi et al.[39] determined about 356 mg/100 g and 198 mg/100 g of dried carotenoid fruit for goji berries grown in Italy and Asia respectively. Zeaxanthin, mainly as a dipalmitate, is considered as the predominant carotenoid in goji fruits, with a concentration of 31–56% (1/2 of the total carotenoid content). β-carotene, β-cryptoxanthin, violaxanthin and lutein are found in lower concentrations.[2,49]

The group of phenolic compounds is quite heterogeneous due to the wide variety of metabolic routes from which these compounds can be generated. The most commonly found phenolics in goji fruits are phenolic acids and flavonoids. Phenolic acids are present in free or bonded form,[47,50] while flavonoids are the most structurally diverse and are responsible for the colour of fruits, leaves and seeds.[51]

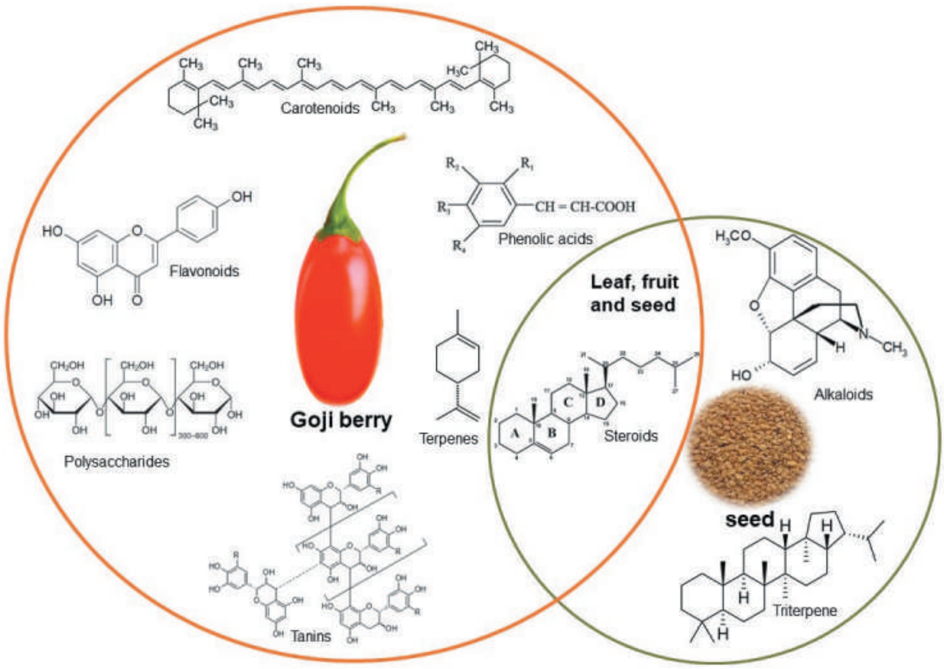


Figure 2. Main phytochemicals present in the fruit, leaf and seed of Lycium plants.

Studies show that goji fruits are important sources of different phenolic acids and flavonoids. The total phenolic content determined by Donno et al.[52] ranged from 199.47 to 502.13 mg/100 g of goji berry. In the study, catechins (59.50%) and cinnamic acid (36.92%) were the main phenolics identified. Benchennouf et al.[53] determined a total phenolic content of 141.30–1097.20 mg/100 g in *Lycium* fruits, with a predominance of cumáric, isoferulic and caffeic acids. Pedro et al.,[48] through statistical optimisation (45°C/162 min/solid:solvent 1:30), obtained a total phenolic content of 1338.80 mg/100 g in goji fruits. The researchers identified syringic and chlorogenic acids as well as rutin and naringin as the main phenolic compounds in goji berries. According to Russo et al.,[25] the main phenolic compounds found in goji fruits were catechin and cinnamic acid.

More than 50 sterols and steroids have been identified in goji fruits, with campesterol predominating in the fruit and seed, lanosterol in flowers and diosgenin in leaves. Alkaloids have a greater number of compounds, with about 72 types identified, and are classified into five categories, namely nortropane, imidazole, piperidine, pyrrole and spermine.[54]

Therefore, the composition of phytochemicals present in goji fruits can vary widely. The different concentrations determined can be related to various factors, such as species, geographic location, cultivation methods, degree of maturation, extraction, processing and storage techniques. Thus, these factors have a strong influence on the associated biological activities.[55]

Goji berry's *in vitro* and *in vivo* biological activities

Recent studies demonstrate different biological activities promoted by goji berries (Table 3). However, the mechanisms responsible for the interactions that promote these activities should be investigated. *In vitro* and *in vivo* tests enable this examination[56] as shown in Figure 3.

Table 3. In vitro and in vivo biological activities of goji berry phytochemicals.

Test	Biological effects	Mechanism of action and properties*	References
In vitro	Antihyperglycemic, anti-aging and antioxidant properties	Presence of bioactive compounds and high antioxidant, anti-diabetic and anti-aging activity demonstrated by *in vitro* tests	[11]
	Anticoagulant and antiplatelet effect	The polysaccharide with the highest degree of degradation has greater inhibitory activity than aspirin against platelet aggregation	[12]
	Anti-inflammatory effect	Treatment of ulcerative colitis by inhibiting the secretion of the main pro-inflammatory cytokines	[13]
	Anti-cancer effect	Inhibition the growth of cancer cells by cell cycle arrest and apoptosis	[14]
	Therapeutic agent	The treatment of Alzheimer's disease against synaptic plasticity, amyloid-β pathology and neuropathology	[43]
	Prebiotic effects	Increasing the growth of Lactobacillus acidophilus and *Bifidobacterium longum*	[20]
	Chemoprotective effect	Effect against Beauvericin induced cytotoxicity in SH-SY5Y neuroblastoma cells	[49]
In vivo	Analgesic effect, and protection against liver and nephrological toxicities	Decreased levels of serum biomarkers in the liver (GGT, LDH and AST) and in the kidney (urea, creatinine, and uric acid)	[17]
	Macular protection	Increased the MPOD, by increasing serum levels of lutein and zeaxanthin	[18]
	Effect against "dry eye disease"	Lacrimal flow induction, with a decrease in corneal and conjunctival lesions	[19]
	Gastrointestinal effect	Decreased levels of inflammatory markers in the colon ulcerative process, pro-inflammatory TNF-α and MPO	[20]
	Treatment of anxiolytic processes	Decreased levels of the marker for brain oxidative stress (MDA), due to increased activities of the SOD and GPX	[16]
	Neuroprotection	Increase in dendritic morphology of neurons and decrease in markers of neural inflammation (reactive astrogliosis, caspase-3, 3-NT and Nrf2)	[15]
	Effects on skeletal muscle	Increase in muscle mass, by regulating the proportion of type IIa oxidative muscle fibers	[21]

*GGT: gamma glutamyltransferase; LDH: lactate dehydrogenase; AST: aminotransferases; MPOD: macular pigment optical density; TNF-α: cytokine; MPO: myeloperoxidase enzyme.

In vitro studies with goji berry

Different cultivars of goji berry can be sources of bioactive compounds with anti-hyperglycemic, anti-aging and antioxidant activities. Wojdyło et al.[11] analysed the potential components of new goji cultivars. Some new cultivars contained large amounts of polyphenols (phenolic and flavonoid acids) and carotenoids and showed antioxidant, anti-diabetic and anti-aging activities in *in vitro* tests. Such activities characterise a significant potential for application in functional foods, medicines and/or cosmetic products.

Among other biological functions of *L. barbarum* are its anticoagulant, antiplatelet and anti-inflammatory potentials. Lin et al.[12] investigated the effect of the degradation of goji berry polysaccharides (LBP) on the inhibition of platelet aggregation, controlling thrombogenesis. *In vitro* experiments showed that LBP degradation significantly increased anticoagulant and antiplatelet activities, induced by arachidonic acid and thrombin. Zu et al.[13] studied the effects of goji in the treatment of ulcerative colitis (UC). The results showed that the lipids present in goji fruits can inhibit the secretion of the main pro-inflammatory cytokines and regulate the expression of the typical anti-inflammatory factor of UC. Thus, they are considered promising for the treatment of UC when used as edible lipid nanoparticles.

Natural compounds with high efficacy, low toxicity and minimal side effects attract the attention of researchers in the study for treatment of cancerous tumours. For example, Gong et al.[14] examined the effects of LBP on the growth of cancer cells. The mechanism studied inhibited the growth of cancer cells by interrupting the cell cycle and apoptosis. Moreover, it demonstrated that LBP can be potential functional ingredients for cancer prevention, whose study *in vitro* showed non-toxicity to normal cells.

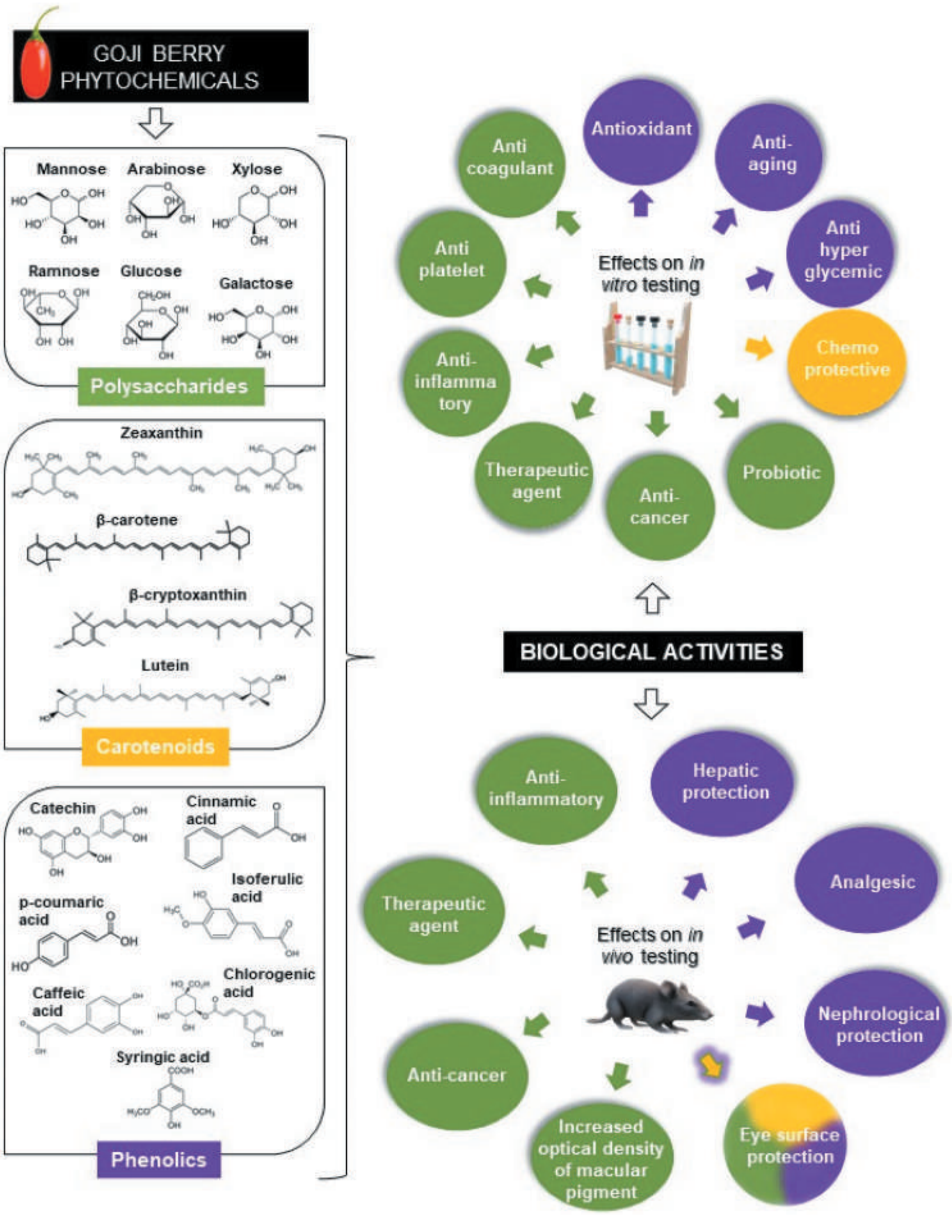


Figure 3. In vitro and in vivo biological activities of goji berry phytochemicals.

Besides promoting these benefits, LBP have a potential therapeutic agent for the treatment of Alzheimer's disease (AD), especially against synaptic plasticity, β-amyloid plaque pathology and neuropathology. This potential was evidenced by Zhou et al.[43] whose *in vitro* study demonstrated a reduction in the generation of β-amyloid levels in neuronal cells, also responsible for AD.

Quality in health is also one of the factors that encourages the scientific community to investigate the benefits of compounds from natural sources introduced into the human diet. Based on this objective, Zhu et al.[20] evaluated the prebiotic effect of LBP. As a result, LBP were able to increase the growth of beneficial probiotic bacteria *in vitro*, such as *Lactobacillus acidophilus* (8.23 log10 CFU/mL) and *Bifidobacterium longum* (6.62 log10 CFU/mL), providing beneficial effects to the gastrointestinal tract.

Eventually, metabolites of fungal origin are found in some foods, such as mycotoxins, which can have negative health impacts. To solve this issue, Montesano et al.[49] demonstrated that carotenoids from goji berry extracts have potential for cytoprotection against cytotoxicity induced by beauvericin (emerging mycotoxin) in SH-SY5Y neuroblastoma cells.

Thus, based on *in vitro* studies, it is possible to support *in vivo* investigations on whether individuals with clinical problems or participants to disease prevention studies will benefit from the consumption of goji berries.

In vivo studies with goji berry

Studies show that goji berry flavonoids may be associated with analgesic effects and protection against liver and kidney toxicities. Rjeibi et al.[17] induced hepatotoxicity and nephrotoxicity in rats, with the administration of doses of tetrachloromethane (CCl_4). The pre-treatment with extracts of *Lycium europeaum* (150 mg/kg) restored biological parameters to normal levels and improved histopathological changes by decreasing the levels of serum biomarkers in the liver (gamma glutamyltransferase, lactate dehydrogenase and aminotransferases) and kidney (urea, creatinine and uric acid). Furthermore, they showed analgesic effects by inhibiting the synthesis of prostaglandins and decreasing the activation of the receptors responsible for the sensation of pain.

Li et al.[18] administered doses of lutein (~ 0.22 μmol/mL) and zeaxanthin (0.03 μmol/mL) extracted from goji berry in a group of people with age-related macular degeneration which is a progressive disease of the retina that results in severe visual impairment. The study showed that goji berry carotenoid supplementation increased the macular pigment optical density (MPOD) by augmenting the serum levels of lutein and zeaxanthin. As a result, the visual function of patients with macular degeneration was improved. Such effects are related to the greater availability of these carotenoids in the liver, which when incorporated into lipoprotein molecules, are transported to the retina more effectively.

The study performed by Chien et al.[19] with Sprangue-Dawley rats showed that the administration of goji berry extract (250–500 mg/kg) doses decreased the severity of the keratoconjunctival condition, reducing corneal and conjunctival lesions caused by 'dry eye' disease. The study showed that the high concentration of LBP in the extracts induced the tear flow and protected the ocular surface. LBP have also been associated with the protection against ulcerative colitis (inflammatory bowel disease) and have reduced anxiolytic processes. Zhu et al.[13] treated rats ulcerated with LBP from goji berry (1–2 mg/kg) and observed, through fluorescence imaging in the near infrared (NIRF), the accumulation of LBP in the ulcerated cells. This fact caused a decrease in the levels of inflammatory markers (TNF-α cytokine and myeloperoxidase enzyme (MPO)) in the colon ulcerative process. Thus, LBP can reduce inflammation, promote healing of the intestinal mucosa and decrease the propensity for the development of colorectal tumours. Besides, goji fruit LBPs can be used as natural medicinal sources in the treatment of anxiolytic processes. According to Karakas et al.,[16] the administration of LBP (20–200 mg/kg) of goji in ovariectomised rats with anxious behaviour decreased the levels of the marker for cerebral oxidative stress (MDA) due to the increased antioxidant activity of the SOD and GPX enzymes.

Goji fruits have been associated with decreased inflammatory processes that affect neurons and dendritic morphology and cause gradual memory loss. As a result, goji berries participate in neuroprotection during brain aging. Ruíz-Salinas et al.[15] demonstrated that the treatment of rats with goji berry extract (3 g/kg) decreased the markers of neural inflammation (reactive astrogliosis, caspase-3, 3-NT and Nrf2) and increased the functional capacity of neurons.

Goji fruits are also associated with the effects on skeletal muscle as shown by Meng et al.[21] Their study demonstrated that goji berry extract (1–2 mg/mL) significantly increased the tibial and gastrocnemius muscle mass of rats. Moreover, it improved the average running distance due to the regulation of the proportion of oxidative muscle fibres of type IIa that assist in mitochondrial biogenesis and fatty acid oxidation. The authors also related the administration of goji berry extract to the reduction of fat levels in the human body.

The polysaccharide of goji berry is related to the relieved of tissue damage and mucosal inflammation. Goji associated with fermenting microorganisms (*Lactobacillus plantarum, Lactobacillus reuteri* and *Streptococcus thermophilus*) can act as probiotics. modulating the intestinal microbiota by decreasing levels of pro-inflammatory cytokines, increased function of anti-ulcerative colitis and regulation of oxidative stress.[57]

Processing and technological applications of goji berry

Goji berries are used in the development of different food, cosmetic and pharmaceutical formulations. The different parts of the *Lycium* plant, such as the roots, leaves, shoots, seeds and fruits, are used. The main application of the root bark and leaves of the goji berry is for medicinal purposes such as the production of teas. Sprouts and seeds are added to food preparation and used in cosmetic products.[1] Fruits are the most consumed and employed parts in the form of nutraceuticals for medicinal purposes and in the formulation of foods and cosmetics.[58]

Goji fruits are often sold in dehydrated form. The purpose of drying is to improve export logistics and increase shelf life since the fruits are collected in summer and autumn. Researchers relate the short shelf life of goji fruits *in natura* with the presence of a thin layer of amorphoceras that accelerates the perspiration of the fruits and shortens their shelf life.[22]

In the conventional drying process, the fruits are initially dried in the shade to wrinkle the skin and then exposed to the sun. In the food industry, convective drying with hot air is one of the most employed techniques. Heat transfer occurs due to the movement of a heated fluid, which causes the transfer of energy from the hot fluid to cold regions.[59] Another industrial method is the electrohydrodynamic drying (EHD) which enables the vaporisation of water from a food without the use of high temperature. Drying occurs through a high-intensity electrical charge, submitted between two points of different dimensions, which generates an electrical effect ('Corona effect') that allows water to vaporise.[60]

However, drying processes can be slow and consume large amounts of energy. In addition, the long period of exposure to high temperatures can cause damage to the characteristics of the fruit. In this context, studies have used physical and chemical pre-treatments to reduce drying time and maintain the nutritional, sensory and phytochemical properties of goji fruits (Figure 4).

Dermesonlouoglou et al.[57] applied osmotic dehydration (OD) (55°C/60 min) to goji fruits (solutions of glycerol, maltodextrin, ascorbic acid and sodium chloride) associated with convective drying (60°C/300 min). OD reduced the drying time by 120 min. It also improved the characteristics of colour, texture and phytochemicals (increased antioxidant activity and total phenolic content). The shelf life of fruits was extended, being 206 days for fruits treated with OD and 99 days for untreated fruits. The OD (55°C/60 min) associated with the pulsed electric field (2.8 kV/cm, 750 pulses) was studied byDermesonlouoglou et al.[57] The research showed a 180 min decrease in drying time (~ 60°C), colour enhancement and greater antioxidant capacity and total phenolic content when compared to drying methods without pre-treatments.

Also using convective drying, Hui et al.[61] treated goji berries with 2, 4 and 6% sodium carbonate (Na_2CO_3) solutions. The results showed that the wax layer of the fruits broke after the treatment with Na_2CO_3, increasing the water diffusion and reducing the drying time. The optimum conditions were: pre-treatment with 2% Na_2CO_3 solution and convective drying at 40°C for 2 h.

Ni et al.[62] applied the ultrasound method as a pre-treatment for drying EHD of *Lycium* fruits. The ultrasonic effect of 200 W and an exposition at 35°C for 20 min influenced the rate of drying and rehydration and the diffusion coefficient. As a result, there was an increase in speed and decrease in drying time in addition to the preservation of the phytochemicals present in goji fruits. Using other pre-treatments, Ni et al.[62] showed that potassium hydroxide (KOH) was the most effective treatment, followed by sodium hydroxide (NaOH), Na_2CO_3, ultrasound and sucrose ester.

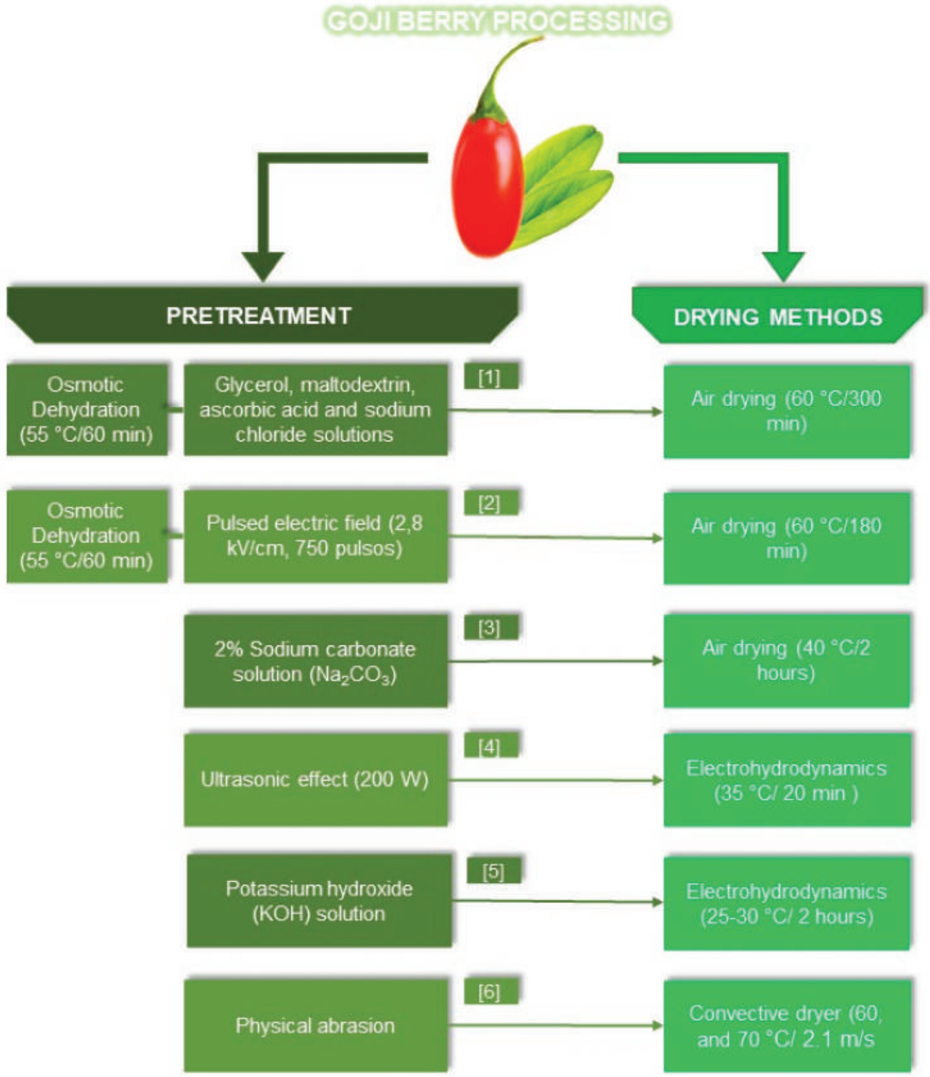


Figure 4. Processes involved in drying goji fruit. Source:[1] Dermesonlouoglou et al.[2,57] Dermesonlouoglou et al.[3,23] Song et al.[4,61] Ni et al.[5,24] Ni et al.[62] and[6] Russo et al.[25].

Russo et al.[25] showed that in addition to the chemical methods mentioned, the physical pretreatment of abrasion in fruits *Lycium barbarum* L. increased the effective diffusion coefficient in the drying process with hot air. This caused a reduction in the time and energy needed for drying. Moreover, fruits subjected to abrasion showed better appearance and nutritional quality.

As mentioned, the processing methods of goji berry are important mainly for maintaining nutritional, sensory, and phytochemical characteristics. These characteristics contribute strongly to the diversification in the development of innovative, functional, healthy, and convenient products and formulations. Ye and Jiang[63] explain the different foods and functional formulations based on goji berry traditionally developed in Asian countries, and that are expanding to other regions of the world. The main applications mentioned are in the form of beverages, such as teas, liquors, and wines, as well as soups and other hot dishes.

Research shows that the development of products based on goji fruits has excellent sensory, nutritional, and phytochemical characteristics. The study by Ducruet et al.[26] showed that 'Ale' beers with goji berry in addition to less turbidity, high colour intensity and high levels of bioactive substances (rutin and ascorbic acid) and antioxidant activity were well accepted by consumers. The study by Ferreira et al.[64] also showed excellent sensory acceptance for a functional white chocolate with the addition of goji berry and prebiotics. Goji berry extracts were also applied to a 'kombucha' (sweetened tea fermented drink)[65] and a yogurt sample,[66] which led to an increase in the total phenolic content, antioxidant capacity and bioaccessibility of the drink.

Balabanova et al.[67] showed that besides their desirable sensory and functional characteristics, the addition of goji berry in a fortified dairy drink prolonged the product's life. This has been shown by Pedro et al.[10] through the addition of organic goji berry (500–3000 mg/kg) extracts in soybean oil. The researchers showed that the extracts were more effective against oxidative deterioration of soybean oil than synthetic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). The study suggests that the extract produced organically can be applied as a safe and natural antioxidant in several food products. Antonini et al.[68] also verified desirable functional and sensory effects, and an extended shelf life in beef burgers with the addition of chia and goji berry (2% and 5%, respectively). Furthermore, both ingredients based on natural sources increased the concentration of phenolic compounds and antioxidant activity and demonstrated an increase in the bioaccessibility of these compounds after an *in vitro* digestion process. With the same objective, Çoban and Ergür[69] studied the effect of coating trout fillets with chia and goji berry mucilage (at concentrations of 1 and 2%). The results showed that the fish coating with chia and goji berry extracts increased shelf life by 5 times compared to the control group (without coating). In addition, 2% goji berry extract was considered the most effective for inhibiting lipid peroxidation and bacterial growth.

The by-products from the manufacture of goji berry juice also have potential as a functional ingredient in food formulations. These by-products have promising added value and low cost which attract the attention of the scientific academy. Bora et al.[70] investigated the replacement of wheat flour by goji berry (WGB) by-products (0, 10, 20, 30 and 40 g/100 g) in muffins and cookies. The incorporation of WGB increased the contents of total phenolics, proteins and dietary fibres. Muffins and cookies showed good sensory characteristics at replacement levels of 30 and 20 g of WGB respectively. The authors suggest that WGB can be incorporated into other products such as breads, tortillas and snacks.

In addition to applications in the food industry, goji berry has recently been incorporated in the formulation of cosmetic products, mainly for dermatological use, due to its anti-aging potential.[44,71] The study by Leite et al.[72] shows formulations emulsified with goji berry extracts have excellent properties related to thermal and rheological stability, bioadhesion to the skin and antioxidant activity. These results suggest that this formulation based on a natural source has promising potential for use in topical treatments against skin aging.

Final considerations

In this review, studies on the botanical and morphological characteristics as well as the technological applications of goji berry were presented and discussed. Among the countless species of goji berry, the *Lycium barbarum* L. is the most cultivated. In general, the morphology of goji is composed of thorny shrubs of up to 3 m high. The flowers are small with a purple or violet blue corolla, and the fruits have a red-orange to black colour and a bitter sweet taste.

According to the research presented in this review, determining the geographic location of goji fruits is extremely important for their traceability, commercial value and forms of application. Hence, spectrophotometric, and chromatographic methods associated with statistical analysis have been applied. These methods enable the differentiation by determining the composition of flavonoids, fatty acids, phytosterols, anthocyanins, stable isotopes, minerals, carotenoids, and polysaccharides. Moreover, the antioxidant activity and morphological characteristics are efficient for the geographic differentiation of goji fruits.

LBP are cited as the main phytochemicals in goji berry, followed by carotenoids, phenolics, sterols and steroids. These compounds have been linked to biological activities *in vitro* and *in vivo*, such as anti-hyperglycemic, anti-aging, antioxidants, anticoagulant, antiplatelet, anti-inflammatory, anti-carcinogenic, in addition to neuroprotection, cytoprotection, hepatoprotection, nephroprotection, prebiotic, eye protection, reduced anxiety and increased muscle mass.

Goji fruits are commonly consumed and sold in dehydrated form. However, some forms of processing can cause damage to the nutritional, sensory and phytochemical properties of the fruit. Therefore, studies presented in this review show that pre-treatments, such as osmotic dehydration, pulsed electric field, ultrasound, and physical abrasion, significantly decrease the damage caused to the dehydration process. Such processes play a significant role in maintaining the desirable characteristics of goji berries for the development of products and formulations. Furthermore, the application of goji fruits has been mentioned in the development of formulations of several products (antioxidant and functional properties in soybean oil, sweets, beverages, meat or dairy and bakery products).

The detailed study of goji berry is important for a better understanding of its benefits to human health, in addition to demonstrating alternatives for the development of new products with antioxidant and functional characteristics. It will instigate the study of new biological effects and applications by the scientific academy and attract several industrial sectors such as food, pharmaceutical and cosmetic.

Highlights

- Phytochemicals determine the geographic location and the ways of applying goji berry.
- Biological effects of goji are important for the development of innovative products.
- Physical and chemical pre-processing of goji berry are essential for quality products.
- Goji berry has wide application potential in different industrial segments.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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ARTIGO 2 - Goji Berry effects on hamburger quality during refrigerated display time

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Goji berry effects on hamburger quality during refrigerated display time

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Abstract

Research on natural additives to replace or reduce synthetic ones in meat products is still being highlighted. The effect of goji berry (GB) (extract, powder and incorporated in an edible coating) in hamburgers during 10 days of refrigerated (2 °C) and illuminated (fluorescent lamp, 1200 lux, 12 h day⁻¹) display were evaluated. Four treatments were studied: control (CONT) – hamburger without GB; GBEX – hamburger with GB extract, GBPW – hamburger with GB powder and GBEC – hamburger with GB edible coating. pH, color, water losses, shear force, total phenolic compounds, antioxidant activity (DPPH and ABTS assays) and lipid oxidation (TBARS) were evaluated. The use of GB positively affected the quality of hamburger during display and the coated samples presented better results, with reduced weight and color losses, lower pH, and shear force values. The inclusion of GB in hamburgers increased the antioxidant activity and inhibited lipid oxidation. GB has potential application in hamburgers to maintain or improve their characteristics during the shelf-life.

Keywords: natural antioxidant; additives; oxidation; bioactive compounds; food quality.

Practical Application: Goji berry has potential application in hamburgers to maintain or improve their characteristics during the shelf-life.

1 Introduction

The search for new products similar to *in natura* ones and with high quality still brings challenges for the meat industry (Vital et al., 2018a; Vital et al., 2018b; Monteschio et al., 2020; Ornaghi et al., 2020). Meat and meat products with a high fat content are normally affected by lipid oxidation (Vital et al., 2021). However, various factors, such as oxygen, light, temperature, the presence of metals and others can accelerate the oxidation process (Domínguez et al., 2019). Lipid oxidation is one of the main significant causes of quality loss in meat products, leading to the appearance of undesirable compounds resulting in quality deterioration, especially with sensorial changes (odor, flavor, and texture), and loss of nutritional value, reducing product shelf life (Ortuño et al., 2014). Oxidative processes are often more intense in processed products associated with the larger surface area in contact with oxygen, and with to the processing itself, as in the case of the hamburger where the meat is ground, which anticipates lipid oxidation and reduces the quality of the product (Cleveland et al., 2014; Özvural et al., 2016).

Aiming to reduce oxidative process in food industry, synthetic antioxidants have already been used. However, due to an increase in demand for natural, healthier, and safe food by consumers', the replacement of synthetic antioxidants by natural products has been evaluated in different studies (Vital et al., 2016, 2021; Kempinski et al., 2017; Fachinello et al., 2018). Among the natural sources of bioactive compounds, the fruits have received considerable attention, in special the berries, which are consumed often by its attractive color and taste; but are also considered as one of the richest natural sources of bioactive compounds with antioxidant activity (Cardoso et al., 2021; Lorenzo et al., 2018). The goji berry (GB) for example has different active compounds such as caffeic acid, chlorogenic acid, coumaric acid, ferulic acid), hyperoxide, gallic acid, catechin, epicatechin, phellandrene, sabinene, γ-terpinene, citric acid, malic acid, oxalic acid, quinic acid and tartaric acid, and vitamin C (Donno et al., 2015). Their consumption was also associated with the prevention of degenerative and chronic diseases (Manganaris et al., 2014). These natural compounds can be used directly in the formulation of foods, processed, or added to the packaging/edible coatings and although these fruits have been widely studied as antioxidants, few studies have shown the effects of the addition of goji berry on meat product quality during its shelf life (Cardoso et al., 2022).

This study was conducted to verify the effect of goji berry on hamburger quality (pH, color, water losses, shear force, antioxidant activity and lipid oxidation) during 10 days of refrigerated display.

2 Materials and methods

2.1 Material

All reagents used were of analytical grade. 200 g of the ripe Goji berry fruit (*Lycium barbarum L.*) was purchased at a local market (Maringá, Paraná, Brazil) and they were used in full.

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2.2 Preparation of hamburger and treatments

Meat - semimembranosus muscle (SM) was obtained from *Longissimus dorsi* of young bulls (½ Angus vs. ½ Nellore; with 443.5 ± 26.2 kg) finished in a feedlot and slaughtered at 12 months old. After that, was vacuum-packaged and frozen at -20 °C until analysis (1 month). Before analysis, SM were thawed (4 °C/24 h), minced, used for hamburger production, and randomly distributed for treatment and analysis.

The GB was dried in a 55 °C greenhouse with air circulation for 48 h, crushed in a grain crusher (80393BZ, Hamilton Beach) with regulation. Different treatments were elaborated: control (CONT) – hamburger without GB extract; GBEX – hamburger with GB extract, GBPW – hamburger with GB powder and GBEC – hamburger with GB + edible coating. For GBPW, GB powder was added directly in minced meat (3% w/w). For the GBEX, 30 g of the GB were stirred with 1 L of water (70 °C) for 30 min. and the mixture was chilled to room temperature, added to minced meat and homogenized. For GBEC, 20 g of alginate was dissolved in 1 L of the so-obtained GB extract (at 70 °C). The hamburgers were, then, submerged in alginate solution for 1 min, allowed to drain (to remove coating excess) for 1 min, submerged in calcium chloride solution (2% w/v) used as a crosslinking for 30 s (Vital et al., 2016, p. 3) to result in the GBEC samples with ~3% GB w/w. Each hamburger was packaged (polystyrene tray), covered with a retractile film and stored with an illuminated display (2° C) (fluorescent lamp, 1200 lux, 12 h day^{-1}). Samples (four replicates per treatment for each analyze/day) were analyzed at 1, 3, 7 and 10 days of display. All samples were analyzed in triplicate.

2.3 pH measurements

The pH was determined at 1, 3, 7 and 10 days of storage time, using a digital pH meter with a penetration electrode as describe by Vital et al. (2016).

2.4 Water losses

The individual weights of hamburgers were recorded each day of analyses. Results were expressed as a percentage relative to hamburger's initial weight (day 0).

2.5 Shear force

Shear force (N) was analyzed using TA.XT Plus (texturometer - Texture Technologies 15 Corp., UK) with a Warner–Bratzler blade. The parameters used were a 5 kg load cell and a speed of 1 mm/s. Four samples were grilled at 200 °C on an electric grill (Grill Philco Jumbo Inox, Philco SA, Brazil) until 72 °C. Then, samples were cooled (25 °C), cut and analyzed in the center.

2.6 Color

Color was evaluated using a Minolta CR-400 (10° view angle and a D65 illuminant) as describe by Vital et al. (2016). Four measurements were recorded in each hamburger. Lightness (L*), redness (a*) and yellowness (b*) were obtained and Chroma and hue values were calculated in the Equations 1 and 2 bellow:

$$Chroma = \sqrt{a^2 + b^2} \quad (1)$$

and

$$Hue = \arctan \frac{b*}{a*} \quad (2)$$

2.7 Lipid oxidation

Malonaldehyde (MDA) content was quantified using TBARS assay (Vital et al., 2016). 5 g of each hamburger was mixed with TCA solution (0.1% EDTA, 0.1% gallic acid and 7.5% TCA) (10 mL), homogenized with an Ultra Turrax, and centrifuged (4° C/15 min/4.000 rpm). The supernatant was filtered and mixed (1:1 v/v) with TBARS solution (562.5 µM HCl, 15% TCA and 1% thiobarbituric acid). The mixture was boiled (100° C/15 min), then cooled, and measured against an MDA standard (535 nm). Results were expressed as mg MDA kg^{-1} of hamburger.

2.8 Total phenolic compound content (TPC) by Folin–Ciocalteu assay

The TPC was determined with modifications (Singleton & Rossi, 1965). The samples of hamburgers on 1 day of display were placed in the proportion of 1:1 (w/v) in methanol. The extracts (supernatant) were obtained by homogenization, centrifugation (15 min/4.000 rpm) and filtration. An aliquot of the supernatant (125 µL) was mixed with Folin– Ciocalteu (1:1 deionized water, 125 µL) and sodium carbonate (28 g/L, 2250 µL). Samples were incubated in the dark (25 °C/30 min) and measured at 725 nm (EvolutionTM 300 spectrophotometer; Thermo Scientific, Madison, USA). Results were expressed as mg gallic acid equivalent (GAE)/g of hamburger.

2.9 ABTS radical scavenging assay

ABTS was analyzed as demonstrated by Re et al. (1999). ABTS·+ was produced by the interaction of 5 mL (7 mM ABTS) with 88 µL potassium persulfate (140 mM), incubated in the dark (25 °C/16 h). Then, ABTS radical was diluted with ethanol (absorbance of 0.70 ± 0.02). The samples of hamburgers on 1 day of display were placed in the proportion of 1:1 w/v in methanol. The extracts (supernatant) were obtained by homogenization, centrifugation (15 min/4.000 rpm) and filtration. An aliquot of the supernatant (40 µL) was mixed with ABTS·+ radical (1960 µL) and absorbance was recorded after 6 min at 734 nm. The radical scavenging activity (%) was calculated as (Equation 3):

$$ABTS\ \ radical\ \ scavening\ \ activity\ \ (\%) = \left(1 - \left(\frac{A_{sample,t=0}}{A_{sample,t}}\right)\right) * 100 \quad (3)$$

where: $A_{sample,\ t=0}$: sample absorbance at time zero and $A_{sample,\ t}$: sample absorbance at 6 min.

2.10 DPPH radical scavenging assay

DPPH scavenging activity was analyzed as demonstrated by Li et al. (2009), with modifications. The samples of hamburgers on 1 day of display were placed in the proportion of 1:1 w/v in methanol. The extracts (supernatant) were obtained by homogenization, centrifugation (15 min/4.000 rpm) and filtration. An aliquot of the supernatant (150 μL) was mixed with s methanolic solution of DPPH (2850 μL; 60 μM), reacted during 30 min and absorbance was read at 515 nm. Antioxidant activity was calculated as (Equation 4):

$$DPPH\ radical\ scavening\ activity\ (\%) = \left(1 - \left(\frac{A_{sample,t=0}}{A_{sample,t}}\right)\right) * 100 \quad (4)$$

where: $A_{sample,\ t\ =\ 0}$: sample absorbance at time zero and $A_{sample,\ t}$: sample absorbance at 30 min.

2.11 Statistical analyses

Data obtained from hamburgers analyzes were evaluated by analysis of variance using the GLM - general linear model (SPSS, v.20.0) (IBM SPSS Statistics, SPSS Inc., Chicago, USA). Means and standard error were calculated for each variable. Display time and type of goji berry application were considered fixed factors (factorial design) with four replicates per treatment. The experiment was repeated two times. When differences were significant, Tukey test was applied (P < 0.05). All samples were analyzed in triplicate.

3 Results and discussion

3.1 pH measurement, water losses and shear force of hamburger with goji berry during display

The values found for pH of hamburger are presented in Table 1. Generally, GBEC presented a lower pH value (P < 0.05), followed by GBEX, GBPW and CONT. The lower value observed in the GBEC treatments may be due to the coating pH (Approximate pH 6) as observed by Vital et al. (2016) in a study with edible coatings. Still, the lower value for treatment with GB addition may be associated with the lower pH of the fruit (around 3). During storage, pH increased for CONT, GBEX and GBPW treatments, while a decrease was observed to GBEC treatment (Table 1).

The increase in pH at the end of display can be caused by the production of volatile basic components such as ammonia and trimethylamine by endogenous or microbial enzymes (Herrera-Mendez et al., 2006; Zarei et al., 2015). Coating decreased water losses (P < 0.001), specially related to GBPW treatment, and in general, no differences were found for the other treatments (Table 2). The grater difference between samples was observed at 10 days of display, when the GBEC retained a significant quantity of water (P < 0.016). The weight losses increased for all treatments during display, as observed by Vital et al. (2016).

Regarding shear force, no interaction between display time and treatments were observed (P > 0.083). In this way, the data are not presented in tables. The shear force reduced with time (P < 0.001), ranging from 1.69 to 1.15 kgf, and the coating made the hamburgers tender (1.11 kgf). The higher shear force for samples without coating, 1.66, 1.43 and 1.42 respectively for CONT, GBEC and GBPW, may be related to the higher weight loss during display time. Water was maintained in the samples with a coating, providing a tender hamburger. This behavior was also observed by Vital et al. (2016), with coated beef.

3.2 Hamburger color

The use of different ingredients and an edible coating can change the appearance of hamburger since the additive color can be different related to its constituents. The color of hamburgers (L*, a*, b*, Hue and Chroma) is provided in Table 3. The lightness values (L*), was stable during time display (P > 0.05) for all treatments, except for GBEC. GBEC presented the lower L* value, probably associated with the lower oxidation (Table 4). Łopacka et al. (2016) also observed an increase in L* value when comparing packaging systems with high content of oxygen compared to lower oxygen. Related to the hamburgers with coating, the maintenance of exudates, as observed by Vital et al. (2016), darkens the color in this study. For GBEX and GBPW, no differences (P > 0.05) were observed during display time. About the redness values (a*), GBEC presented the highest values (P < 0.05) until the 7th day, at the 10th day, no differences were observed between the treatments. Meat pigment, without oxygen (deoxyMb) has a purple-red color. With oxygen (MbO_2), has a bright red (Insausti et al., 1999), the highest value for GBEC may be associated with the exudate that remains attached to the system, intensifying the red color.

Regarding b* value, a little variation was observed during display, and GBEC presented a higher value (P < 0.05), associated with the coating color (yellow).

Table 1. pH measurements of hamburgers with goji berry (GB) during refrigerated an illuminated display.

Treatments	Days				SEM[1]	P < Value
	1	3	7	10		
CONT	6.05^{aB}	6.04^{aB}	5.97^{aB}	6.60^{aA}	0.0423	< 0.001
GBEX	5.99^{aB}	5.91^{bBC}	5.83^{cC}	6.13^{bA}	0.0196	< 0.001
GBPW	5.99^{aB}	6.02^{aB}	5.87^{bC}	6.48^{aA}	0.0355	< 0.001
GBEC	5.97^{bA}	5.92^{bB}	5.79^{dD}	5.85^{cC}	0.0115	< 0.001
SEM[2]	0.0025	0.0104	0.0105	0.0498		
P < Value	< 0.001	< 0.001	< 0.001	< 0.001		

Note: $^{a-c}$ Means followed by the same lowercase letter in the same column do not differ statistically among themselves by Tukey test (P < 0.05). $^{A-D}$Means with different uppercase letters in the same line are significantly different (P < 0.05). CONT: hamburger without GB extract, GBEX: hamburger with GB extract, GBPW: hamburger with GB + powder and GBEC: hamburger with GB + edible coating. SEM[1]: Standard error of means from the treatments. SEM[2]: Standard error of means from storage time.

Table 2. Water losses of hamburgers with goji berry (GB) during refrigerated an illuminated display.

Treatments	Days				SEM[1]	P < Value
	1	3	7	10		
CONT	1.26^{A}	1.63^{A}	2.62^{abB}	3.01^{abB}	0.16	< 0.001
GBEX	1.10^{D}	1.82^{C}	2.66^{abB}	3.60^{aA}	0.26	< 0.001
GBPW	0.97^{C}	1.51^{C}	3.04^{aB}	4.01^{aA}	0.20	< 0.001
GBEC	1.23^{B}	1.64^{AB}	2.09^{bAB}	2.49^{bA}	0.15	0.016
SEM[2]	0.04	0.06	0.12	0.17		
P < Value	0.050	0.416	0.038	0.005		

Note: a-c Means followed by the same lowercase letter in the same column do not differ statistically among themselves by Tukey test (P < 0.05). A-D Means with different uppercase letters in the same line are significantly different (P < 0.05). CONT: hamburger without GB extract, GBEX: hamburger with GB extract, GBPW: hamburger with GB + powder and GBEC: hamburger with GB + edible coating. SEM[1]: Standard error of means from the treatments. SEM[2]: Standard error of means from storage time.

Table 3. Color of hamburgers with goji berry during display refrigerated an illuminated time.

Treatments	Storage days					
Lightness, L*	1	3	7	10	SEM[1]	P < Value
CONT	47.81^{a}	47.81^{a}	49.13^{a}	47.95	0.48	0.704
GBEX	46.63^{a}	47.53^{a}	48.03^{a}	47.71	0.42	0.680
GBPW	46.05^{ab}	48.95^{a}	47.01^{a}	45.88	0.51	0.201
GBEC	43.41^{bAB}	40.19^{bB}	39.74^{bB}	44.61^{A}	0.59	0.005
SEM[2]	0.46	0.67	0.64	0.59		
P < Value	0.006	< 0.001	< 0.001	0.153		
Redness, a*						
CONT	15.14^{bA}	12.96^{abB}	7.69^{bD}	9.60^{C}	0.47	< 0.001
GBEX	15.83^{abA}	9.87^{cB}	7.48^{bC}	9.04^{B}	0.45	< 0.001
GBPW	17.40^{aA}	11.15^{bcB}	7.62^{bC}	10.35^{B}	0.53	< 0.001
GBEC	17.36^{aA}	14.67^{aB}	13.02^{aB}	9.95^{C}	0.44	< 0.001
SEM2	0.27	0.38	0.33	0.27		
P < Value	0.004	< 0.001	< 0.001	0.400		
Yellowness, b*						
CONT	16.34^{cA}	16.14^{bcA}	13.74^{bB}	16.28^{abA}	0.44	< 0.001
GBEX	16.88^{cA}	14.30^{cBC}	13.68^{bC}	15.62^{bB}	0.21	< 0.001
GBPW	18.77^{bA}	17.09^{bAB}	14.83^{bC}	16.90^{aB}	0.30	< 0.001
GBEC	21.08^{aA}	19.40^{aAB}	18.69^{aBC}	17.08^{aD}	0.34	< 0.001
SEM2	0.32	0.39	0.30	0.22		
P < Value	< 0.001	< 0.001	< 0.001	0.015		
Chroma						
CONT	22.23^{bA}	20.74^{bAB}	15.79^{bC}	18.97^{abB}	0.42	<0.001
GBEX	23.17^{bA}	17.41^{cB}	15.61^{bC}	17.78^{bB}	0.41	<0.001
GBPW	25.63^{aA}	20.42^{bB}	16.71^{bC}	19.90^{aB}	0.51	<0.001
GBEC	27.37^{aA}	24.36^{aB}	22.83^{aB}	19.79^{abC}	0.62	<0.001
SEM2	0.36	0.51	0.42	0.28		
P < Value	< 0.001	< 0.001	< 0.001	0.027		
Hue						
CONT	47.18^{abB}	51.31^{cB}	60.92^{aA}	59.81^{A}	1.01	< 0.001
GBEX	46.87^{bC}	55.42^{abB}	61.32^{aA}	59.56^{A}	0.85	< 0.001
GBPW	47.21^{abC}	56.96^{aB}	62.70^{aA}	58.57^{B}	0.91	< 0.001
GBEC	50.57^{aC}	52.99^{bcBC}	55.29^{bB}	59.90^{A}	0.50	< 0.001
SEM2	0.49	0.54	0.54	0.66		
P < Value	0.019	0.001	< 0.001	0.895		

Note: a-c Means followed by the same lowercase letter in the same column do not differ statistically among themselves by Tukey test (P < 0.05). A-D Means with different uppercase letters in the same line are significantly different (P < 0.05). CONT: hamburger without GB extract, GBEX: hamburger with GB extract, GBPW: hamburger with GB + powder and GBEC: hamburger with GB + edible coating. SEM[1]: Standard error of means from the treatments. SEM[2]: Standard error of means from storage time.

Samples that present low Chroma are considered pale (Cardoso et al., 2016) and in this study, CONT and GBEX showed lower values than GBEC until the 7th day, which may not be desirables to consumers at purchase time. Generally, GBEC had higher values during display time. Fresh meat normally becomes less light and red during display. Regarding Hue, the values increased with display time, and on the 10th day no differences between treatments were observed ($P > 0.05$). GBEC presented less variation in Hue value until the 7th day.

Thus, an additive or technology, as a powder, an extract, or an edible coating, that can intensify or maintain meat color, especially the redness, could lead to an extension in meat color display-life (Vital et al., 2016), favoring consumer choice at the time of purchase. Hue value was not different between treatments and increased with display time.

Fresh hamburgers are susceptible to fast deterioration especially due to the high level of moisture, protein, lipid, and processing. Thus, food industry wish/search new alternatives to extend the shelf-life of meat products and maintain their quality, especially the color, which is one of the principal attributes for the consumers on the purchase moment (Vital et al., 2016).

3.3 Lipid oxidation

The oxidation leads to degradation of lipids, pigments, proteins, and is one of the major mechanisms of quality for deterioration in meat products (Liu et al., 2010). The inclusion of GB in different ways significantly reduced TBARS values (Table 4). Lipid oxidation increased significantly ($P < 0.001$) during display only for CONT, while the other treatments maintained the oxidation stabilized. At 10 days, the TBARS values reached approximately 1.48, 0.99, 0.95 and 1.02 mg MDA kg^{-1} for CONT, GBEX, GBPW and GBEC, respectively. Oxidation is one of the major factors responsible deteriorations in foods leading to consumer rejection (Pouzo et al., 2016).

Thus, this study shows that the use of goji berry could be effective in reduce/inhibit the lipid oxidation in meat products during refrigerated display. Besides, other studies have also demonstrated the effectiveness of using natural compounds with antioxidant activity in coatings or films to prevent the lipid oxidation, as also in the isolated form. Bulambaeva et al. (2014) observed that GB powder effectively in inhibits the lipid oxidation of sausages. Amiri et al. (2019) evaluated the corn starch films with nanoemulsion of *Zataria multiflora* essential oil with cinnamaldehyde on fresh beef patties and observed that TBARs' values in samples containing EO and cinnamaldehyde were significantly lower than that in the control. Vital et al. (2021) observed that the lipid oxidation of lamb patties was reduced by the use of essential oil in coating and modified atmosphere. Cardoso et al. (2016) showed that a coating of chitosan and gelatin could reduce TBARS values, related to the chitosan antioxidant activity. Borella et al. (2019) evaluated the effect of rosemary antioxidant in hamburgers during shelf life and the natural antioxidants used were efficient in maintaining the oxidative stability of the product during the frozen storage time. In this way, natural compounds isolated or incorporated in a coating, such as the GB, can improve the quality and shelf-life of meat products by preventing its lipid oxidation.

3.4 Polyphenol compounds and antioxidant activity

Related to total phenolic compounds (TPC), treatments with GB had higher values ($P < 0.001$; Table 5), as expect, due to the bioactive compounds of the fruit. The antioxidant activity was measured using the DPPH and ABTS assays, and also hamburger with GB had a higher radical scavenging activity ($P < 0.001$).

Table 4. Lipid oxidation (TBARs) of hamburgers with goji berry during refrigerated an illuminated display time.

Treatments	Days				SEM[1]	P < Value
	1	3	7	10		
CONT	0.79^{C}	1.07^{aB}	1.43^{aA}	1.48^{aA}	0.30	< 0.001
GBEX	0.86	0.83^{b}	0.93^{b}	0.99^{b}	0.08	0.050
GBPW	0.80	0.80^{b}	0.96^{b}	0.95^{b}	0.11	0.073
GBEC	0.86	0.89^{b}	1.01^{b}	1.02^{b}	0.13	0.096
SEM[2]	0.07	0.13	0.23	0.24		
P < Value	0.316	0.002	< 0.001	< 0.001		

Note: [a-c] Means followed by the same lowercase letter in the same column do not differ statistically among themselves by Tukey test ($P < 0.05$). [A-D]Means with different uppercase letters in the same line are significantly different ($P < 0.05$). CONT: hamburger without GB extract, GBEX: hamburger with GB extract, GBPW: hamburger with GB + powder and GBEC: hamburger with GB + edible coating. SEM[1]: Standard error of means from the treatments. SEM[2]: Standard error of means from storage time.

Table 5. Total phenolic compounds (TPC) and antioxidant activity (ABTS and DPPH assays) of hamburger with goji berry.

Analyses	Treatments				P < Value
	CONT	GBEX	GBPW	GBEC	
TPC (mg EAG/ kg hamburger)	0.24 ± 0.003^{c}	0.28 ± 0.004^{ab}	0.26 ± 0.005^{b}	0.29 ± 0.006^{a}	< 0.001
ABTS radical scavenging (%)	69.52 ± 0.56^{c}	78.39 ± 0.72^{b}	85.05 ± 0.90^{a}	84.62 ± 0.24^{a}	< 0.001
DPPH radical scavenging (%)	30.98 ± 1.21^{c}	49.66 ± 0.30^{a}	37.95 ± 1.50^{b}	42.89 ± 1.70^{b}	< 0.001

Note: [a-c] Means followed by the same lowercase letter in the same line do not differ statistically among themselves by Tukey test ($P < 0.05$). CONT: hamburger without GB extract, GBEX: hamburger with GB extract, GBPW: hamburger with GB + powder and GBEC: hamburger with GB + edible coating.

Samples with GB presented little differences between analyses (mythology employed), and these differences were because the bioactive compounds can act by different mechanisms with different radicals, such as reactions with electron transfer processes (ABTS), or by hydrogen atom transfer (DPPH) (Gülçin, 2010). Nevertheless, natural compounds with antioxidant activity as TPC can be used in the meat industry to minimize the degradation during display time (Kumar et al., 2015; Zhang et al., 2016).

4 Conclusion

The use of goji berry had positive effects on hamburger quality during display time. Generally, samples with coating presented better results, with less weight and color losses, lower pH values, and greater tenderness. Also, the use of goji berry in hamburgers increased the product antioxidant activity and inhibited its lipid oxidation. Thus, GB has potential application in the meat industry to maintain/improve final product characteristics during the shelf-life.

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ARTIGO 3 - Computer vision as the golden tool: mathematical models for evaluating color and storage time of hamburgers with Goji BeRry natural additive

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Computer vision as the golden tool: mathematical models for evaluating color and storage time of hamburgers with goji berry natural additive

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Abstract

A new approach to meat color and shelf-life assessment is reported here for hamburgers mixed with goji berry. Samples with powdered or aqueous extract of goji berry were monitored, compared with hamburgers without the additive for ten days and its L*, a* and b* properties were determined by computer vision using a homemade setup. Noteworthy results, in particular the luminosity, for all samples with goji berry – only or with salt and pepper – were perceived, and predictive mathematical models for these food mixtures are here presented. The addition of goji berry in hamburgers, pioneered in this study, present advantageous characteristics both in terms of storage time and a positive effect on human health owing to the polyphenols in the natural additive. This study also offers insights into a low-cost and reliable method for the quality control of meat with potential application in the food industry.

Keywords: CIE; color evaluation; meat color; computer vision system; RGB.

Practical Application: Computer vision can be replaced by the conventional colorimeter, already validated as a methodology for color, since it quickly an analyzes whole sample and does not allow human interference on the choice of points to be collected. Thus, for the industry, this method would be ideal, quick, low-cost and interference less.

1 Introduction

In 2019, beef cattle farming business in Brazil accounted for approximately 130 billion dollars, which represents a 3.5% increase compared with the previous year. Such amount includes all inputs for agriculture, investments in genetics, animal health improvement, food, nutrition, and sales within the country's domestic market (Associação Brasileira das Indústrias Exportadoras de Carnes, 2021). In 2021, Brazil exported 1.8 million tons of beef, which is equivalent to a free on board (FOB) value of US$9.2 million (Food and Agricultural Policy Research Institute, 2021). Due to this growing demand, meat industry seeks to offer higher quality products and meet safety criteria that encompass both human health and environmental issues (Teixeira & Rodrigues, 2019).

Efficient ways to ensure the quality of meat products are provided by retarding its oxidative processes through physical barriers, or by adding chemical additives directly (Al-Hijazeen, 2022; Lishtanawati et al., 2022; Monteschio et al., 2020; Vital et al., 2016; Vital et al., 2018a; Vital et al., 2018b). Salt and pepper are meat preservatives and their addition dates back to many centuries. Besides these two substances, the food industry has incorporated nitrites – inorganic species that prevent bacterial growth and maintain the food color (Azeem et al., 2019) – and monosodium glutamate, a flavor enhancer responsible for the umami taste. However, in large amount intake, these additives are proven to be harmful for health (Bhat et al., 2020).

Natural antioxidants are highlighted as a good additive in foods since they are safe for food products and improve their nutritional, physical-chemical and visual characteristics during storage time (Al-Hijazeen, 2022; Alexandre et al., 2021; Guerrero et al., 2018; Huang et al., 2022; Monteschio et al., 2020; Ornaghi et al., 2020). Many authors have addressed the addition of these compounds to various types of food products such as beef burgers (Carvalho et al., 2020), packaging and edible coatings (Lourenço et al., 2019), beef steaks (Vital et al., 2018a), lamb meat (Lima et al., 2022), fish fillet (Vital et al., 2018b), among others.

The goji berry (*Lycium barbarum L.*) is a plant from Asia, whose fruits are rich in phenolic antioxidants, widely used in the East hemisphere for medical formulations, fresh consumption, preparation of teas, or even as a food supplement (Frattaruoli et al., 2018; Huang et al., 2022; Lu et al., 2019). Five classes of polyphenols are found in goji berry- benzoic acids, catechins, cinnamic acids, flavonoids and tannins, in addition to terpenes, organic acids and

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vitamin C (Donno et al., 2015; Huang et al., 2022; Wang et al., 2010) These phenols are responsible for ensuring the integrity of food and its natural appearance.

Color is the first sensory impact caused to human eyes and an essential feature to be considered by consumers during meat evaluation, since it indicates freshness, and plays a major role on shelf life (Eiras et al., 2017; Mancini & Hunt, 2005; Monteschio et al., 2020; Udomkun et al., 2018). Meat color is usually determined by colorimeters. However, only a small portion of the product can be assessed, often intuitively chosen by researchers, leading to inaccurate measurements and results (Tomasevic et al., 2019).

Computer vision has been exploited as a reliable, quick, low-cost, and non-invasive alternative for meat product analysis and requires only a single measurement to evaluate an entire sample. Digital images are able to capture the overall information and store data for transforming into a multivariate color measurement system (Lima et al., 2022; O'Sullivan et al., 2003; Passetti et al., 2017, 2019; Tarlak et al., 2016a, b).

In this study, we propose a mathematical model based on computer vision to investigate the performance of goji berry (powdered or in aqueous extract) as a natural preservative for maintaining the color of hamburger-type products. The color space parameters of these samples were determined using computer vision at different storage times.

2 Materials and methods

2.1 Ethic committee, local, animals, and diets

This experiment was approved by the Department of Animal Production and Research Ethic Committee at the State University of Maringá (UEM) in Maringá, Paraná, Brazil and it followed the guiding principles of biomedical research with animals (CAAE: 44460020.3.0000.0104 protocol number).

This study was conducted at the Rosa & Pedro Sector of the Experimental Farm Station at UEM. The sensorial evaluation was performed at the Meat Quality Laboratory at UEM. A total of 24 crossbred bulls (*Bos taurus taurus x Bos taurus indicus*) at 24 ± 3.2 months of age and weighting mean initial body weight of 385.5 ± 3.84 kg were used in a completely randomized design.

2.2 Samples and additives

The hamburgers were prepared by using the *Longissimus dorsi* of bulls, collected from the left half-carcass between from 6th to 13th ribs.

Goji berry (*Lycium barbarum L.*), black pepper (*Piper nigrum L.*) and commercial salt (Ajisal®) were purchased from local market in Maringá, Paraná, Brazil. The goji berry was dried in an oven at 55° C for 72 h and finely ground (Hamilton Beach®) for 2 min. The goji berry aqueous extract was prepared by dissolving the powder in distilled water and then added in different concentrations to the hamburgers.

2.3 Mass and sample proportion

Using a piece of meat, a set of 10 hamburgers was prepared in the following proportions (% mass/mass), which were analyzed in triplicate: Control sample (1): 80% meat and 20% fat; Sample 2: 98.9% control sample + 1% commercial salt + 0.1% pepper; Sample 3: 95.9% control sample + 1% commercial salt + 0.1% pepper + 3% goji berry powder; Sample 4: 92.9% control sample + 1% commercial salt + 0.1% pepper + 6% goji berry powder; Sample 5: 95.9% control sample + 1% commercial salt + 0.1% pepper + 3% goji berry aqueous extract; Sample 6: 92.9% control sample + 1% commercial salt + 0.1% pepper + 6% goji berry aqueous extract; Sample 7: 97% control sample + 3% goji berry powder; Sample 8: 94% control sample + 6% goji berry powder; Sample 9: 97% control sample + 3% goji berry aqueous extract; Sample 10: 94% control sample + 6% goji berry aqueous extract. All samples were placed on white Styrofoam trays, sealed with flexible polyvinyl chloride (PVC) film and random stored under refrigeration (2-4° C), free from contamination, under conditions comparable to commercial/home ones.

Ten hamburgers (~50 g, ~2 cm high) were prepared with a hamburger-maker in the following proportions (% w/w): Control sample (1): 80% meat and 20% fat; Sample 2: 98.9% control sample + 1% commercial salt + 0.1% pepper; Sample 3: 95.9% control sample + 1% commercial salt + 0.1% pepper + 3% goji berry powder; Sample 4: 92.9% control sample + 1% commercial salt + 0.1% pepper + 6% goji berry powder; Sample 5: 95.9% control sample + 1% commercial salt + 0.1% pepper + 3% goji berry aqueous extract; Sample 6: 92.9% control sample + 1% commercial salt + 0.1% pepper + 6% goji berry aqueous extract; Sample 7: 97% control sample + 3% goji berry powder; Sample 8: 94% control sample + 6% goji berry powder; Sample 9: 97% control sample + 3% goji berry aqueous extract; Sample 10: 94% control sample + 6% goji berry aqueous extract. All samples were placed on white Styrofoam trays, sealed with flexible polyvinyl chloride (PVC) film and random stored under refrigeration (2-4 °C), free from contamination, under conditions comparable to commercial/home ones.

2.4 Apparatus for the photographs

The homemade setup for photographs (Figure 1) was based on the reported by Girolami et al. (2013) with adaptations. A cardboard box (w = 60 cm, h = 30 cm, d = 32 cm) was fully covered of black paint and two drills were made on the upper part of the box: one of them to place a camera, and the other for a light source (T9 LED light bulb, 7000 lumens/12 V).

The photography images were obtained by using a cell phone camera (Xiaomi mi 8, 12MP, 4,000 x 3,000 pixels, sensor size 1/2.55" + 1/3.4" and stabilization) with focus stabilization. Zoom and flash resources were not used. Both the camera and the light source were placed orthogonally and ~30 cm (box height) from the samples for image acquisition with the LED placed 2 cm on the right side of the camera. The photographs were taken at intervals of 1, 3, 7 and 10 days (d).

Figure 1. Homemade setup for photographs: front (a) and upper (b) view.

2.5 Color instrumental measurement/mathematical modeling

Data were collected following the studies reported by (Tarlak et al., 2016a) and analysis was performed by using the IEC (International Electrotechnical Commission) Lab method by transforming components from RGB (red, green and blue) into the XYZ model. RGB images generally contain 8 bits of data per color channel and can be named as 24-bit RGB (8 bits x 3 channels) with values ranging from 0 to 255. The relationship between the RGB and XYZ models is defined in the Equations 1-3 below (International Electrotechnical Commission, 1999).

$$R'_{RGB} = \frac{R_{8bit}}{255} \tag{1}$$

$$G'_{RGB} = \frac{G_{8bit}}{255} \tag{2}$$

$$B'_{RGB} = \frac{B_{8bit}}{255} \tag{3}$$

If R'_{RGB}, G'_{RGB} and $B'_{RGB} \leq 0.04045$, then (Equations 4-6):

$$R_{RGB} = \frac{R'_{RGB}}{12.92} \tag{4}$$

$$G_{RGB} = \frac{G'_{RGB}}{12.92} \tag{5}$$

$$B_{RGB} = \frac{B'_{RGB}}{12.92} \tag{6}$$

yet, if R'_{RGB}, G'_{RGB} and $B'_{RGB} > 0.04045$, then (Equations 7-9):

$$R_{RGB} = \left(\frac{R'_{RGB}+0.055}{1.055}\right)^{2,4} \tag{7}$$

$$G_{RGB} = \left(\frac{G'_{RGB}+0.055}{1.055}\right)^{2,4} \tag{8}$$

$$B_{RGB} = \left(\frac{B'_{RGB}+0.055}{1.055}\right)^{2,4} \tag{9}$$

Thus (Equation 10),

$$\left\langle \begin{array}{l} X = 0.4124 R_{RGB} + 0.3576 G_{RGB} + 0.1805 B_{RGB} \\ Y = 0.2126 R_{RGB} + 0.7152 G_{RGB} + 0.0722 B_{RGB} \\ Z = 0.0193 R_{RGB} + 0.1192 G_{RGB} + 0.9505 B_{RGB} \end{array} \right. \tag{10}$$

From the XYZ model it is possible to obtain the L*, a* and b* components according to the following equations (Equations 11-12) (Azad & Hasan, 2017; Lima, 2020):

$$\left\langle \begin{array}{l} L = \left[116 * f * \left(\frac{y}{y_0} \right) \right] - 16 \\ a* = 500 * \left[f * \left(\frac{x}{x_0} \right) - f * \left(\frac{y}{y_0} \right) \right] \\ b* = 200 * \left[f * \left(\frac{y}{y_0} \right) - f * \left(\frac{z}{z_0} \right) \right] \end{array} \right. \tag{11}$$

Where,

$$f(q) = \left\langle \begin{array}{ll} \sqrt[3]{q}, & q > 0.008856 \\ \left(\frac{841}{108} * q \right) + \frac{4}{29} & q \leq 0.008856 \end{array} \right. \tag{12}$$

In this case, $x_0 = 94.81$, $Y_0 = 100$ and z0 = 107.3 are the tristimulus values for the CIE illuminant D65.

The Adobe Photoshop CS3 software was used to collect forty color pixels from each photograph, ten points from each quadrant (quadrants are defined as four equal parts of an image already segmented). Parameters L*, a* and b* were, accordingly, determined through Equation 4 using the collected points.

3 Results

3.1 L, a* and b* values*

The L* values represent the luminosity perceived by an observer and can range from zero to one hundred, referring to black and white, respectively. The a* and b* values are vectors that represent the four colors perceived by the human eye, i.e., red, green, blue, and yellow; a* expresses the range of green to red (where green lies on a negative quadrant on a Cartesian coordinate axis, and red on a positive quadrant). The b* value, orthogonally to a*, represents the variation between blue (negative quadrant) and yellow (positive quadrant) (Hardeberg, 2001). In

Table 1 the L*, a* and b* parameters determined for hamburger samples with and without goji berry additives are shown.

On the first day of experiment (Table 1), sample 1 (control) clearly depicted a substantially higher luminosity than the other samples (L* > 35) whereas sample 4 presented the lowest value (L* < 25), while the luminosity parameters of the other trials remained on the 20 < L* < 30 range (Figure 2).

Parameter a*, which represents the red-green color variation, and parameter b*, related to the yellow-blue coloration. In both cases, sample 5 values outstand from the other tests, with color matching the red (a*) and yellow (b*) regions (Figure 3e and Figure 4e).

The scatter diagrams in Figure 3 show the correlation between a* and time (d) for all tests (refer to Table 1). The obtained data properly fit to a linear function, with a negative slope, which indicates that the color of the samples is changing from red to green. A coefficient of determination, R^2, is above 0.92 for the majority of the tests, except for samples 1 (control) and 3, with R^2 ~ 0.774 and 0.893, respectively. In this case it is possible to predict the value of a* for days not collected.

The correlation between variables b* and time (d) (refer to Table 1) is depicted in Figure 4. For the most essays, a coefficient of determination, R^2, is above 0.96, except for samples 3, 4, and 5, with R^2 ranging from 0.793 to 0.813. The obtained data

Table 1. Hamburger sample composition and its *L**, *a**, and *b** values.

	Sample composition	Time/d	*L**	*a**	*b**
Sample 1 Control	Meat (80%) + fat (20%)	1	36.7395	33.4875	15.1556
		3	24.2213	27.1952	14.2165
		7	24.9043	15.8341	6.5339
		10	26.9181	19.1876	3.4858
Sample 2	Control (98.9%) + commercial salt (1%) + pepper (0.1%)	1	27.2587	30.2940	15.4520
		3	26.6016	25.4270	12.3193
		7	29.6966	19.2123	6.2352
		10	29.6409	15.7982	1.9146
Sample 3	Control (95.9%) + commercial salt (1%) + pepper (0.1%) + ***goji berry* powder (3%)**	1	25.9158	19.9669	11.5624
		3	24.5696	20.2331	15.1192
		7	23.9979	11.9544	7.1578
		10	27.2371	11.1287	2.2293
Sample 4	Control (92.9%) + commercial salt (1%) + pepper (0.1%) + ***goji berry* powder (6%)**	1	21.5084	15.5809	10.3795
		3	22.9119	15.7700	13.6817
		7	23.3351	12.2468	6.2336
		10	25.7918	11.2936	-0.2875
Sample 5	Control (95.9%) + commercial salt (1%) + pepper (0.1%) + ***goji berry* aqueous extract (3%)**	1	29.1833	32.4025	15.9567
		3	29.8107	33.4108	19.9517
		7	28.1985	25.1634	10.3987
		10	28.4956	17.6180	3.7438
Sample 6	Control (92.9%) + commercial salt (1%) + pepper (0.1%) + ***goji berry* aqueous extract (6%)**	1	28.5228	30.0545	15.1932
		3	25.4791	27.5221	14.3142
		7	26.0462	19.7245	5.8566
		10	25.5686	15.4490	0.1994
Sample 7	Control (97%) + ***goji berry* powder (3%)**	1	27.6347	30.8648	14.8750
		3	25.4584	27.0803	14.4471
		7	26.6910	19.5457	5.4769
		10	27.7989	15.2978	0.8726
Sample 8	Control (94%) + ***goji berry* powder (6%)**	1	27.5359	31.1793	15.4848
		3	23.2544	26.6779	13.6280
		7	27.1658	19.3980	5.8213
		10	27.1469	15.3625	0.8307
Sample 9	Control (97%) + ***goji berry* aqueous extract (3%)**	1	27.5981	30.3229	15.5786
		3	25.2170	26.9568	14.5755
		7	26.8538	19.5201	5.5733
		10	26.6185	15.3038	0.3180
Sample 10	Control (94%) + ***goji berry* aqueous extract (6%)**	1	27.8646	31.0699	15.4290
		3	25.6173	26.9120	14.1944
		7	25.7669	19.3189	5.6258
		10	27.9565	15.8604	0.6773

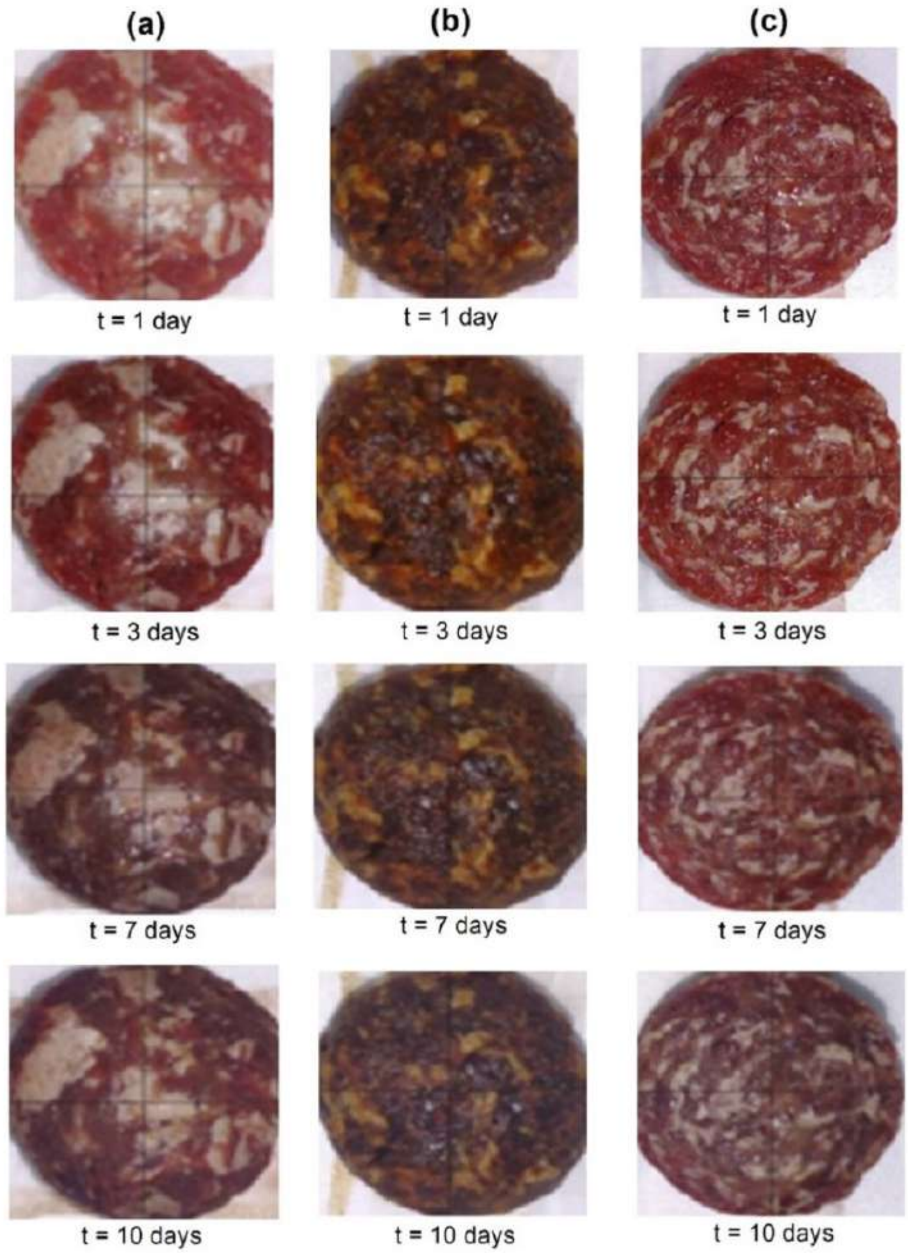

Figure 2. Hamburger color variation over time. (a) Sample 1: Meat only, (b) Sample 4: Meat + ajisal + pepper + 6% goji powder, (c) Sample 5: Meat + ajisal + pepper + 3% goji in extract.

properly fit to a linear function, with a negative slope, which indicates that the color of the samples is changing from yellow to blue. Choosing, for example, samples 1, 4 and 5, it is possible to estimate the parameters a* and b* when t = 5 and t = 9 days. (Table 2).

The meat color indicates salubriousness or freshness. The L*, a* and b* values for samples 7-10, comprising only those hamburgers containing goji berry powder or aqueous extract, exhibited a promising color preservation behavior. For all these samples the luminosity values tend to remain in the $25 < L^* < 30$ interval, in the same luminosity range as for control sample, or even with higher values in some cases.

4 Discussion

4.1 L, a*, and b* values*

A color space is both a specification of a coordinate system and a subspace in that same system where each color can be represented by a single point. In terms of digital image processing, the most commonly used models are RGB (red, green, blue), CMY (clay, magenta, yellow) and CMYK (cyan, magenta, yellow and black), consistent to how humans describe and interpret primary colors (Azad & Hasan, 2017). The RGB model is based on a Cartesian coordinate system. Each color pixel can be represented by a vector since a color image has, at least, three components. The main limitations of this model are

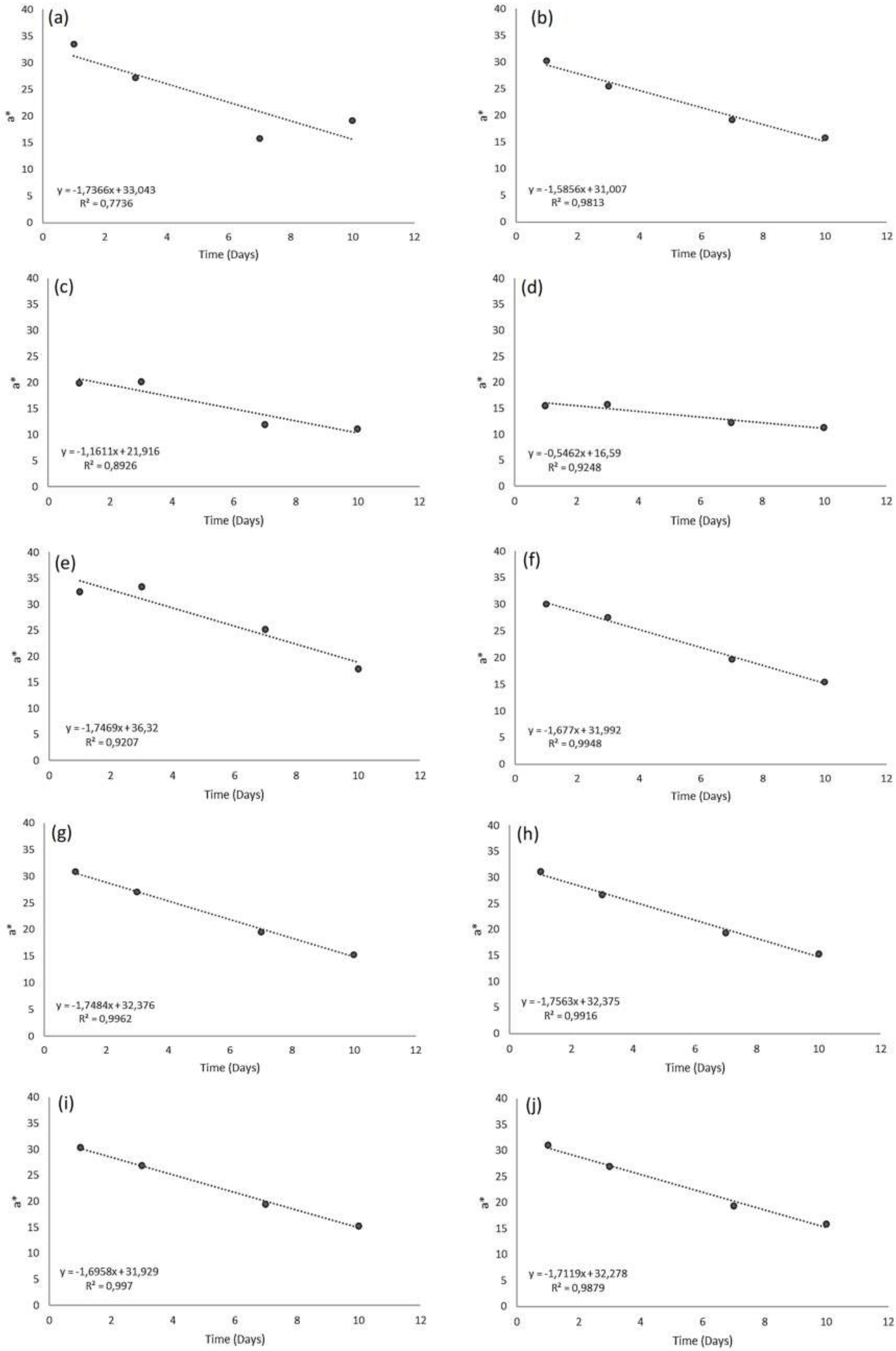

Figure 3. a* scatter diagrams. (a) Sample 1: Meat only, (b) Sample 2: Meat + ajisal (1%) + pepper (0.1%), (c) Sample 3: Meat + ajisal + pepper + 3% goji powder, (d) Sample 4: Meat + ajisal + pepper + 6% goji powder, (e) Sample 5: Meat + ajisal + pepper + 3% goji in extract, (f) Sample 6: Meat + ajisal + pepper + 6% goji in extract, (g) Sample 7: Meat + 3% goji powder, (h) Sample 8: Meat + 6% goji powder, (i) Sample 9: Meat + 3% goji in extract and (j) Sample 10: Meat + 6% goji in extract.

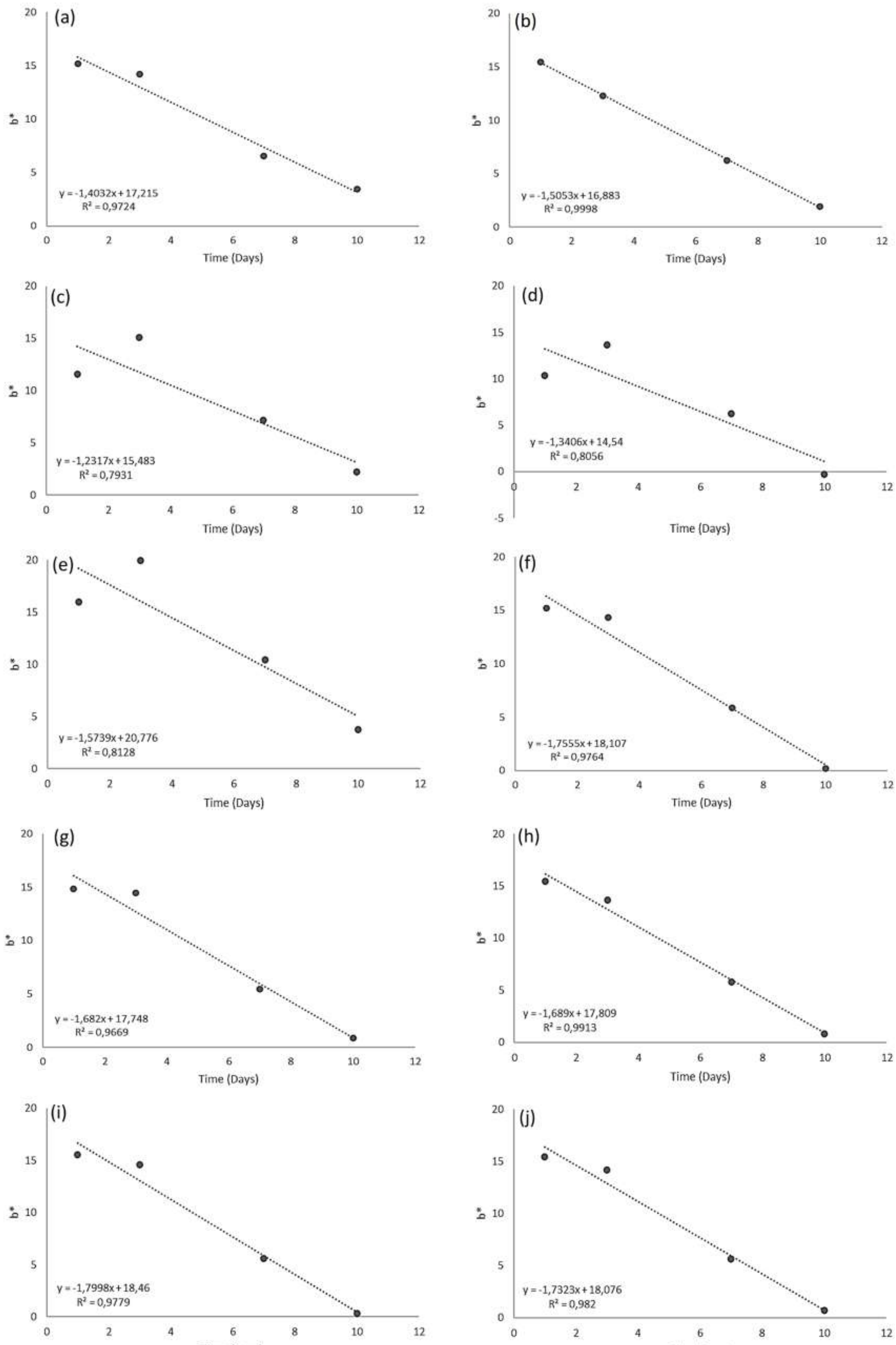

Figure 4. b* scatter diagrams. (a) Sample 1: Meat only, (b) Sample 2: Meat + ajisal (1%) + pepper (0.1%), (c) Sample 3: Meat + ajisal + pepper + 3% goji powder, (d) Sample 4: Meat + ajisal + pepper + 6% goji powder, (e) Sample 5: Meat + ajisal + pepper + 3% goji in extract, (f) Sample 6: Meat + ajisal + pepper + 6% goji in extract, (g) Sample 7: Meat + 3% goji powder, (h) Sample 8: Meat + 6% goji powder, (i) Sample 9: Meat + 3% goji in extract and (j) Sample 10: Meat + 6% goji in extract.

Table 2. Prediction and validation of mathematical models for samples 1, 4 and 5.

Samples	Sample composition	Time/d	*a* observed*	*a* mathematical model*	*b* observed*	*b* mathematical model*
Sample 1 Control	Meat (80%) + fat (20%) a*(t) = -1,7366t + 33,043 b*(t) = -1,4032t + 17,215	1	33.4875	31.3064	15.1556	15.8118
		3	27.1952	27.8332	14.2165	13.0054
		5		24.3600		10.1990
		7	15.8341	20.8868	6.5339	7.3926
		9		17.4136		4.5862
		10	19.1876	15.6770	3.4858	3.1830
Sample 4	Control (92.9%) + commercial salt (1%) + pepper (0.1%) + ***goji berry* powder (6%)** a*(t) = -0,5462t + 16,59 b*(t) = -1,3406t + 14,54	1	15.5809	16.0438	10.3795	13.1994
		3	15.7700	14.9514	13.6817	10.5182
		5		13.8590		7.8370
		7	12.2468	12.7666	6.2336	5.1558
		9		11.6742		2.4746
		10	11.2936	11.1280	-0.2875	1.1340
Sample 5	Control (95.9%) + commercial salt (1%) + pepper (0.1%) + ***goji berry* aqueous extract (3%)** a*(t) = -1,7469x + 36,32 b*(t) = -1,5739x + 20,776	1	32.4025	34.5731	15.9567	19.2021
		3	33.4108	31.0793	19.9517	16.0543
		5		27.5855		12.9065
		7	25.1634	24.0917	10.3987	9.7587
		9		20.5979		6.6109
		10	17.6180	18.8510	3.7438	5.0370

characterized by its dependence on the sensitivity of the catching image device and intolerance to light variations (International Electrotechnical Commission, 1999; Lima, 2020).

The CIE Lab model is a suitable tool for various color management systems (CMS) provided that it does not depend on the device used. Temporal evaluation covering 10 d of experiments shows that samples 2 and 5 presented the highest luminosity values, with an apparent contrast to the other samples from day 3 onwards. Sample 5 (L* ~ 30) displayed a noteworthy luminosity value, clearly far from the luminosity region that encompasses the remaining samples.

Table S1 (supporting information) indicates that experimental errors for L* did not exceed 25%, when comparing all the hamburgers containing additives with control. Sample 4, however, presented a considerable percentage error of 41.46%. The highest error percentages are detected in the first day of data collection and decrease over time. On the tenth day, the highest error value of the samples did not exceed 10.12% (Sample 2).

A well-adjusted mathematical model might be able to represent all the systematic information contained in the sample space and the deviations must relate only to the random errors embedded in the measurements (Bona et al., 2002; Coppo et al., 2014). The mathematical model we propose here can be validated for predictive purposes, i.e., the L* values for non-monitored days can be predicted, since the R^2 values indicate good quality of linear fit and the error values are low.

Studies developed by Minz et al. (2020) applied to the powder bed, an algorithm was developed to process images in high-definition (HD) and provide better accuracy in measurements. Considering that the HD image matrix makes image processing complex and with high utilization of machine resources, algorithms were used for pre-processing and reduction of the image matrix, in addition to the CIE lab colorimeter parameters obtained in the RGB image transformation.

According to Pathare et al. (2013) computer vision has been used to objectively measure the color of different foods since they provide some obvious advantages over a conventional colorimeter, namely, the possibility of analyzing each pixel of the entire surface of the food and quantifying surface characteristics and defects.

4.2 Coloring and antioxidant activity

Myoglobin is the main protein responsible for meat color, although other heme proteins, such as hemoglobin and cytochrome C, also play important roles. According to Mancini & Hunt (2005), the mechanisms of meat coloration and discoloration are:

1. Oxygenation: there is a predominance of deoxy hemoglobin, a substance that belongs to the heme group, with a Fe^{2+} metallic center and its sixth coordination site is vacant. In this case, the color of meat is red/purple or purple/pink; when dioxygen binds to the empty Fe^{2+} site, the color changes to cherry-red, but the valence of iron is maintained.
2. Oxidation: oxidation of myoglobin derivatives, where Fe^{2+} is oxidized to Fe^{3+}.
3. Oxidation + reduction: the color of meat is preserved due to the reduction of metmyoglobin, which is possible by reducing enzyme systems and NADPH. However, after animal death, this supply is no longer available.
4. Formation of carboxy-hemoglobin: this is a not fully elucidated mechanism, but it is known that carbon monoxide (CO) binds to the vacant site of deoxy-hemoglobin, leading to a bright red color.

As detected by computer vision, goji berry is capable of maintaining color parameters at the same intensity as for the control sample or samples containing salt and pepper. In other words, these additive-hamburgers present a color already widely accepted by consumers. During the period of 10 days covered by the experiment, no visual color degradation was observed, indicating a reliable storage time.

The color preservation is a result of rate decrease or inhibition of meat protein oxidation (O'Sullivan et al., 2003). The phenolic antioxidants found in goji berry donate hydrogens and stabilize oxidizable substances likely through mechanistic pathways ii and/or iii (discussed above). Therefore, the antioxidants do not compete with the substrate for oxygen absorption, instead, they deactivate oxidizing species.

It is widely known that oils and fats are susceptible to oxidation, through radical reactions, when in contact with atmospheric oxygen. Oxidation is accelerated by the presence of metal ions, light, temperature, ionizing radiation (Bondioli et al., 2003; Ferrari & Souza, 2009; Galvan et al., 2013) and leads to the formation of acids, aldehydes, esters, ketones, peroxides, hydroperoxides, and alcohols, as well as to polymerization products (Coppo et al., 2014; Xin et al., 2009). It is worth emphasizing the possibility of rancidification provided by fat, that accounts for 20% hamburger composition. Degradation products can damage flavor, but the goji berry phenolics may act as free radical terminators, leading to protection from both fat and meat oxidation (Borsato et al., 2014; Coppo et al., 2014).

Besides both meat color and freshness preservation, the goji berry also depicts other beneficial effects over consumer health, as it neutralizes the effects of free radicals in the body. As a matter of fact, the hamburgers here studied can act as functional food, preventing aging and promoting neuroprotection, diabetes control and cytoprotection, including antitumor activity (Donno et al., 2015).

5 Conclusion

The evaluation of hamburgers with goji berry additives demonstrated a distinguished L*, a*, and b* results for sample 5 (meat + commercial salt + pepper + goji berry aqueous extract) and samples 7-10 (only goji berry, powdered or in aqueous extract). The meat colors of these trials remained visually acceptable during a 10 day-evaluation, proving the role of the antioxidants in goji berry as a good preservative. The addition of goji berry in hamburgers, pioneered in this study, present advantageous characteristics both in terms of storage time and positive effect on human health owing to the polyphenols in the natural additive.

This study also provided an appealing insight on the predictive mathematical model presented, with a fast, low-cost, and reliable homemade system to assess meat color and stipulate a realistic storage time, when the visual characteristics are maintained. In computer vision, some adjustments are necessary to obtain the results with greater accuracy, such as using software with environment control, automating the process of obtaining images and using machine learning.

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Supplementary Material

Supplementary material accompanies this paper.

Table S1. Experimental errors for L* of all the samples of hamburgers.

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CONSIDERAÇÕES FINAIS

A Goji Berry apresenta um amplo potencial de aplicação na indústria, com destaque especial para a indústria de alimentos devido às suas propriedades nutricionais e funcionais. Além de seus inúmeros efeitos benéficos à saúde (devido à sua rica composição nutricional e propriedades bioativas), também é considerado de alto interesse para a indústria, por ser capaz de agregar valor aos produtos.

Especificamente na indústria de cárneos, a sua adição é direcionada, principalmente, à melhoria da conservação e manutenção, tendo como resultado o aumento da vida de prateleira. Na análise realizada com a utilização da Goji Berry como aditivo natural aos hambúrgueres, verificou-se que os antioxidantes presentes no fruto contribuem para a estabilidade dos hambúrgueres durante o tempo de exposição, retardando a oxidação de lipídios, resultando em uma ampliação na vida de prateleira. Observou-se ainda que as amostras que foram revestidas com a cobertura contendo Goji Berry apresentaram melhores resultados do que aquelas que foram incorporadas com Goji Berry, apresentando menores perdas de peso, cor, menores valores de pH e maior maciez.

Em geral, um dos principais atributos analisados pelos consumidores de produtos cárneos é a cor, porém, se realizada por testes convencionais como por exemplo pelo colorímetro, sua análise é considerada demorada e nem sempre muito efetiva. Dessa forma, após a análise da atuação da Goji Berry como um aditivo natural (que se mostrou funcional e com resultados satisfatórios), a abordagem seguinte foi a predição da cor dos hambúrgures através de um sistema de visão computacional.

Nessa análise, o modelo proposto demonstrou ser um método rápido, viável, barato, confiável e de fácil manuseio. Esses aspectos foram analisados através de um modelo matemático preditivo que foi capaz de avaliar a mudança da coloração da carne e estipular um tempo de armazenamento realista.

Durante a investigação, foi possível visualizar que amostras com extrato aquoso de Goji Berry se comportaram de forma diferente quando comparadas ao controle (*sample 1*) e às amostras adicionadas de sal e pimenta (*sample 2*). De forma que, as cores da carne permaneceram visualmente aceitáveis durante 10 dias. Entretanto, para a obtenção de resultados com maior precisão seria necessário a utilização de softwares com controle de ambiente, automatização do processo de obtenção de imagens e até mesmo a utilização da ferramenta de *Machine Learning* para extrapolar os resultados.

Sendo assim, através dos diferentes estudos realizados e os resultados encontrados, foi possível comprovar a eficácia do Goji Berry para a indústria de alimentos, em especial na conservação dos alimentos do tipo hambúrgueres e na preservação de sua cor. Essa capacidade foi observada tanto pelos métodos convencionais, como também pelo modelo matemático preditivo estabelecido.