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TESE

“Desenvolvimento, caracterização e aplicação de concentrados proteicos para o mercado *plant-based* a partir de grão-de-bico e lentilha”

RODRIGO FERNANDES CALDEIRA

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“E aprendi que se depende sempre

De tanta, muita, diferente gente

Toda pessoa sempre é as marcas

Das lições diárias de outras tantas pessoas

E é tão bonito quando a gente entende

Que a gente é tanta gente onde quer que a gente vá

E é tão bonito quando a gente sente

Que nunca está sozinho por mais que pense estar.”

(Caminhos do Coração – Gonzaguinha)

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RESUMO GERAL

CALDEIRA, Rodrigo Fernandes. **Desenvolvimento, caracterização e aplicação de concentrados proteicos para o mercado *plant-based* a partir de grão-de-bico e lentilha**. 2024, 119p. Tese (Doutorado em Ciência e Tecnologia de Alimentos). Instituto de Tecnologia, Departamento de Tecnologia de Alimentos, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 2024.

Com o crescimento da demanda mundial por fontes alternativas de proteínas, notadamente as de origem vegetal, o objetivo deste estudo foi obter concentrados proteicos de grão-de-bico e lentilha por via úmida clássica, avaliando uma primeira etapa de escalonamento e analisando os concentrados proteicos dos grãos quanto às propriedades nutricionais e tecnológicas. Para a etapa de extração alcalina dos grãos, foram avaliados os parâmetros de pH, tempo de extração e relação sólido:líquido. Já para a fase de precipitação isoelétrica dos extratos alcalinos, foram avaliados os parâmetros de pH e tempo de precipitação sob agitação. Os melhores resultados para cada etapa do processo de cada grão foram escalonados para um aumento de 10 vezes e os mesmos resultados foram observados quando comparados aos processos em escala laboratoriais. O melhor resultado para a obtenção do concentrado proteico de lentilha foi com extração alcalina em pH 9,0, por 10 min e com relação soluto:solvente 1:10. Já na precipitação ácida a melhor resposta obtida foi no pH 5,0 e tempo de 10 minutos. A recuperação proteica foi de 14% em massa e o concentrado apresentou 85g/100g de proteína. Os resultados observados para a obtenção do concentrado de grão-de-bico, foram com extração alcalina em pH 8,5, por 20 min e com relação soluto:solvente 1:12. Já na precipitação ácida a melhor resposta obtida foi no pH 4,5 e tempo de 10 minutos. Após a seleção dos processos para a obtenção dos concentrados proteicos, os mesmos foram avaliados para cinco diferentes propriedades tecnológicas e, de forma geral, todas as farinhas e concentrados analisados mostraram-se aptos para serem aplicados em diferentes categorias de alimentos e bebidas. Pôde-se observar que a farinha (LF) e o concentrado proteico de lentilha (LPC) apresentaram solubilidade maiores nos pH 3 e 9, enquanto a LF apresentou maior retenção de água e óleo. Em relação as respostas para formação de espuma, estabilidade de espuma, capacidade emulsificante e estabilidade emulsificante o LPC, apresentou maiores valores. Já os resultados para farinha (CF) e concentrado proteico de grão-de-bico (CPC) mostraram que a farinha e o concentrado proteico apresentaram valores de solubilidades bastante inferiores em comparação com a lentilha em extremos de pH. A CF apresentou melhores resultados para a capacidade de retenção de água, capacidade de formação de espuma, capacidade emulsificante, enquanto o CPC teve melhores respostas para as propriedades tecno-funcionais de capacidade de retenção de óleo, estabilidade da espuma, estabilidade emulsificante, menor grau de gelificação. Por fim, os concentrados proteicos e as farinhas de lentilha e grão-de-bico e lentilha foram avaliados quanto às propriedades nutricionais, sendo realizadas, a composição centesimal, quantificação de minerais e de aminoácidos, quantificação de fatores antinutricionais e digestibilidade dos ingredientes. Os resultados mostraram que os ingredientes de lentilha contêm maior teor de proteína se comparados aos de grão-de-bico. Potássio, fósforo e magnésio foram os minerais com maior concentração nos ingredientes, houve aumento de sódio, ferro e aminoácidos essenciais nos concentrados se comparados às farinhas. O inibidor de tripsina foi aumentado no concentrado de lentilha, mas reduzido no grão-de-bico, enquanto o ácido fítico foi reduzido nos concentrados e todas as amostras apresentaram baixos níveis de oligossacarídeos promotores de flatulência. Os concentrados proteicos apresentaram maior digestibilidade quando comparados às farinhas. De acordo com todos os resultados observados nesse estudo, os concentrados proteicos de lentilha e grão-de-bico mostraram ser uma alternativa proteica promissora para o mercado *plant-based*, podendo vir a ser produzidos como ingredientes para abastecer a indústria nacional desse segmento de mercado.

Palavras-chave: *Pulses*, concentrado proteico, *Lens culinaris*, *Cicer arietinum*, propriedades tecnológicas e nutricionais, fatores antinutricionais, digestibilidade de proteínas *in vitro*, composição mineral.

ABSTRACT

CALDEIRA, Rodrigo Fernandes. **Development, characterization and application of protein concentrates for the plant-based market from chickpeas and lentils**. 2024, 121p. Thesis (PhD in Food Science and Technology). Institute of Technology, Department of Food Technology, Federal Rural University of Rio de Janeiro, Seropédica, RJ, 2024.

With the growth in the global demand for alternative protein sources, especially those of plant origin, the objective of this study was to obtain chickpea and lentil protein concentrates by the classical wet process, evaluating a first scaling up stage and analyzing the protein concentrates for their nutritional and technological properties. For the alkaline extraction of the grains, the parameters of pH, extraction time and solid:liquid ratio were evaluated. For the isoelectric precipitation of the alkaline extracts, the parameters of pH and precipitation time under agitation were evaluated. The best results for each step of the process for each grain were scaled up to a 10-fold increase and the same results were observed when compared to laboratory-scale processes. The best results for the lentil protein concentrate for the alkaline extraction was at pH 9.0 for 10 min and with a solute:solvent ratio of 1:10. For the acid precipitation, the best response was obtained at pH 5.0 and a time of 10 minutes. Protein recovery was of 14% of mass and the concentrate presented 85 g/100 g of protein. The results observed for obtaining the chickpea concentrate were for the alkaline extraction at pH 8.5, for 20 min and with a solute:solvent ratio of 1:12. For the acid precipitation, the best response was obtained at pH 4.5 and a time of 10 minutes. After selecting the processes for obtaining the protein concentrates, they were evaluated for five different technological properties and, in general, all flours and concentrates analyzed were suitable for application in different categories of food and beverages. It was observed that lentil flour (LF) and protein concentrate (LPC) presented higher solubility at pH 3 and 9, while LF presented greater water and oil retention. Regarding the responses for foam formation, foam stability, emulsifying capacity and emulsifying stability, LPC presented higher values. The results for chickpea flour (CF) and protein concentrate (CPC) showed that the flour and protein concentrate presented much lower solubility values when compared to lentils at extremes pH. CF presented better results for water retention capacity, foam formation capacity and emulsifying capacity, while CPC had better responses for the oil retention capacity, foam stability, emulsifying stability and lower degree of gelation. Finally, the lentil and chickpea protein concentrates and flours were evaluated for their nutritional properties by the centesimal composition, minerals, amino acids and antinutritional factors quantifications and digestibility of the ingredients. The results showed that lentil ingredients contain higher protein contents when compared to chickpea ingredients. Potassium, phosphorus and magnesium were the minerals with the highest concentration in the ingredients; there was an increase in sodium, iron and indispensable amino acids in the concentrates when compared to the flours. The trypsin inhibitor was increased in the lentil concentrate, but reduced in the chickpea protein, while phytic acid was reduced in the concentrates and all samples presented low levels of flatulence-promoting oligosaccharides. Protein concentrates presented greater digestibility when compared to flours. According to all the results observed in this study, lentil and chickpea protein concentrates proved to be a promising protein alternative for the plant-based market and, may be produced as ingredients to supply the national industry in this market segment.

Keywords: Pulses, protein concentrate, *Lens culinaris*, *Cicer arietinum*, technological and nutritional properties, antinutritional factors, in vitro protein digestibility, mineral composition.

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LISTA DE ABREVIAÇÕES, SIGLAS E SÍMBOLOS

Ala – alanine;
ANOVA - analysis of variance;
AOAC – Association of Official Analytical Chemists;
AOCS - American Oil Chemists' Society;
Arg – arginine;
Asn – Asparagine.
Asp – asparagine;
Asp - aspartic acid;
CF - chickpea flour;
CPC – chickpea protein concentrate;
CSPC - control solution of each ingredient;
CW - cell wall fragments;
Cys – cysteine;
DF - dilution factor;
DNA - deoxyribonucleic acid;
EAI – emulsifying capacity index;
ESI - emulsion stability;
FAO - Food and Agriculture Organization of the United nations;
FBC- fava bean concentrate;
FC - foaming capacity;
FS - foam stability;
g – gram;
Glu - glutamic acid;
Gly – glycine;
h – hour;
HCl - hydrochloric acid;
His – histidine;
His – histidine;
Ile – isoleucine;
kDa - Kilodaltons;
L – Liter;
Leu – leucine;
LF - Lentil flour;
LPC – lentil protein concentrate;
LSTD - low molecular protein standard solution;
Lys – lysine;
M_0 - initial mass of the sample;
M_1 - mass of the microtube with the dry sample;
M_2 - mass of the tube with the sample after discarding the oil or water;
Met – methionine;
mg – milligram;
min – minutes;
mL – milliliter;
mm – millimeter;
NaCl - sodium chloride;
NaOH - sodium hydroxide;
OHC – oil holding capacity;
PB – protein bodies;
pH – hydrogen potential;
Phe – phenylalanine;
Pro – proline;
SDS - sodium dodecyl sulfate;
Ser – serine;
SG – starch granule;
SPC - sample protein concentration;
The – threonine;
TPLF - total protein in lentil flour;
TPLPC - total protein in lentil protein concentrate;
Try – tryptophan;
Tyr – threonine;
Tyr – tyrosine;
V_0 - initial volume before stirring;
V_1 - total volume after stirring;
V_2 - total volume after each tested time;
Val – valine;
WHC – water holding capacity;
θ - oily volume of the emulsion;
μm – micrometer;
°C – celsius degree.

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INTRODUÇÃO GERAL

O consumidor está cada dia mais exigente em relação às características dos alimentos que consome, inclusive a origem das matérias-primas utilizadas, sendo cada vez mais importante acompanhar as mudanças nas preferências e expectativas dos consumidores, bem como a análise das inovações alimentares e seu impacto no mercado global (Huebbe & Rimbach, 2020; Jeske et al., 2018). No decorrer das últimas décadas, tornou-se crucial para as indústrias a busca pela adequação, melhorando a segurança, o prazo de validade, o valor nutricional, a conveniência e palatabilidade dos alimentos.

Nos últimos anos, uma boa parte dos consumidores passou a desenvolver a mentalidade de uma alimentação voltada para uma dieta à base de plantas e de proteínas alternativas, adotando inovações no mercado de alimentos, que exploram essa tendência de consumo (Aschemann-Witzel et al., 2020a). É notória a explosão de produtos alimentícios, base de plantas encontrados nas prateleiras dos supermercados mundo afora. Destaque tem sido dado às alternativas a carne, laticínios, aves, bebidas e frutos do mar. Esses produtos atendem a demanda existente para consumidores vegetarianos, flexitarianos e veganos (Noguerol et al., 2021a; Rosenfeld et al., 2020a).

A indústria de alimentos e os setores empresariais interessados precisam estar cientes dos desafios e oportunidades de investir na tendência de alimentos e proteínas à base de plantas. Para tanto, faz-se necessário uma revisão do mercado e da disponibilidade de ingredientes, o que pode ajudar a traçar o caminho para os próximos anos (Aschemann-Witzel et al., 2020a).

O mercado brasileiro já oferece diversos produtos à base de plantas, e sua expansão iniciou-se a partir de 2019, sendo encontrados nas gôndolas dos supermercados diversos produtos análogos a carne, como: almôndega, carne moída, embutidos fatiados, empanados, hambúrgueres, quibe, linguiça, empanados de frango e de carne, salsichas, pratos prontos, tiras de frango, extratos vegetais em substituição ao leite, iogurte, manteiga, queijo, requeijão, maionese, ovo vegetal para panificação e a tendência é aumentar ainda mais a oferta (The Good Food Institute Brazil, 2020).

As proteínas de origem animal, em geral, suprem as necessidades dos aminoácidos essenciais, porém, ao se consumir somente proteínas vegetais, deve-se associar diferentes fontes, de forma a garantir a oferta dos mesmos, já que essas proteínas nem sempre apresentam todos os aminoácidos essenciais em boas quantidades. Por exemplo, os cereais são geralmente deficientes em lisina e ricos em aminoácidos sulfurados (metionina). Por outro lado, as leguminosas são ricas em lisina, leucina, ácido aspártico e arginina, mas geralmente apresentam baixo ou nenhum conteúdo de aminoácidos sulfurados (metionina e cisteína) e triptofano. Logo, a associação desses alimentos permite uma boa complementaridade em termos da ingestão de aminoácidos (Bessada et al., 2019a; J. I. Boye et al., 2010; Chardigny & Walrand, 2016).

Além da relevância nutricional das proteínas de origem vegetal, elas também são utilizadas em diversos produtos alimentícios para melhorar as características tecnológicas deles e seu uso apoia-se em uma ou mais propriedades como: solubilidade, capacidade de absorção de água e óleo, capacidade de formação de espuma, capacidade emulsificante e/ou capacidade de formação de gel (J. I. Boye et al., 2010; Kumar & Pandey, 2020).

O padrão de proteína vegetal mais comumente utilizado no mercado é extraído da soja, porém existe um grande interesse em explorar proteínas de outras fontes, especialmente de leguminosas. Isso se deve ao seu alto valor nutricional e alto teor proteico, disponibilidade de culturas no Brasil e em outros países, baixo custo de produção, sustentabilidade e funcionalidade (Johnston et al., 2015).

No entanto, as proteínas de leguminosas ainda não são bem aceitas pelo consumidor devido aos *off-flavors* que apresentam, especialmente um sabor conhecido como *beanny*, se remetendo à grãos verdes. Assim, apresentam-se como desafios a superação desses gargalos sensoriais e o aumento da solubilidade dessas proteínas, para que possam ser mais amplamente aplicadas em diferentes alimentos e bebidas (Chardigny & Walrand, 2016; Magrini et al., 2018).

Nas últimas décadas ocorreram diversos avanços nessa direção, porém, vários caminhos ainda podem ser explorados, especialmente no que diz respeito às rotas tecnológicas para a obtenção dessas proteínas e desenvolvimento de ingredientes de base nacional e a funcionalização das proteínas, para que possam entregar melhores aspectos sensoriais, tecnológicos e nutricionais.

Diante do exposto, este trabalho tem por objetivo estudar os processos de obtenção das proteínas de leguminosas não oleaginosas (pulses) de grão-de-bico e lentilha, bem como o desempenho tecnológico e nutricional dos ingredientes obtidos, além de avaliar se estes processamentos afetariam o off-flavor, bem como outros fatores anti-nutricionais, de modo a subsidiar informações para o mercado de ingredientes proteicos de base vegetal no Brasil, tendo um olhar para a diversificação de matérias-primas dessas culturas produzidas no país com alto potencial de expansão para o abastecimento interno e potencial para futura exportação.

ESTRUTURAÇÃO DA TESE

A tese foi dividida em quatro capítulos e gerou 5 publicações que são citadas no anexo.

Os capítulos da Tese dividem-se em:

CHAPTER I – Revisão da Literatura

CHAPTER II - Processing parameters, techno-functional properties and potential food application of lentil protein concentrate as an ingredient for the *plant-based* market – Artigo publicado no periódico Food Research International.

CHAPTER III - Proteins from chickpea: obtaining process, techno-functional properties and potential application.

CHAPTER IV - Nutritional composition and *in vitro* digestibility of flours and protein concentrates from kabuli chickpeas and green lentils.

CHAPTER I

REVISÃO DE LITERATURA

2 REVISÃO DE LITERATURA

2.1. Leguminosas não oleaginosas – Pulses

Pulses ou leguminosas não oleaginosas é derivada de uma palavra latina “puls ou pultis” que significa pasta espessa. São sementes secas de leguminosas, pertencentes à família *Fabaceae* (*Leguminoseae*). A Organização das Nações Unidas para Alimentação e Agricultura (FAO) reconhece 11 tipos de pulses cultivados globalmente e seus representantes mais conhecidos são o feijão-comum (*Phaseolus vulgaris*), feijão-caupi (*Vigna unguiculata*), ervilha (*Pisum sativum*), lentilha (*Lens culinaris*) e o grão-de-bico (*Cicer arietinum*) que são utilizados normalmente como fontes proteicas na indústria alimentícia e contém, além das proteínas, fibras dietéticas, minerais, vitaminas e vários fitoquímicos (Bessada et al., 2019a; Danihelová & Šturdík, 2012; Foschia et al., 2017; Kumar & Pandey, 2020; Temba et al., 2016). A leguminosa é “qualquer fruto seco ou vagem” que contenha sementes ou grãos secos e que fixam nitrogênio no solo, é bastante utilizada para consumo humano ou ração animal devido a uma característica comum em todas, são altamente nutritivas (Asif et al., 2013; Proserpio et al., 2020).

A conscientização e a demanda por leguminosas não oleaginosas continuam crescendo, incentivando o desenvolvimento e o lançamento de novos produtos a cada ano com intuito de atender a demanda por produtos sem glúten, ricos em proteínas, fibras, amido resistente e baixo teor de gordura (Bogahawaththa et al., 2019; Bresciani & Marti, 2019; Chung et al., 2008; Lam et al., 2018).

O alimento do futuro terá de ter a capacidade de fornecer diversos benefícios uteis a saúde dos consumidores, como saciedade mais longa (ou seja, útil para controle de peso), liberação de energia mais eficiente (ou seja, crucial para atletas), captação controlada de glicose (ou seja, vital para diabéticos), e liberação e absorção otimizada de drogas e/ou nutrientes (Bornhorst & Singh, 2014).

2.2. Pulses em estudo

2.2.1. Grão-de-bico (*Cicer arietinum*)

É um membro da família das leguminosas da estação fria *Fabaceae* (*Leguminosae*). Acredita-se que registros da planta datam há mais 7.450 anos no Oriente Médio. Desse período em diante passou a ser cultivado em regiões temperadas e semi-áridas do mundo, como Ásia, Europa, Austrália e América do Norte (Roy et al., 2010). Mais de 50 Países cultivam o grão-de-bico, sendo que sua maior produção encontra-se no Sul e Sudeste Asiático com mais de 80% de contribuição regional (Ghoshal & Kaushal, 2020; Hirdyani, 2015; Merga & Haji, 2019a). Acredita-se que o grão-de-bico tenha surgido no Sudeste da Turquia e a vizinha Síria, devido a três espécies selvagens serem encontrada lá (C.

bijugum, C. echinospermum e C. reticulatum), sendo que a C. reticulatum é considerada progenitora do grão-de-bico cultivado mundialmente (Cicer arietinum) (Sajja et al., 2017).

A Índia é o maior produtor de grão-de-bico do mundo, respondendo por mais de 65% (9,075 milhões de toneladas em 2019) da produção, porém também é o maior consumidor, não sendo autossuficiente quanto ao abastecimento interno. Já o segundo maior produtor e exportador é a Austrália com 14% de participação na produção (Merga & Haji, 2019a; Muehlbauer & Sarker, 2017; Queiroga et al., 2021; Roy et al., 2010).

A variedade Desi, que apresenta camada pigmentada (castanho a preto), espesso tegumento e tamanho pequeno da semente é responsável por 80% da produção, enquanto que os outros 20% são produzidos com os tipos Kabuli, que apresenta as sementes com cascas de cor branca a creme e varia em tamanho de pequeno a grande (Hevryk et al., 2020; Jukanti et al., 2012; S. Sharma et al., 2020).

Figura 01 – Características das variedades de grão-de-bico. **Fonte:** (Queiroga et al., 2021)

A produção, do grão-de-bico em 2018 foi de 17,2 milhões de toneladas, fazendo com que fosse a segunda leguminosa não oleaginosa mais produzida no mundo. Em primeiro lugar encontra-se o feijão com 30,4 milhões de toneladas (R. Kaur & Prasad, 2021a; Merga & Haji, 2019a; Santos et al., 2021).

A produção, brasileira é em torno de 2.500 a 3.500 kg/ha, tendo um custo de produção bem menor se comparado com o feijão (30 a 40%). No ano de 2019 o Brasil deixou de importar grão-de-bico, devido ao aumento da área plantada para 9.000 hectares, naquele período foi até exportado o seu excedente para paises asiáticos, porém voltou a importar devido a alta demanda pelas indústrias produtoras de alimentos. (Queiroga et al., 2021; Rodrigues, 2019).

No Brasil, o grão-de-bico foi introduzido pelos imigrantes espanhóis e do Oriente Médio no final do século XIX e início do XX, onde os nascidos no Brasil procuraram manter a tradição familiar, com o consumo do grão (Sharma, 1984).

Na atualidade o grão é utilizado cozido, às vezes misturados com alimentos como hortaliças, em carnes, molhos e condimentos. Sua farinha é utilizada como ingrediente na fabricação de pães e bolos ou na formulação de alimentos infantis, já seus grãos descascados são triturados e empregados para fazer sopas, pastas ou sobremesas.(Nascimento et al., 1998).

2.2.1.1 Composição química do grão-de-bico

O grão-de-bico é uma excelente fonte de carboidratos, especialmente os de baixa digestibilidade, além de ser rico em proteínas (12,4 a 31,5%), Possui quantidades significativas de todos os aminoácidos essenciais, exceto os sulfurados.

O amido (41% a 50%) é o principal carboidrato de armazenamento, seguido pelo teor de fibra alimentar (6 a 10%), já os lipídios (2,70 a 6,48%) estão presentes em maior quantidade de comparado a outros pulses, como feijões, ervilha e lentilha, porém em menor quantidade que a soja, enquanto leguminosa. Os lipídeos estão presentes na forma de ácidos graxos insaturados como os linoléico e oléico.

Já quanto à composição de oligonutrientes e minerais, o grão-de-bico apresenta valores elevados de cálcio, magnésio, fósforo e, principalmente, potássio. Em quantidades mais modestas encontram-se as vitaminas solúveis como a riboflavina (B_2), ácido pantotênico, (B_5), piridoxina (B_6), além da niacina, tiamina, folato e o precursor da vitamina A β-caroteno (Hirdyani, 2015; Jukanti et al., 2012; Kishor et al., 2017).

2.2.1.2 Proteínas

O grão-de-bico apresenta-se equiparado com outras leguminosas como a soja e o feijão em teor de proteína, apresentando alta biodisponibilidade e boa digestibilidade (48–89,01%). As globulinas e albuminas são as principais proteínas de armazenamento. As globulinas são compostas de legumina e vicilina (Y. W. Chang et al., 2012; Faridy et al., 2020). A legumina (360 kDa) é a principal proteína de armazenamento e representa 97% do total de globulinas (Faridy et al., 2020; Serrano-Sandoval et al., 2019; Yust et al., 2003). As proteínas do grão-de-bico apresentam um adequado equilíbrio de aminoácidos destacando-se Glu, Asp, Arg, Leu, Phe, Lys, Ser, em menor proporção apresenta a His, Gly, Tre, Ala, Tyr, Val, Ile; sendo deficientes em aminoácidos sulfurados como Met e Cys, sendo o oposto dos cereais, que são ricos em aminoácidos contendo enxofre e a lisina é o aminoácido limitante, daí a importância de uma dieta balanceada contendo leguminosas e cereais (Cortés-Giraldo et al., 2016; Faridy et al., 2020; Kaur & Prasad, 2021a).

2.2.1.3 Carboidratos e fibras alimentares

Os carboidratos correspondem ao componente mais abundante do grão-de-bico (62–70%) e são formados principalmente por oligossacarídeos (α-galactosídeos) e polissacarídeos como o amido, sendo 35% resistente e 65% disponível (Faridy et al., 2020). Os oligossacarídeos são compostos por 2–10 unidades de monossacarídeos e a quantidade de oligossacarídeos (com base na massa seca) é de

cerca de 10,4–17,0% no grão-de-bico. O grão-de-bico é uma boa fonte de α-galactooligossacarídeo (α-GOS), principalmente ciceritol, rafinose, estaquiose e uma pequena quantidade de verbascose, que são carboidratos não digeridos no intestino delgado. Além deles, é encontrado também amido resistente, pectina, hemicelulose, celulose (Faridy et al., 2020; R. Kaur & Prasad, 2021a; Rachwa-Rosiak et al., 2015; Y. Zhang et al., 2017). Os oligossacarídeos não digeríveis são capazes de modificar a microbiota intestinal, auxiliando no crescimento de bactérias benéficas (bifidobactérias e lactobacilos) e inibindo o crescimento de bactérias patogênicas e putrefativas. Além de serem fermentados pela microbiota colônica, produzindo uma mistura de ácidos graxos de cadeia curta, principalmente os ácidos acético, propiônico e butírico. Contudo, esses mesmos oligossacarídeos podem gerar flatulência em alguns indivíduos, dificultando o consumo por parte da população sensível à esse efeito (Y. Zhang et al., 2017).

As fibras solúveis são partes comestíveis das plantas ou carboidratos análogos que são resistentes à digestão e absorção no intestino delgado humano com fermentação completa ou parcial no intestino grosso (Wang & Toews, 2011). São compostas de poli/oligossacarídeos, lignina e outras substâncias vegetais. Alguns autores (Murty et al., 2010; Nestel et al., 2004) compararam uma dieta com grão-de-bico com uma dieta habitual, os mesmos detectaram que as fibras alimentares do grão-de-bico, tornavam de uma maneira geral o intestino mais saudável acompanhado por facilidade e aumento na frequência da defecação, com consistência das fezes mais macias. As fibras alimentares promovem o relaxamento na função intestinal, auxiliando na movimentação do material através do sistema digestivo (Jukanti et al., 2012).

2.2.1.4 Minerais

Devido à importância dos minerais na saúde humana, o grão-de-bico apresenta uma boa fonte de minerais tais como, ferro, zinco, magnésio e cálcio, fornecendo uma quantidade média de 5,0 mg, 4,1 mg, 138 mg e 160 mg por 100 g de sementes de grão-de-bico cru, respectivamente. As necessidades nutricionais diárias de ferro e zinco podem ser atendidas consumindo 100 g de grão-de-bico e 200 g de grão-de-bico podem atender às necessidades diárias de magnésio. Já para os outros minerais, as quantidades são menores e há a necessidade de consumo de outros alimentos para que se alcance a necessidade mínima diária (Jukanti et al., 2012; Kaur & Prasad, 2021a)

2.2.1.5 Vitaminas

O organismo humano necessita de pequenas quantidades de vitaminas, que podem ser supridas por meio de uma alimentação diária bem balanceada, com inclusão de leguminosas, cereais, vegetais, frutas, carnes e laticínios. O grão-de-bico consumido com outros alimentos poderá suprir

essas necessidades por vitaminas. Em sua composição é encontrado ácido fólico em quantidade bem considerada e tocoferóis. Em quantidades mais modestas, encontram-se as vitaminas hidrosolúveis como a riboflavina (B_2), ácido pantotênico, (B_5) e piridoxina (B_6) (Hirdyani, 2015; Jukanti et al., 2012; Kishor et al., 2017).

2.2.2. Lentilha (*Lens culinaris*)

É da família das *Leguminaseae*, também conhecidas como dhal vermelho, masur ou ervilha partida, além de ser considerada uma das mais antigas do mundo 7.000 aC, originária do sudoeste da Ásia. Na safra de 2019 foi produzido 5.734.201 toneladas, tendo como maior produtor o Canadá, seguido por Índia, Turquia, Austrália, Estados Unidos e Nepal, que juntos representam 57,5% da produção mundial, porém é cultivada em mais de 70 países (Benayad & Aboussaleh, 2021; Khazaei et al., 2019a).

As variedades de lentilhas são diferenciadas através do seu padrão, tamanho das sementes e sua cor. As lentilhas verdes são mais consumidas nos Estados Unidos e Canadá, enquanto as lentilhas vermelhas são mais comuns em países do sul da Ásia, como Índia e Nepal (Osemwota, 2021).

2.2.2.1 Composição química da lentilha

Apresenta excelentes características nutricionais e por esse motivo é considerada “a carne dos consumidores de baixa renda” (Benayad & Aboussaleh, 2021; Tharanathan & Mahadevamma, 2003). A lentilha tem baixo teor de gordura, alto teor de proteína e fibras dietéticas, além de possuir carboidratos complexos e micronutrientes essenciais, como ferro, zinco e vitaminas do complexo B (Jarpa-Parra, 2018a; Joehnke et al., 2021a; Khazaei et al., 2019b).

2.2.2.2 Proteínas

Dependendo da variedade de lentilha, o teor de proteína está entre 20,6% e 31,4%, sendo as proteínas de armazenamento encontradas no cotilédone da planta, com baixa porcentagem de aminoácidos sulfurados (Alrosan et al., 2022; Jarpa-Parra, 2018b). As proteínas são classificadas de acordo com seu comportamento de solubilidade, como globulinas (70%) que são solúveis em sal, albuminas (16%) solúveis em água, prolaminas (3%) solúveis em álcool e glutelinas (11%) solúveis em ácido e base diluídas. As globulinas que apresentam a maior concentração na lentilha são tradicionalmente conhecidas como 7S (vicilina) e 11S (legumina) (J. Boye et al., 2010a; Jarpa-Parra, 2018a; Joehnke et al., 2021a). A proteína da lentilha contém todos os aminoácidos essenciais e fornece uma quantidade suficiente de alguns aminoácidos essenciais, como , lisina, treonina e fenilalanina (Hang et al, 2022). No entanto, como muitas outras sementes de leguminosas, a lentilha geralmente

carece de aminoácidos sulfurados (metionina e cisteína) e triptofano (Hang et al, 2022).

Carboidratos e Fibras Alimentares

O carboidrato é o macronutriente de maior proporção nas sementes de lentilha (70%), dentre esses carboidratos o amido representa de 35 a 53%, enquanto o restante é representado pelos mono-, di-, tri- e oligossacarídeos que podem variar de 5 a 9%, celulose e hemicelulose (10%) e lignina (2 a 3%). O amido, principal carboidrato encontrado na lentilha está como grânulos dispersos na matriz de proteína no cotilédone (Bhatty, 1988; Joshi et al., 2017a). A lentilha apresenta uma proporção considerável de amido resistente, amido esse, que não é digerido no intestino delgado e, portanto, é classificado como uma fibra alimentar para sofrer fermentação pelos microrganismos no intestino grosso, que produz ácidos graxos de cadeia curta. Independentemente dos métodos de processamento utilizados, o conteúdo de amido resistente em lentilhas processadas ainda permanece em um nível relativamente alto quando comparado a outras fontes alimentares, especialmente cereais e batata, que após processamento, apresentam conteúdo de amido resistente geralmente reduzido (Perera et al, 2010, Kaale et al, 2022).

A lentilha também apresenta excelente quantidade de fibras (11 a 31%) consideradas carboidratos prebióticos, como os oligossacarídeos fermentáveis, sendo que os principais são os galacto-oligossacarídeos (GOS), também chamados de oligossacarídeos da família da rafinose (RFO). Estes são derivados de α-galactose (1→6 ligados) da sacarose (α-glicose 1→2 ligada a ß-frutose), com rafinose (trissacarídeo), estaquiose (tetrassacarídeo) e verbascose (pentassacarídeo) sendo os representantes mais abundantes (Ispiryan et al., 2019; Joehnke et al., 2021, Dhull et al, 2022).

Assim como observado no grão-de-bico, esses carboidratos não digeríveis no intestino humano são fermentados pela microbiota colônica e são considerados precursores biossintéticos, pois produzem no processo de fermentação ácidos graxos de cadeia curta e gases. Os gases podem ser percebidos como flatulência por parte dos consumidores, como já mencionado previamente (Joehnke et al., 2021; Johnson et al., 2020).

2.2.2.5 Minerais

As lentilhas são uma fonte importante de Fe e Zn. O ferro, é utilizado no corpo na fabricação de glóbulos vermelhos. Essas células são importantes para o transporte de oxigênio dos pulmões para as células sendo usadas na geração de energia e, portanto, ajudam a prevenir a fadiga, enquanto o zinco é um elemento importante para os humanos e está amplamente implicado no metabolismo de proteínas, lipídios, ácidos nucleicos e transcrição genética. Sua função dentro do corpo humano é extensa na reprodução, reparo de feridas, função imunológica e, no nível microcelular, macrófagos, neutrófilos entre outros (Dhull et al, 2022, Benayad & Aboussaleh 2021). Além do Fe e Zn, as

lentilhas contêm outros minerais nos quais suas concentrações variam entre 6–11, 9–17, 387–490, 808–1092 μg/g, para Cu, Mn, Ca e Mg (Ramírez-Ojeda et al, 2018,).

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CHAPTER II

Processing parameters, techno-functional properties and potential food application of lentil protein concentrate as an ingredient for the *plant-based* market

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Processing parameters, techno-functional properties and potential food application of lentil protein concentrate as an ingredient for the *plant-based* market

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Abstract

Lentil (Lens culinaris) is a protein-rich legume consumed worldwide and it also has the potential to become an alternative source of protein ingredient for human nutrition. The aim of this study was to determine the best processing parameters for the whole grain protein wet extraction, as well as to analyze the techno-functional properties, and physical characteristics of the protein concentrate and its flour. It was also evaluated the application of the concentrate into a fish-like croquette. The processing route was carried out by alkaline extraction and acid precipitation of the proteins where the pH, stirring time and solute:solvent ratio were evaluated. The final dried protein concentrate presented 85% protein on dry basis and a mass yield of 14%. The results were reproducible when tested on a first scaling up test. For the techno-functional properties, solubility, water and oil retention capacities, emulsification and foaming capacities and stability, and gelling capacity were tested. As for the food application into fish-like croquettes, the lentil protein showed similar scores for sensory acceptance, flavor and texture when compared to a commercial clean-taste concentrate. The results

observed in this study were compatible to other alternative pulse-protein ingredients on the market, positioning lentil protein as a promising alternative protein source to produce ingredients for the *plant-based* market.

1. Introduction

The global demand for protein is expected to more than double by 2050, according to projections of the population increase to approximately 10 billion people in the world (Henchion et al., 2017; Joehnke et al., 2021; Lonnie & Johnstone, 2020). In recent years, many consumers have developed a mindset in their food geared towards a *plant-based* diet and alternative proteins (Aschemann-Witzel et al., 2020b). There is a noticeable increase of *plant-based* food products on supermarket shelves around the world. Alternatives to meat, dairy, poultry, beverages, and seafood products stand out. These products may be targeted to vegetarian, vegan, and mainly flexitarian consumers (Noguerol et al., 2021b; Rosenfeld et al., 2020b).

Plant-based proteins are key ingredients in the formulation of *plant-based* food products and the ingredient choice depends on the protein source, its availability as well as its performance in the product. The diversification of raw materials is essential, as it will allow meeting the world growing demand for protein ingredients, as well as directing the use of different ingredients for different products through their techno-functional and nutritional properties (Hoehnel et al., 2022).

Lentil (Lens culinaris) is a pulse of the leguminosae family and it is considered one of the oldest grain in the world remounting to 8,500 years, originating in southwest Asia (Joshi et al., 2017b; Kaale et al., 2022). In the 2021 harvest, 5,610,104 tons were produced, with Canada as the largest producer followed by India and Australia. Although these three main countries represent 70.4% of world production (FAOSTAT, 2023), lentil is a legume cultivated in more than 70 countries and globally consumed in different forms, being a great source of protein and other important nutrients in the diet (Khazaei et al., 2019, Shrestha et al., 2023a). In addition, lentil protein concentrates and isolates may offer a great potential for alternative protein ingredients to be used in the food industry.

Brazil has an incipient lentil production up to the present, as the culture of the grain consumption is not largely developed in the country. However, Brazil is considered a major grain producer and has the potential become a relevant producer of lentil and its ingredients to the food industry, thus meeting the growing global demand for alternative proteins.

Lentil seeds are rich in protein (21-31%), dietary fiber (5-20%), and essential micronutrients such as iron (7.5 mg/100 g), zinc (4.8 mg/100 g) and vitamins, mainly vitamin B9/folate (Romano et al,, 2021).

In terms of proteins, animal sources tend to afford a more complete distribution and amounts of indispensable amino acids added to a better digestibility rate when compared to plant proteins. Vegetable proteins have different protein folding added to the presence of fibers and antinutritional factors (tannins, phytates, chymotrypsin and trypsin inhibitors), what explains a poorer digestibility (Berrazaga et al., 2019, Grela et al., 2017, Henchion et al., 2017, Ismail et al., 2020).

Although legume proteins lack some sulfur amino acids (methionine and cysteine), they have good proportions of leucine/isoleucine and leucine/lysine, presenting a good nutritional quality (Jarpa -Parra, 2018, Joehnke et al., 2021, Lan et al., 2016;), especially when combined with cereal sources, as they are rich in the sulfur ones. The most abundant amino acids found in lentil grain are lysine, leucine, arginine, aspartic acid and glutamic acid (Gunes & Karaca, 2021, Kaale, et al., 2022,).

Despite all the available literature concerning studies on lentil and different processes to obtain lentil protein, there was is a lack of studies that have valuated the variable processing parameters altogether when using the classical alkaline protein extraction followed by acid precipitation. Although this is a classic method, it could be the choice for the short-term production of lentil protein in Brazil, given the country's industrial facilities, as being a large producer of soy-based ingredients.

In this sense, the aim of this study was to determine the best processing parameters for the protein extraction in a lab-scale followed by a first scaling up test, as well as to analyze the techno-functional properties and physical characteristics of lentil protein concentrate and its flour. Furthermore, an application test of the lentil protein concentrate in a fish-like croquette was also carried out, in order to evaluate the sensorial aspects of the obtained ingredient.

2. Material and methods

2.1. Material

Commercial green lentil grains (Lens culinaris) were purchased in the local market, Rio de Janeiro, Brazil. The grains were ground in an LM3100 hammer mill (Perten Instruments AB, Huddinge, Sweden) equipped with a 0.8 mm sieve to obtain the lentil flour (LF) that was stored under refrigeration at 6-8°C until use. The soybean oil used to prepare the oil-in-water emulsions was purchased from a local supermarket. Bovine serum albumin (BSA), sodium hydroxide (NaOH), hydrochloric acid (HCl), sodium dodecyl sulfate (SDS), phenol reagent from Folin and Ciocalteu, monobasic potassium phosphate and dibasic potassium phosphate were purchased from Sigma - Aldrich was purchased from the Rio de Janeiro, in Brazil. Sodium tartrate dihydrate and copper (II) sulfate pentahydrate were purchased from Merck. Tris,β-mercaptoethanol buffer and 2× Laemmli sample buffer were purchased from Bio-Rad. All chemicals were of analytical grade.

2.2. Chemical composition of the ingredients

The chemical composition of lentil flour (LF) and lentil protein concentrate (LPC) was measured according to the "Analysis of the Association of Official Analytical Chemist" (AOAC, 2010) in triplicate, including moisture, ash, protein, fat, dietary fiber. Carbohydrates were calculated by difference.

2.3. Obtaining lentil protein concentrate (LPC)

LPC was obtained through alkaline extraction followed by acid precipitation. The following variable parameters related to the alkaline extraction step of proteins were analyzed: pH, time, and solute-solvent ratio. The variables analyzed in the acid precipitation step were pH and time.

2.3.1. Alkaline extraction of proteins

The pH testing values of 8.0, 8.5, 9.0, and 9.5 were achieved by adjusting the pH with 0.1 M NaOH solution. In the first stage, the LF was mixed with water observing a 1:10 ratio (3 g/30 mL) in a 50 mL falcon tube. The pH was adjusted to each tested value, and the mixture was gently stirred (150 rpm) for 60 min using an orbital shaker (Alcacer, Paraná, Brazil). Then, the material was centrifuged at 5600*g* for 15 min (Thermo Scientific Heraeus Multifinge-R, Osterode, Germany). The supernatant was collected and the soluble protein content was determined (Bradford, 1976). The pH value that resulted in the highest soluble protein content was selected for the next step of alkaline extraction testing.

Time was the second variable to be tested and the extractions were performed by testing 10, 15, 30, 60, 90, and 120 min of stirring. It was used the same proportion of 1:10 of LF and water as mentioned above and the best extraction pH observed in the first step. Centrifugation and protein determination conditions were the same as in the previous test. The time that resulted in the highest soluble protein content was selected for the next step of alkaline extraction testing.

In a third step, the solute-solvent ratio was evaluated. For this purpose, the LF was mixed with water in different proportions: 1:6 (5 g/30 mL), 1:8 (4 g/32mL), 1:10 (3 g/30mL), 1:14 (2g/28mL), 1:16 (2 g/32mL), and 1:20 (1.5 g/30mL). The other process variables were kept fixed, using the best previously defined pH and extraction time. Centrifugation and protein determination conditions were the same as in the previous steps. The proportion of LF and water that resulted in the highest soluble protein content was selected for the extraction process.

2.3.2. Acid precipitation of proteins

Acid precipitation tests were performed in the extracts by using the conditions previously defined for the alkaline extraction. 100g of lentil flour was subjected to acid precipitation by using 0.1 M HCl. The pH values tested were 4.0, 4.5, 5.0, and 5.5. The suspensions were maintained for 30 minutes under agitation (150 rpm) using a magnetic stirrer. Then, the samples were centrifuged

(5600g, 15min) and the precipitates were dried in a forced air oven at 60ºC for 24 h. The dried LPC was then analyzed for moisture content (AOAC, 2005) and total protein by the micro Kjedahl method (AOAC, 2010). The best pH for acid precipitation was determined by comparing the LPC protein content (g/100g) and the protein recovery (%) in dry basis.

Precipitation times (10, 15, 20, and 25 min) were evaluated using the pH defined in the previous test at the same test conditions. The precipitated suspensions were subjected to centrifugation (5600g, 15 min) and the precipitates were dried following the same conditions described above. Again, the definition of the best time was determined by evaluating the protein content and the protein recovery.

2.3.3. Scaling-up the process for obtaining LPC

Based on the conditions determined on the laboratory scale (see figure 3), an experiment was carried out in a first scale-up effort (10 times, 1 kg of LF), in order to evaluate the reproducibility of the process. After obtaining the protein precipitate, a washing step was added to reduce the acidity of the product. For that, the precipitate was re-suspended in water (1:1 in weigh), stirred for 10 min and centrifuged (5600 g, 15 min).

For the scaled up processing, the LPC drying process was performed in a pilot-scale spray dryer (NIRO Atomizer, Soborg, Dinamarca) with inlet air temperature of 160 ºC, outlet air temperature of 85 ºC, air flow of 460 m/s and process flow of 10 L/h.

The total nitrogen compounds in the flour and lentil protein concentrate were determined using the Kjeldahl method (AOAC, 2010), and their protein content was calculated using a correction factor of N x 6.25. The results were expressed in g/100 g of sample. Protein recovery was determined using Equation (1).

Protein recovery (%) = (MassLPC x TPLPC / MassLF x TPLF) x 100 (1)

Where, “MassLPC” is the mass of lentil protein concentrate (g), “MassLF” is the mass of lentil flour (g), “TPLPC” is the total protein in lentil protein concentrate (%) (dry basis) and “TPLF” is the total protein in lentil flour (%) (dry basis).

The protein yield calculated to obtain the protein concentrate was determined according to Equation (2). The masses were weighed by using an analytical balance.

Protein Yield (%) = (MassLPC / MassLF) x 100 (2)

Where, “MassLPC” is the mass of lentil protein concentrate (g) and “MassLF”is the mass of lentil flour (g).

The LPC obtained in this step was analyzed for its techno-functional properties, morphology, particle size, and for food application.

2.4. Characterization of lentil protein concentrate

2.4.1. Morphology

SEM (scanning electron microscope) images were obtained using a TM-3000 benchtop SEM (Hitachi, Tokio, Japan) operated at 15 kV. Neither sputtering nor chemical fixation of the samples were performed. The dried samples were fixed on the stubs using double-sided carbon tape and the images were obtained at 1000x magnification.

2.4.2. Particle size

The particle size was determined according to the methodology cited by Gouvêa et al (2023). A (Microtrac Inc., Montgomery Ville, USA), based on blue ray laser technique was used. The analysis was carried out in duplicate and in three reading cycles, using isopropyl alcohol as dispersant fluid (refractive index 1.376). The sample was dispersed in alcohol and immediately fed into the equipment. For the particle, a refractive index of 1.50 was adopted. The particles were analyzed as the average particle size diameter based on the volume-weighted average results ($D_{(4,3)}$), the distribution with sample particle sizes that are below 10% (D_{10}), the distribution with sample particle sizes that are below 50% (D_{50}), and the distribution with sample particle sizes that are below 90% (D_{90}), as recommended by Horiba (2012).

2.4.3. Techno-functional properties

The lentil flour (LF) and the lentil protein concentrate (LPC) were the ingredients produced and they were tested for all the following properties.

2.4.3.1.**Solubility**

Protein solubility was determined based on the method reported by Silva et al., 2022. The ingredients were solubilized in water (1g/100g) and the pH was adjusted from 3 to 9 with NaOH or HCl. The solutions were kept under constant stirring on an orbital shaker for 30 minutes at room temperature (~25 °C), followed by centrifugation at 7224 × g for 15 minutes. Supernatants were collected and quantification of soluble protein was performed (BRADFORD, 1976). A control solution (1g/100g) of each ingredient was obtained by solubilizing the ingredient in 0.1 M NaOH instead of water and maintained under the same conditions as above. The protein content present in the supernatant of the control solution was considered as 100% of the soluble protein (alkaline medium) and the percentage of solubility of each sample was determined by Equation (3).

Protein solubility (%) = (SPC / CSPC) x 100 (3)

Where, “SPC” refers to sample protein concentration and “CSPC” to the control solution of each ingredient.

2.4.3.2.**Water (WHC) and oil (OHC) holding capacities**

WHC and OHC of protein samples were determined as defined by (Silva et al., 2022). Approximately 0.01 g of each protein ingredient was weighed into microtubes and 1 mL of water or oil was added, followed by vortexing (Vixar-Vortex Mixer EC) for 1 min. After homogenization, they were left to rest for 30 min at room temperature and centrifuged at 10836 × g for 20 min (Eppendorf AG 22331 Hamburg Centrifuge, series 5452 XM 344604). The supernatant was discarded and the excess water or oil on the lid and edge of the tube was removed by tapping the tubes on paper towel. The weight was recorded and the water and oil retention capacities were defined by using Equation (4).

WHC or OHC (g/g of ingredient) = $(M_2-M_1) / M_0$ (4)

Where, "M_1" is the mass of the microtube with the dry sample; "M_2" is the mass of the tube with the sample after discarding the oil or water and "M_0" is the initial mass of the sample.

2.4.3.3. **Emulsion activity (EAI) and stability (ESI)**

EAI and ESI of protein samples were determined as defined by (Silva et al., 2022). Twenty milliliters of soybean oil was added to 60 mL solution of the ingredients (0.5 g/100 g) with pH adjusted to 7 by using 0.1 M NaOH or 0.1 M HCl. The mixture was mechanically homogenized at 9500 rpm for 1 min, using an T25 basic ultra-turrax (IKA, Werke, Germany) with an S25 KV-18 probe. Fifty microliters of the emulsion was removed between the middle and bottom of the beaker and added of 5 mL of sodium dodecyl sulfate (SDS) (0.1 g/100g) at times 0 and 10 min after homogenization. Absorbances were determined at 500 nm with a spectrophotometer (Biospectro, USA) at times 0 (A0) and 10 min (A10) after emulsion formation. EAI and ESI were calculated using equations (5) and (6), respectively.

EAI $m^2/g = 2 \times 2.303 \times DF \times A0 / c \times \Theta \times 10000$ (5)

ESI (min) = $(A_0 / A_0 – A_{10}) \times 10$ (6)

Where, "DF" is the dilution factor (100), "c" is the initial concentration of the protein solution (g/mL), "θ" is the oily volume of the emulsion (0.25), A_0 and A_{10} are the absorbances of the emulsion in times 0 min and 10 min, respectively.

2.4.3.4. **Foam formation capacity (FC) and foam stability (FS)**

FC and FS of protein samples were determined as defined by (Silva et al., 2022). LPC and LF sample solutions (2.5 g/100 g) were prepared and adjusted to pH 7 by using 0.1 M NaOH or 0.1 M HCl. Fifteen milliliters were transferred to a 100 mL beaker and homogenized for 2 min using an ultrasonic turrax (Probe S 25 KV-18 G), following the rotation/time ramp of 6500 rpm/30 s, 9500 rpm/30 s and 13500 rpm/60 s. The foam formed was carefully transferred to a 50 mL graduated

cylinder by using a spatula. FC and FS were calculated according to Equations (7) and (8), respectively:

$$FC\ (\%) = [(V_1 - V_0) / V_0] \times 100 \quad (7)$$

$$FS\ (\%) = (V_2/V_1) \times 100 \quad (8)$$

Where, "V_0" is the initial volume before stirring, "V_1" is the total volume after stirring and "V_2" is the total volume after each tested time (10, 30 and 60 min).

2.4.3.5. **Least gelling concentration**

Least gelling concentration was determined based on the method reported by (Silva et al., 2022). Suspensions of the ingredients varying in concentration (2–20 g/100 g) were prepared in 5 mL of distilled water and transferred to sealed glass test tubes. The tubes were immersed in a water bath (Ultrathermostatic SL 152-Solab) at 100 °C for 1 h, followed by immediate cooling in an ice bath and kept overnight at 4 °C. The samples were then poured and divided into 3 classifications, regarding gel formation: (1) no gel formation, when the solution was liquid and flowed without resistance, (2) weak gel, when the solution flowed with some resistance, (3) gel formation, when the tube was inverted and the solution did not flow. The minimum concentration for gel formation was defined as the lowest concentration to form a firm gel in all three replicates of the same sample.

2.5. Food application

For testing the food application of lentil protein concentration (LPC), fish-like croquettes were prepared by using water (65.2%), textured soybean protein (14.2%), wheat flour (8.9%), tomato paste (3.4%), fresh cilantro (3.2%), salt (1.2%), crude palm oil (1.2%), LPC (1.0%), soy bean oil (1.0%), dehydrated onion (0.4%), dehydrated garlic (0.3%). Control samples were prepared replacing the LPC for commercial fava bean concentrate (Ingredion Vitessence clean taste, 60% protein). Ingredients were mixed and cooked until a homogeneous mass was formed, which was shaped into croquettes with individual weight of 20 g. The fish-like croquettes were packed in polyethylene plastic bags and stored frozen (-18 °C) until testing.

Sensory acceptance test (Meilgaard et al., 2006) was carried out with 101 judges, who were asked to rate their overall, flavor and texture acceptance of the croquettes using a 9-point structured hedonic scale ranging from 1 (I disliked extremely) to 9 (I liked extremely). For the tests, croquettes were prepared in an air fryer at 180ºC for 10 minutes. Samples were served on white disposable plates, coded with random three-digit numbers and presented in a monadic and balanced manner to minimize the effect of sample positioning. A glass of water was offered between samples to eliminate residual taste in the mouth. Results were submitted to analysis of variance (5% probability).

For means of ethical purposes, despite sensory analysis was not registered into an ethical committee, all the appropriate protocols for protecting the rights and privacy of all participants were

utilized during the execution of the research. No coercion to participate, full disclosure of study requirements and risks, verbal consent of participants, no release of participant data without their knowledge, ability to withdraw from the study at any time.

2.6. Statistical analysis

The analyzed parameters were submitted to ANOVA. All analyzes were performed in triplicate, unless otherwise specified, and means and standard deviations were calculated, and when F values were significant ($p < 0.05$), Tukey's test was applied at the significance level of 0.05 for comparison of mean values using STATISTICA software, version 7.0 (StatSoft Inc., Tulsa, OK, USA).

3. Results and discussions

3.1. Chemical composition of lentil flour

The proximate composition of lentil flour was as follows: moisture (9.01%), ash (2.54%), protein (23.50%), fat (1.12%), dietary fiber (12.29%) and carbohydrate (51.54%). The composition found in the used flour was as expected when comparing to other market grains and flours.

3.2. Alkaline extraction

Soluble protein values were higher from pH 8.5 to 10.0, however the tendency curve showed a peak at pH 9.0, which was therefore chosen as the pH for the extraction process (Fig. 1A), reinforcing the findings from other authors, such as in the studies carried out by Jarpa-Parra et al., (2014a) and Lee et al (2021) for red lentil.

No significant differences ($p \geq 0.05$) were observed among protein extraction times (Fig. 1B), thus 10 min should be theoretically enough to extract the proteins form the matrix. However, considering that 10 min is a very short time during an industrial process, where the amounts are much larger, 20 min were chosen for the extraction process. The extraction time of different studies from lentil (H. W. Lee et al., 2021) and other pulses Du et al. (2018) also have considered short periods of extraction, from 10 to 30 min of extraction. In the findings of Jarpa-Parra et al. (2014), time did not influence the extraction of green lentil proteins.

No differences were observed in the extract obtained with the different solid:liquid ratios tested (Fig. 1C), but the curve showed an increasing tendency at higher dilutions. For the alkaline protein extraction process, whole lentil flour was used. As a result, it is likely that the starch absorbed much of the water in the system, meaning that the 1:6 and 1:8 dilutions did not have enough free water in the extraction system. In an industrial process, this could represent a limiting step. However, as in an industrial process, the amount of water used for the extraction directly influences the amount of effluents generated, it was decided to use the 1:10 ratio, thus minimizing this effect and making the process more economical and efficient.

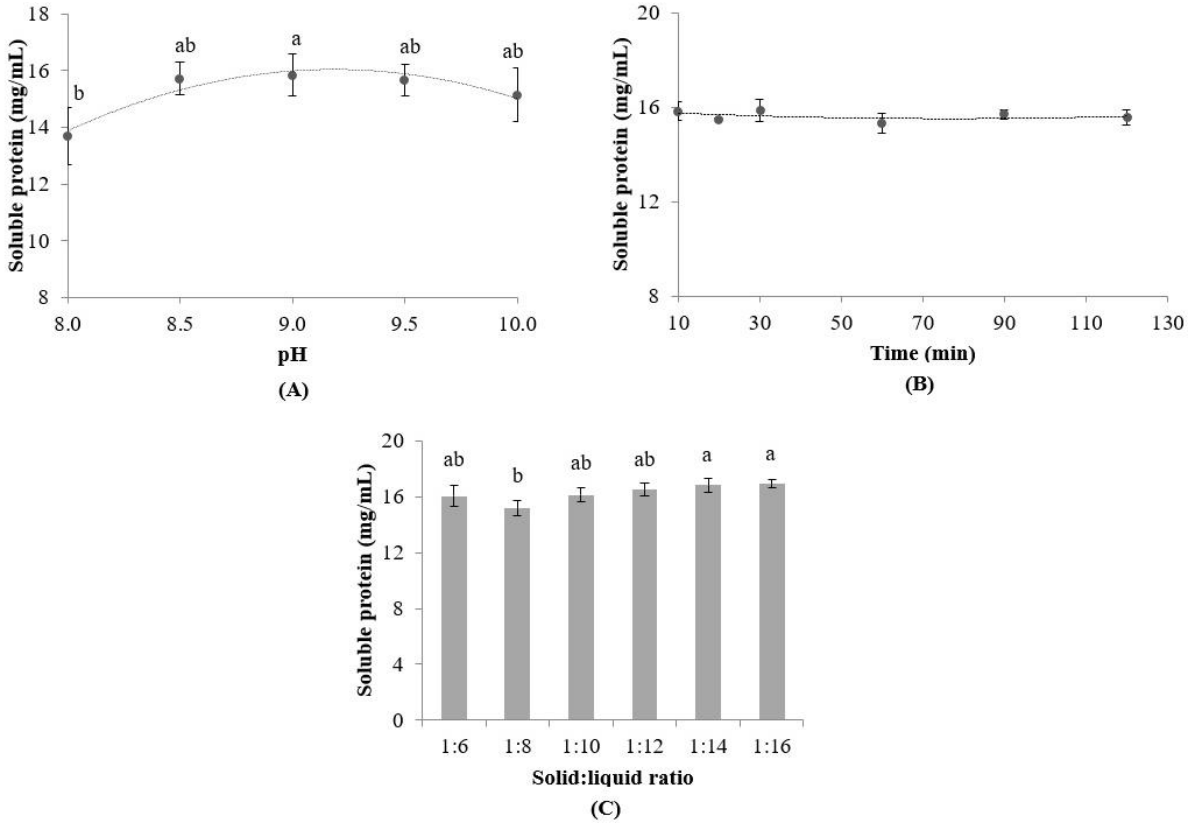

Fig. 1. Alkaline extraction parameters of lentil protein concentrate. (A) Effect of pH on lentil protein extraction; (B) Effect of time on lentil protein extraction, and (C) Effect of solute:solvent ratio on lentil protein extraction. Different letters mean significant difference between samples ($p \leq 0.05$)

3.3. Acid precipitation

No significant differences ($p \geq 0.05$) were observed in the protein content of LPC obtained by precipitation in the different tested pH values, although an increasing tendency was observed with increasing pH when considering protein quantitation data (Fig. 2A). However, analyzing the protein recovery from the process, a different pattern was observed, with the highest value at pH 5.0 and with no differences in the pH range from 4.0 to 5.0. A slight decrease was seen at pH 5.5, showing less precipitation of the protein, which emphasizes the remoteness from the isoelectric point of the protein. Taking into consideration both curves of figure 2A, the pH of 5.0 was chosen as an optimal parameter.

There was also no significant difference between the protein contents in the concentrates obtained at different agitation times during precipitation ($p \leq 0.05$) (Fig. 2B). Thus, the shortest process time (10 min) can be used in this step.

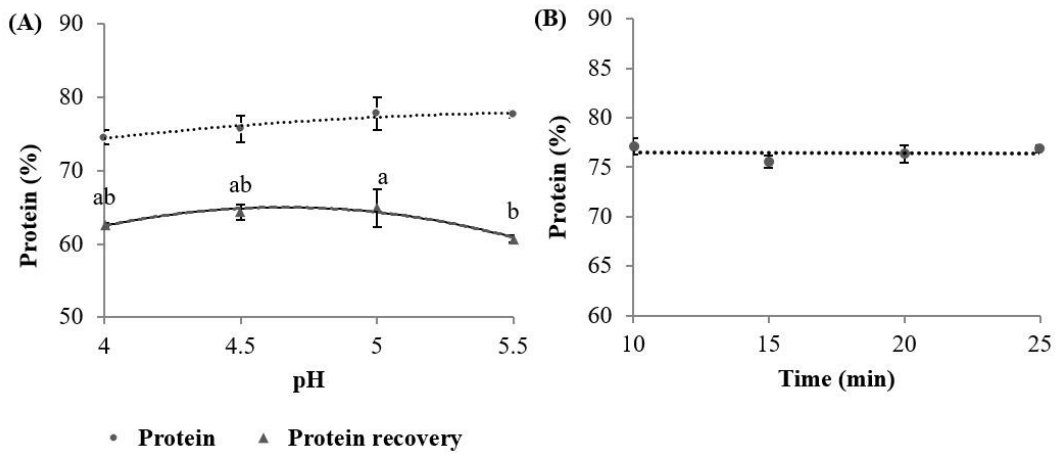

Fig. 2. Acid precipitation parameters for obtaining lentil protein concentrate. (A) Effect of pH on lentil protein precipitation and on protein recovery and (B) Effect of time on lentil protein precipitation. Different letters mean significant difference between samples ($p \leq 0.05$). Protein precipitation refers to total protein mass on the precipitated sample and protein recovery refers to the mass of protein obtained on the concentrate taking into consideration the protein mass from the flour.

3.4. Scaling up process

The LPC obtaining process (Fig. 3) showed a pH of 6.50 after washing, a mass yield of 13.92% and the final product presented a protein content of 84.96% (N x 6.25), on a dry basis, which corresponds to 80.56% on a wet basis with a moisture content of 5.18%. Considering an initial protein content of 23.50% in the LF, the process increased the protein content in 3.4 times. This result is quite high in comparison to other few lentil commercial protein concentrates, that ranges from 50% to 60% in protein. In fact, with the exception of soy proteins, the other pulse proteins that are in the market are massively obtained from air classification, which has economic and environmental advantages, but the low protein yields and purity are still drawbacks from the method and additional "wet methods" are required to enhance the protein content and purity (Boukid et al., 2021).

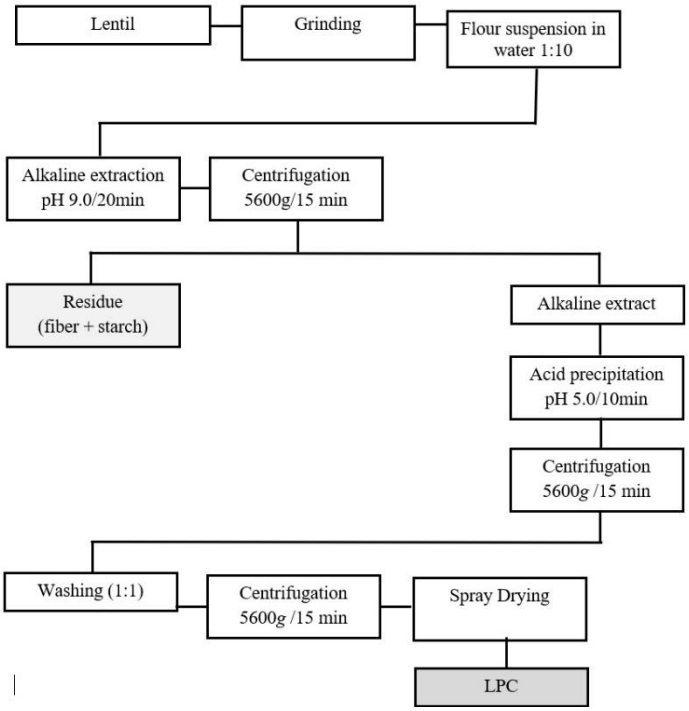

Fig. 3. Flow chart for obtaining lentil protein concentrate (LPC).

3.5. Morphology

Figure 4 shows the micrograph of lentil flour (LF) and its protein concentrate (LPC). In the flour (Fig. 4A), starch granules are observed in ovoid and spherical shape (arrow), and with approximate sizes of 20 to 40 μm (length) and 15 to 25 μm (width). The particles adhered to the granules are the protein bodies (PB) or fragments of the protein cell matrix disrupted during milling. It is also possible to observe components of the cellular matrix around or adhered to the protein bodies and the granule. These finding are typical of pulse flours, as also reported by Ahmed et al. (2016), Li et al. (2019), Sivakumar et al. (2023) and Zhang et al. (2019).

As expected, LPC showed basically protein bodies (~85% protein) with a folded or wrinkled surface with varying sizes up to 20 μm and small impurities that are probably starch or cell wall fragments. Protein wrinkling occurs as a result of spray drying the sample due to the low moisture diffusion capacity of aqueous biopolymer solutions, such as proteins. In the spray drying process there is a high concentration gradient between the droplet/particle and the drying medium. During the moisture evaporation flow, the drying droplet always tries to take the shortest path for water vapor diffusion, which results in a folded or wrinkled surface (Brishti et al., 2020a; Joshi et al., 2011; Rezvankhah et al., 2021). The same was also observed by Gouvêa et al. (2023), Choe et al. (2022) and Vogelsang-O'Dwyer et al. (2020), when analyzing different pulses ingredients.

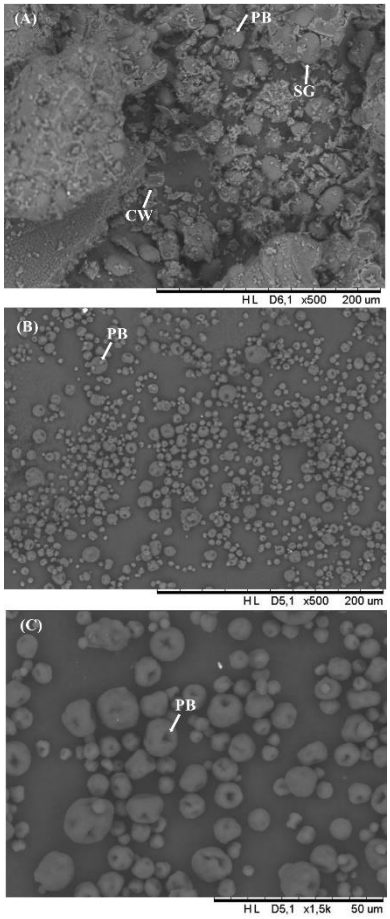

Fig 4. SEM micrographs of the ingredients. (A) lentil flour, (B) lentil protein concentrate, (C) enlarged micrograph of the protein concentrate. SG- Starch granule, PB- Protein bodies; CW- Cell wall fragments.

3.6. Particle size

The particle size is an important parameter that influences some properties of the ingredients, such as the absorption capacity and solubility in water, the stability of emulsion and foaming, among others, affecting the quality of the final product. The values of the mean diameter of the samples [D(4,3)], and their percentiles (D10, D50 and D90) are listed in Table 1.

The LPC showed a particle size of about 8 times smaller than the flour (11.03 μm and 88.30 μm, respectively), with 90% of the particles being smaller than 18.72 μm, which is probably due to the process of obtaining LPC. The LF was obtained directly by grinding the grain, which confers a larger size, while the LPC was extracted in alkaline medium followed by acid precipitation and spray dried, generating smaller and more homogeneous particles (Brishti et al., 2020a; Gouvêa et al., 2023).

In addition, the particle size of microcapsules obtained by spray drying is influenced by the atomizer nozzle, liquid delivery rate, atomization conditions, air pressure and total solids content (McNamee et al., 1998). Shen et al. (2021) reported that drying techniques influenced the particle size in obtaining quinoa protein isolate. Those authors observed percentiles (D50) for spray-dried of 10.43 μm, which were smaller than freeze-dried powders (44.24 μm) and vacuum dried (38.25 μm). Edwards et al (2020), reported a mean particle size diameter for lentil flour (D50) of 25.6 μm, smaller than observed in our studies, which can be related to the technique for obtaining the flour (treatment, type of grinding, sieving). While Bourré et al. (2019) compared the influence of the sieve diameter (0.50, 0.79 and 1.00 mm) in the particle size of red lentil flour and found for D50 83.70, 242.60, and 302.40 μm respectively.

The particle size of lentil flour is larger than that of the concentrate due to its composition as it presents higher levels of cellular fragments (fibers) that are difficult to break down during the grinding process and also due to its higher carbohydrate content, as the starch granules are relatively larger than the protein bodies. As a higher purity of the protein concentrate is achieved, smaller particle sizes will be found. Drying processes will also have a major influence on particle size, and as the concentrate was dried by microencapsulation (spray-drying), it is expected to observe smaller particles (Gouvêa, et al. 2023).

Table 1. Particle size of lentil flour (LF) and lentil protein concentrate (LPC).

Properties	Samples	
	LF	LPC
D(4,3) (μm)	88.30±1.32a	11.03±0.62b
D10 (μm)	8.75±0.46a	2.03±0.02b
D50 (μm)	34.92±1.33a	9.74±0.06b
D90 (μm)	235.90±10.98a	18.72±0.72b

Different letters in the same line mean significant difference between samples (Tukey test, $p \leq 0.05$).

3.7. Solubility

In order to provide useful information for the effective use of lentil flour and protein concentrate in food applications, the solubility of the samples were studied at pH ranging from 3 to 9 (Fig. 5). LF and LPC showed the lowest solubility between pH 4-5, known to be the isoelectric point of pulse proteins, in which protein-protein interactions disfavor solubility when compared to the other pH levels studied (H. W. Lee et al., 2021). At pH 3, LF and LPC showed a solubility of 41.75% and 45.17%, respectively, while in the alkaline pH range, LF was more soluble at pH 9 (92.21%) while LPC showed greater solubility in pH 8 (93.22%).

Other researchers such as Boye, Aksay, et al. (2010), Jarpa-Parra et al. (2014a), Ladjal Ettoumi & Chibane, (2015) reported that the solubility of LF and LPC was lower at pH 4-5 and higher at pH between 1-4 and 8-9, as expected from a pulse protein behavior in a v-type curve of solubility. Aryee et al. (2017) reported that for raw lentil flour and lentil protein isolate obtained by wet processing, low solubility between pH 4-6 and high solubility at pH 9 (92%) was observed. For the flour, there was no difference with our findings, however LPC showed a slightly difference at pH 8. Joshi et al. (2011) reported a solubility of 81% for lentil protein isolate obtained by spray-drying. These differences may be associated with the conditions for obtaining the proteins, varieties, genotype, location and cultivation climate.

Considering the solubility at low pH values, LPC would be a candidate for use in acidic beverages, such as fruit juices and smoothies, for example.

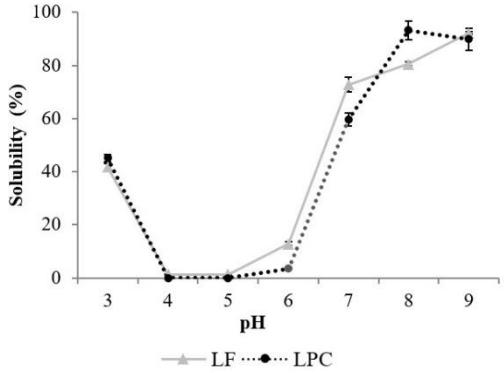

Fig. 5. Solubility curve of lentil flour (LF) and lentil protein concentrate (LPC) at different pH values.

3.8. Water holding capacity (WHC) and oil holding capacity (OHC)

Water and oil holding capacities define the amount of water or oil absorbed per gram of protein, respectively (Shrestha et al., 2023). WHC influences juiciness, which is as desirable as fibrous attributes in meat analogues (Cornet et al., 2021; Shrestha et al., 2023), while OHC determines the protein's ability to interact with the oil phase in emulsions and other foods (Shrestha et al., 2023).

The WHC of LF (2.39 g/g) was significantly higher ($p<0.05$) than that of LPC (1.58 g/g), while the OHC of LF (1.90 g/g) showed no difference ($p<0.05$) from the LPC of 1.54 g/g (Fig. 6). Several studies to obtain lentil protein concentrates or isolates from isoelectric precipitation reported different WHC than our findings. Joshi et al. (2011), reported WHC of 0.43 g/g for green lentil concentrates. Lee et al. (2021) studied protein concentrate from three varieties of red lentil and the WHC ranged from 3.1 to 3.5 g/g, while Aydemir and Yemenicioglu (2013) carried out studies on several varieties of lentils and found a WHC ranging from 1.08 to 1.47 g/g, which are values similar to our findings. Different data found by several authors indicate that the varieties, type of process for obtaining isolates or concentrates directly influence WHC, added to a lack of consensus on the way

of measuring the responses (Kiosseoglou et al., 2021). These results can be correlated to the composition of the samples and the presence of carbohydrates in LF what may enhance WHC, as observed in other commercial ingredients (Gouvêa et al., 2023).

The WHC and OHC of lentil flours and concentrates greatly vary according to the origin of the grain, considering varieties, harvest time, cultural practices, climate and production process (Adebiyi & Aluko, 2011; J. Boye et al., 2010b; H. W. Lee et al., 2021; Shrestha et al., 2023b). The results of this study showed that both the flour and the concentrate have good water and oil holding capacities when comparing the results from other pulses sources, and therefore could be potential ingredients for food applications that require water and fat absorption, such as in breads, cake doughs, pastes, meat product preparations, as well as being an alternative in textured food products and also as a meat substitute or extender to increase flavor retention and improve mouthfeel in meat analogues.

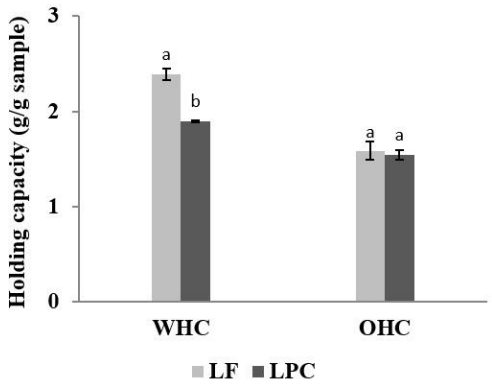

Fig. 6. Water holding capacity (WHC) and oil holding capacity (OHC) of lentil flour (LF) and lentil protein concentrate (LPC). Different letters mean significant difference between samples ($p \leq 0.05$).

3.9. Emulsifying capacity (EAI), emulsion stability (ESI), foaming capacity and foam stability.

High EAI indicates great interfacial area of the formed emulsion and, therefore, great emulsification capacity, while high ESI indicates good ability to maintain the emulsion over time (Hall & Moraru, 2021; Pearce & Kinsella, 1978). EAI for LF was of 19.9 m^2/g and 21.3 m^2/g for LPC (Fig. 7A). The values found for both LF and LPC are close to the findings of other studies who reported values for EAI of commercial pulses protein ingredients (14.03 m^2 /g to 19.39 m^2 /g) (Gouvêa et al., 2023; H. W. Lee et al., 2021; Shevkani et al., 2015). Therefore, considering the EAI values observed, it is possible to apply LF and LPC in the production of solid and semi-solid foods that require emulsification, such as hamburgers, sausages and other meat analogues.

The ESI value of LPC (53.9 min) was more than twice the value for LF (24.1 min), indicating that LPC has more ability to maintain the emulsion over the studied time (0-60min) when compared

to LF (Fig. 7B). The higher emulsion stability rate obtained by LPC, which is spray dried, can be attributed to protein denaturation, because the three-dimensional structure of protein molecules is disrupted after the release of hydrophobic moieties. Denaturation increases emulsification capacity by increasing the rate of protein binding to the interface (Karaca et al., 2011a). Both LF and LPC showed higher ESI values than the findings by (Gouvêa et al., 2023) for pulses protein commercial ingredients.

The foaming capacity of LPC (84.4%) was significantly higher than that of LF (80.0%) (Fig. 7C). LF foam was stable at 96.2% while LPC maintained 97.6% after 60 min. Toews & Wang (2013) reported LPC foam stability from 79 to 81% after 120 min. Kaur & Sandhu (2010), reported for green lentil flour foaming capacity from 33.9 to 47.3%. If compared to our study, the difference is probably related to the type of process for obtaining LPC and variety of lentils. Both LF and LPC were able to sustain the foam structure for more than 60 min probably due to good protein flexibility and electrostatic repulsions.

Those results indicate that both LF and LPC might be applicable in aerated foods (mousses, toppings and confectionery) that need good foam formation and stability.

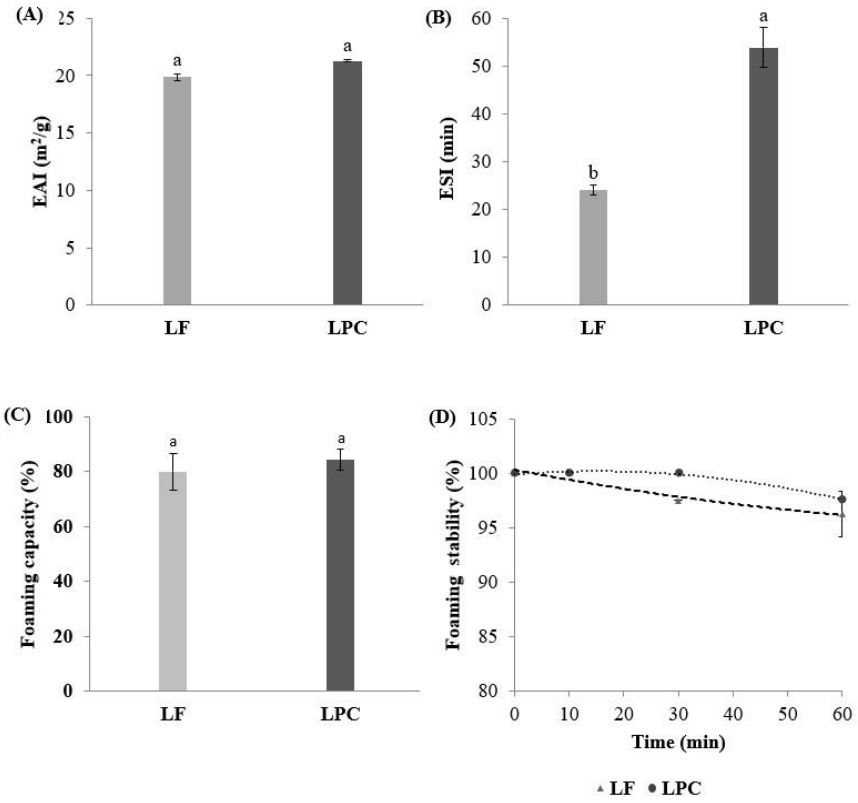

Fig. 7. (A) Emulsifying capacity index– EAI, (B) Emulsion stability – ESI, (C) Foaming capacity and (D) Foam stability for lentil flour (LF) and lentil protein concentrate (LPC). Different letters mean significant difference between samples ($p \leq 0.05$).

3.10. Least gelling concentration

Both LF and LPC showed a least gelling concentration at 0.18 g/mL (Table 2). To form a gel, the protein must be denatured in whole or in part and reorganized to form a three-dimensional network (Gouvêa et al., 2023; Papalamprou et al., 2009a). Testing parameters such as temperature, pH, heating time and cooling, together with lentil variety, climate, time of harvest and processing interferes on the gelling response from the grain and from that, a diversity of results have already been mentioned on the literature, showing better and worse gelling properties from lentil and lentil proteins, going from 0.08 to 0.18 g/mL (Boye, Aksay, et al., 2010; Aydemir & Yemenicioğlu, 2013; Kaur & Sandhu, 2010; Joshi et al., 2011; Jarpa-Parra et al., 2014)

Therefore, results obtained in our study for least gelling concentration are in accordance with studies carried out in legumes in general, although a method standardization would benefit comparisons.

Table 2. Least gelling concentration of lentil flour (LF) and lentil protein concentrate (LPC).

Sample	Replicates	Concentration (g/mL)									
		0.02	0.04	0.06	0.08	0.10	0.12	0.14	0.16	0.18	0.20
LF	1	-	-	-	-	-	-	±	±	+	+
	2	-	-	-	-	-	-	±	±	+	+
	3	-	-	-	-	-	-	±	±	+	+
LPC	1	-	-	-	-	±	±	±	±	+	+
	2	-	-	-	-	±	±	±	±	+	+
	3	-	-	-	-	±	±	±	±	+	+

"-" Did not form gel; "±" Weak gel; "+" Firm gel.

3.11. Food Application

Legume-based protein concentrates and isolates are used to formulate various food products, totally or partially replacing proteins of animal origin. Among meat analogue products, those of "fish type" are the least studied and, in general, they require application tests with very specific ingredients in order to simulate a similar sensory experience to the animal reference product. In this sense, it was formulated fish-type croquettes in order to evaluate the sensorial behavior of LPC in a product that undergoes a thermal processing in its manufacture and compared it to a similar product, containing a clean-taste protein concentrate from fava bean as a pulse ingredient.

There was no significant difference ($p<0.05$) in the sensory acceptance tests analyzed between fish croquettes made with the in-house lentil protein concentrate (LPC) and commercial fava bean concentrate (FBC). The attributes evaluated were general acceptance (LPC 6.5 and FBC 6.6), flavor

(LPC 6.6 and FBC 6.6) and texture (LPC 5.9 and FBC 6.0). Both products showed a good general acceptance by the consumers, and the presence of off-flavors where not detected.

4. Conclusions

Despite all the literature information about obtaining lentil protein of different varieties for different purposes, there was a lack of information on evaluating the variable parameters of wet processing of lentil grains all together. The results from this study showed the best conditions for the alkaline extraction of lentil protein to be at pH 9.0, solute:solvent ratio of 1:10 and stirring time of 10 minutes, followed by acid precipitation at pH 5.0 and stirring time of 10 minutes. By processing the grain flour under these conditions, it was possible to obtain a very high protein concentrate with 85% protein with a mass yield of 14%. The lentil concentrate was evaluated for its techno-functional properties and it presented a similar behavior to other pulses proteins, with a high emulsifying capacity and foam formation and stability.

In terms of potential applications of the obtained ingredients, the flour may be indicated to compose solid food products, especially the ones from to the meat-like or bakery categories to enhance the products bulk, as the flour is richer in carbohydrates (starch and fiber). On the other side, the protein concentrate can be indicated to be used in a larger variety of products. In the solid ones (meat-like, bakery or pastas) it may enhance the protein content and the favors the interfacial characteristics of foods. For products that need emulsification and or foaming, such as mayonnaise and creams it may also be applicable.

Finally, with the increased demand for alternative sources of protein, lentil can be used as a rich source, with its concentrate being suitable for the food market, including the plant based one.

Author statement

The authors declare that the submitted manuscript has not been published elsewhere and it is not under consideration for publication elsewhere.

Ethical Statement

Participants in sensory tests gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this survey" where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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CHAPTER III

Proteins from chickpea: obtaining process, techno-functional properties and potential application

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Proteins from chickpea: obtaining process, techno-functional properties and potential application

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Abstract

Chickpea (Cicer arietinum L.) is the third most cultivated and consumed legumes (Pulse) worldwide, after bean and pea, and is commonly commercialized in the form of seeds, flour or canned food. Grains are rich in proteins with the potential to be an alternative source of protein for human nutrition. The use of legume proteins is highly dependent on composition, functional and structural properties. The objective of this study was to determine the best processing parameters for the wet extraction of protein from grains chickpea, as well as to analyze the technical-functional properties and physical characteristics of the protein concentrate. The application of the concentrate in fish-like croquette was also evaluated. The processing route was carried out by alkaline extraction and acid precipitation of proteins where pH, stirring time, and solute:solvent ratio were evaluated. The best results for alkaline extraction were at pH 8.5, solute:solvent ratio of 1:12, and stirring time of 20 minutes. For acid precipitation, the best results were at pH 4.5 and stirring time of 10 minutes. The final dry protein concentrate presented ~78% protein (dry basis) and a mass yield of ~12%. Regarding techno-functional properties, the results for flour (FC) and protein concentrate (CPC) were as follows: solubility (FC pH 3 = 7.60% and pH 9 = 83.82%; CPC pH 3 = 51.45% and pH 9 = 90.72 %), water holding capacity (CF = 2.18 g/g and CPC = 1.23 g/g), oil holding capacity (CF = 1.69 g/g and CPC = 1.59 g/g), foaming capacity (CF = 82.22% and CPC = 77.78%), foam stability up to 60 min (CF= 92.67% and CPC= 93.82%), emulsifying capacity (CF = 18.79 m^2/g and CPC = 16.49 m2/g),

emulsifying stability (CF = 28.69 min and CPC = 36.64 min) and the lowest degree of gelation for CF (0.10 g/mL) and CPC (0.18 g/mL). As demonstrated in the sensory test, the global acceptance, flavor, and texture of croquettes added CPC showed no differences when compared to croquettes added with commercial fava bean concentrate. CPC proved to be a promising protein alternative for the *plant-based* market.

keywords: Pulse protein; Plant protein; Protein extraction; Protein characterization; Food ingredients Sensory analysis.

1. Introduction

The forecast is that global demand for proteins will increase by 943.5 million metric tons by 2054, which means it is necessary to introduce protein alternatives into the market, including those of plant origin (Hewage et al., 2022). This increase in protein is expected to more than double by 2050, in line with projections of population growth to approximately 10 billion people worldwide (Boukid, 2021; Henchion et al., 2017b; Joehnke et al., 2021c; Lonnie & Johnstone, 2020b).

Several *plant-based* food products are considered the main innovations in the sector, possible causes would be the demand from consumers for restrictions on products of animal origin, lifestyle, the popularization of veganism, vegetarianism and also the increase in the number of flexitarians (Batista et al., 2023). Chickpeas (Cicer arietinum L.), a member of the Fabaceae family, contain 18% to 29% protein, 4% to 7% lipids, and 50% to 60% starch, (Boukid, 2021; Espinosa-Ramírez & Serna-Saldívar, 2019; Gupta et al., 2021). In 2021, approximately 15.9 million tons of dry grains were produced, with the Asian continent responsible for 84.4% of world production, followed by Oceania 5.5%, Africa 5.1%, the Americas 2.9%, and Europe 2.1%. India is the largest producer of chickpeas in the world, with 11.9 tons in 2021, which represents 74.84% of world production, followed by Australia and Turkey (FAOSTAT 2022).

Two varieties are the most explored worldwide, Desi, which is characterized by relatively small angular seeds, with varied and sometimes spotted colors, and Kabuli, which is characterized by larger, smoother, and generally light-colored seeds (Merga & Haji, 2019b). Kabuli is the most used in Brazil, while Desi has a greater demand in Asian countries (Nascimento, W. M. 2016). Brazil imports chickpeas from Argentina and Mexico to meet its domestic demand, as its annual production is almost non-existent (Queiroga; G. A., et al., 2021). Cultivars adapted to the Brazilian climate have recently been launched, such as IAC Morocco, BRS Aleppo, BRS Cristalino, BRS Toro, BRS Cícero, and BRS Kalifa, which will allow the country to be an important producing country in the future.

The biggest challenge for producing vegetable proteins and their derived ingredients is ensuring that they can provide structural qualities, texture to foods and have a diversity of ingredients

to serve the market. Their main functions are in the stabilization of emulsions and foams, production of gels, protein drinks, and meat analogues (Aschemann-Witzel et al., 2020c; Jimenez-Munoz-2021). Therefore, the objective of this study was to determine the best processing parameters for extracting protein from whole chickpea grains using the classic wet method, as well to analyze the technical-functional properties and physical characteristics of chickpea protein concentrate. The application of the concentrate in fish-like croquettes was tested to verify its suitability as a replacement for animal protein.

2. Material and methods

2.14.Materials

Commercial chickpea grains (*Cicer arietinum L.*) were purchased in the local market, Rio de Janeiro, Brazil. The grains were ground in an LM3100 hammer mill (Perten Instruments AB, Huddinge, Sweden) equipped with a 0.8 mm sieve to obtain chickpea flour (CF). The flour was defatted in a soxhlet extractor for 48 h using petroleum ether, dried in an oven at 40 °C, and stored under refrigeration at 6-8 °C until use.

2.15.Obtaining chickpea protein concentrate (CPC)

CPC was obtained through alkaline extraction followed by acid precipitation. The following parameters related to the alkaline extraction step of proteins were analyzed: pH, time, and solute-solvent ratio. Stirring time and pH were the variables analyzed in the acid precipitation step. All experiments were performed in triplicate and submitted to analysis of variance and comparison of means by Tukey's test (5% probability) to define the best response parameters.

2.2.1. Alkaline extraction of proteins

The pH testing values of 8.0, 8.5, 9.0, and 9.5 were achieved by adjusting the pH with 0.1 M NaOH. In the first step, the defatted CF was mixed with water observing a 1:10 ratio (3 g/30 mL) in a 50 mL falcon tube. The pH was adjusted and the mixture was gently stirred (150 rpm) for 60 min using an orbital shaker (Alcacer, Paraná, Brazil). The material was centrifuged at 5600 x *g* for 15 min Thermo Scientific Heraeus (Multifinge-R, Osterode, Germany). The supernatant was collected and the soluble protein content was determined (Bradford, 1976). The pH value that resulted in the highest soluble protein content was selected for the next step of alkaline extraction testing.

Stirring time (10, 15, 30, 60, 90, and 120 min) was the second variable studied in protein solubilization (second step). The same proportion of defatted CF and water as mentioned above (1:10) and the best extraction pH in the first step were used. The time that resulted in the highest soluble protein content was selected for the next step of alkaline extraction testing.

In the third step, the solute-solvent ratio was evaluated. For this purpose, the defatted CF was mixed with water in different proportions: 1:6 (5 g/30 mL), 1:8 (4 g/32 mL), 1:10 (3 g/30 mL), 1:14 (2 g/28 mL), 1:16 (2 g/32 mL), and 1:20 (1.5 g/ 30 mL). The other process variables were kept fixed, using the best previously defined pH and extraction time. The proportion of deffated CF and water that resulted in the highest soluble protein content was selected for the extraction process.

2.2.2. Acid precipitation of proteins

Acid precipitation tests were performed in the extracts by using the conditions previously defined for the alkaline extraction. Deffated CF (100 g) was subjected to acid precipitation by using 0.1 M HCl. The pH values tested were 4.0, 4.5, 5.0, and 5.5. The suspensions were maintained for 30 minutes under agitation (150 rpm) using a magnetic stirrer. Then, the samples were centrifuged (5600 x g, 15 min) and the precipitates were dried in a forced air oven at 60 ºC for 24 h. The dried CPC was then analyzed for moisture content (AOAC, 2005) and total protein by the micro Kjeldahl method (AOAC, 2010). The best pH for acid precipitation was determined by comparing the CPC protein content (%) and the protein recovery (%) in dry basis.

Precipitation times (10, 15, 20, and 25 min) were evaluated using the pH defined in the previous test at the same test conditions. The precipitated suspensions were subjected to centrifugation (5600 x *g*, 15 min) and the precipitates were dried following the same conditions described above. Again, the definition of the best time was determined by evaluating the protein content and the protein recovery.

2.2.3. Scaling-up the process for obtaining CPC

Based on the conditions determined on the laboratory scale, an experiment was carried out to increase the production scale (by 10 times, 1 kg of CF), to evaluate the reproducibility of the process. After obtaining the protein precipitate, a washing step was added to reduce the acidity of the product. For that, the precipitate was re-suspended in water (1:1 in weigh), stirred for 10 min, and centrifuged (5600 x *g*, 15 min).

For the scaled-up processing, the CPC drying process was performed in a pilot-scale spray dryer (NIRO Atomizer, Soborg, Dinamarca) with inlet air temperature of 160 ºC, outlet air temperature of 85 ºC, air flow of 460 m/s, and process flow of 10 L/h.

The CPC obtained in this step was analyzed for its techno-functional properties, morphology, particle size, and for food application.

2.3.Characterization of chickpea protein concentrate

2.3.1. Morphology

SEM (scanning electron microscope) images were obtained using a scanning electron microscope TM-3000 (Hitachi High-Tech, Tokyo, Japan) operated at 15 kV. The dried samples were directly placed on aluminum stubs using sticky double-sided conductive carbon tape and the images were obtained at 1000x magnification.

2.3.2. Particle size

The particle size was determined by light scattering according to the methodology cited by Gouvêa et al. (2023) using a S3500 laser diffraction equipment (Microtrac Inc., Montgomery Ville, USA). The analysis was carried out in duplicate and three reading cycles, using isopropyl alcohol as dispersant fluid (refractive index 1.376). The sample was previously dispersed in alcohol and immediately fed into the tank filled with isopropyl alcohol then pumped to the equipment. For the particle, a refractive index of 1.50 was adopted.

2.3.3. Techno-functional properties

The techno-functional characteristics of solubility, water and oil holding capacities, emulsion activity and stability, foam formation capacity and foam stability, and least gelling concentration were determined as described by Silva et al. (2022).

2.3.4. Statistical analysis

The analyzed parameters were submitted to ANOVA. All analyzes were performed in triplicate, unless otherwise specified, and means and standard deviations were calculated, and when F values were significant ($p < 0.05$), Tukey's test was applied at the significance level of 0.05 for comparison of mean values using STATISTICA software, version 7.0 (StatSoft Inc., Tulsa, OK, USA).

2.4. Food application

For testing the food application as ingredient, fish-like croquettes were prepared by using water (65.2%), textured soybean protein (14.2%), wheat flour (8.9%), tomato paste (3.4%), fresh cilantro (3.2%), salt (1.2%), crude palm oil (1.2%), CPC (1.0%), soybean oil (1.0%), dehydrated onion (0.4%), dehydrated garlic (0.3%). Control samples were prepared replacing the CPC by commercial faba bean concentrate (Ingredion Vitessence clean taste, 60% protein). Ingredients were mixed and cooked until a homogeneous mass was formed, which was shaped into croquettes with individual weight of 20 g. The fish-like croquettes were packed in polyethylene plastic bags and stored frozen (-18 °C) until testing.

Sensory acceptance test (Meilgaard et al., 2006) was carried out with 101 judges, who were asked to rate their overall, flavor and texture acceptance of the croquettes using a 9-point structured hedonic scale ranging from 1 (I disliked extremely) to 9 (I liked extremely). The croquettes were prepared in an air fryer at 180 ºC for 10 minutes. Samples were served on white disposable plates, coded with random three-digit numbers and presented in a monadic and balanced manner to minimize the effect of sample positioning. A disposable cup of water was offered between samples to eliminate residual taste in the mouth. Results were submitted to analysis of variance (5% probability).

Participants in sensory tests gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this survey" where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption.

3. Results and discussions

3.1. Alkaline extraction

As reported by some authors, lipids can interfere as a barrier to solvent penetration during protein extraction(Saâ Nchez-Vioque et al., n.d.; Soto-Madrid et al., 2023; Toews & Wang, 2013). Therefore, a deffating procedure was previously applied to the flour. Whole chickpea flour had lipid content of 6.08 g/100 g and the deffated flour 2.37 g/100 g on dry basis.

Regarding the effect of pH on the extracted protein content, no significant differences were observed ($p \leq 0.05$) when pH ranged from 8 to 10 (Fig. 1A). According to Gao et al., (2020), extreme alkaline and acidic pH during extraction and precipitation, together with high temperatures during spray drying, can denature yellow pea proteins. Likewise, Lee et al., (2007) reported that alkaline extraction at pH 9.5 for lentil protein isolate resulted in greater denaturation compared to extraction at pH 8. Furthermore, based on the literature where many studies adopted pH 9 to perform alkaline extraction (Boye et al., 2010c; Kaur & Singh, 2007; Papalamprou et al., 2010) and the present work did not detect a significant difference between the pH values studied, pH 8.5 was chosen for alkaline extraction in order to preserve the quality of the protein as much as possible and reduce the amount of alkali in the process.

No significant differences ($p \leq 0.05$) were observed among protein extraction times (Fig. 1B). However, as 10 min is considered a very strict time for process control, the stirring time of 20 min was defined as the ideal time to perform protein extraction. Jarpa-Parra et al (2014) also found that time did not influence the extraction of proteins from green lentils.

No differences were observed ($p \leq 0.05$) in the protein content of extracts obtained with the solid:liquid proportions of 1:12, 1:14, and 1:16, and the results were higher than the values obtained at lower dilution level (1:6, 1:8 and 1:10 ratios). In the literature, several studies used the 1:10 ratio

in the chickpea protein extraction by isoelectric precipitation, however, none of them evaluated the ideal solid-liquid ratio for extraction (Ghribi et al., 2015; Karaca et al., 2011; Papalamprou et al., 2010; Perović et al., 2022; Sánchez-Vioque et al., 1999).

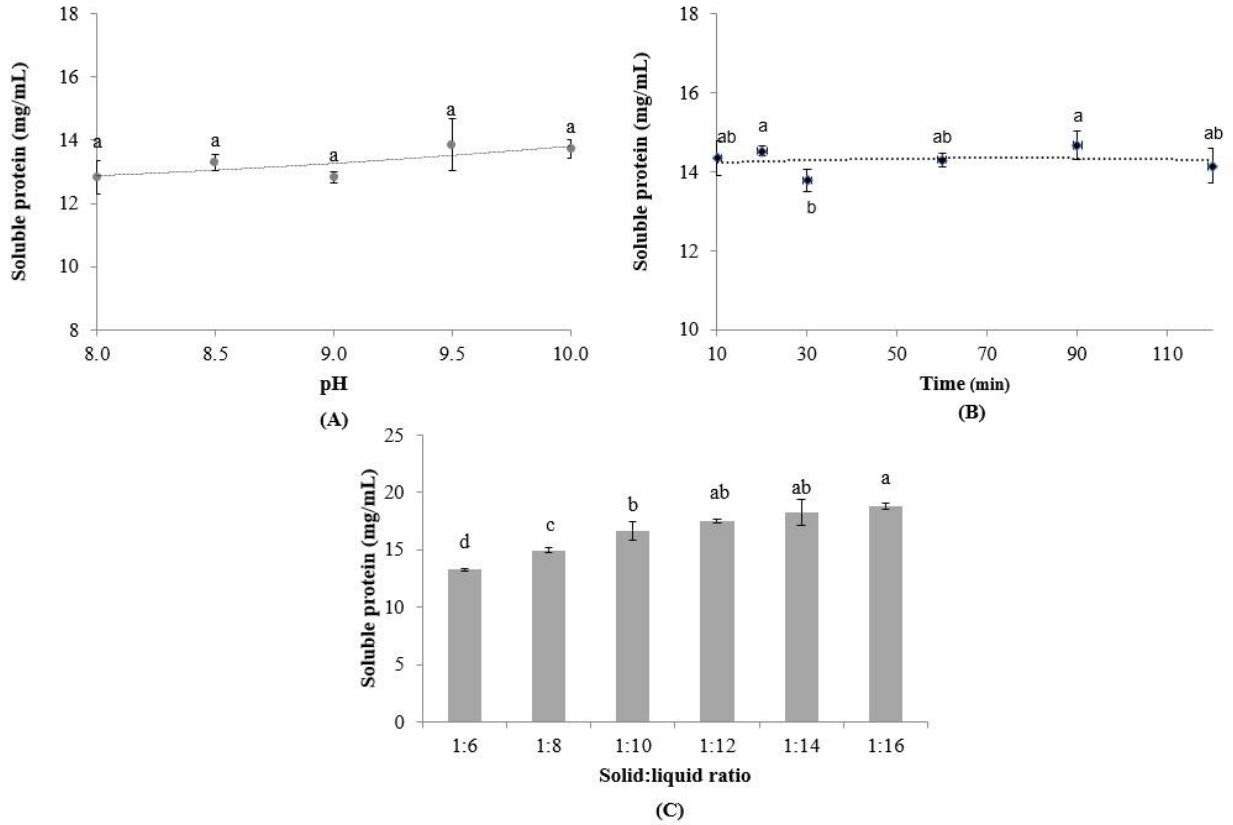

Fig. 1. (A) Effect of pH on chickpea protein extraction; (B) Effect of stirring time on chickpea protein extraction, and (C) Effect of solute:solvent ratio on chickpea protein extraction. Different letters mean significant difference between samples ($p \leq 0.05$).

3.2.Acid precipitation

There were no significant differences ($p \leq 0.05$) in the protein content of the CPC obtained in pH 4.5, 5.0 and 5.5, while protein recovery was greater at pH 4.0 and 4.5 (73.62% and 74.97%, respectively). Thus, pH 4.5 was chosen for the precipitation process due to the higher protein content in the CPC compared to pH 4.0. Other studies have also used pH 4.5 for producing chickpea protein concentrates and isolates (L. Chang et al., 2022; M. Kaur & Singh, 2007; Peyrano et al., 2016; Tontul et al., 2018).

The stirring time during precipitation practically did not affect the protein content of the concentrates (Fig. 2B), so the shortest process time (10 min) was selected for the process.

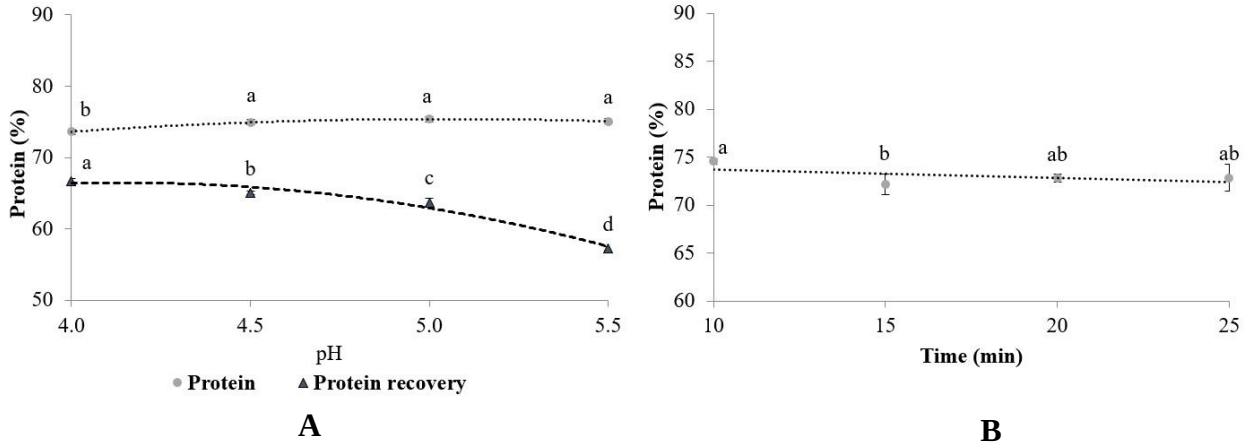

Fig. 2. (A) Effect of pH on chickpea protein precipitation and protein recovery, and (B) effect of time on chickpea protein precipitation. Different letters mean significant differences between samples ($p \leq 0.05$).

3.3.Scaling up process

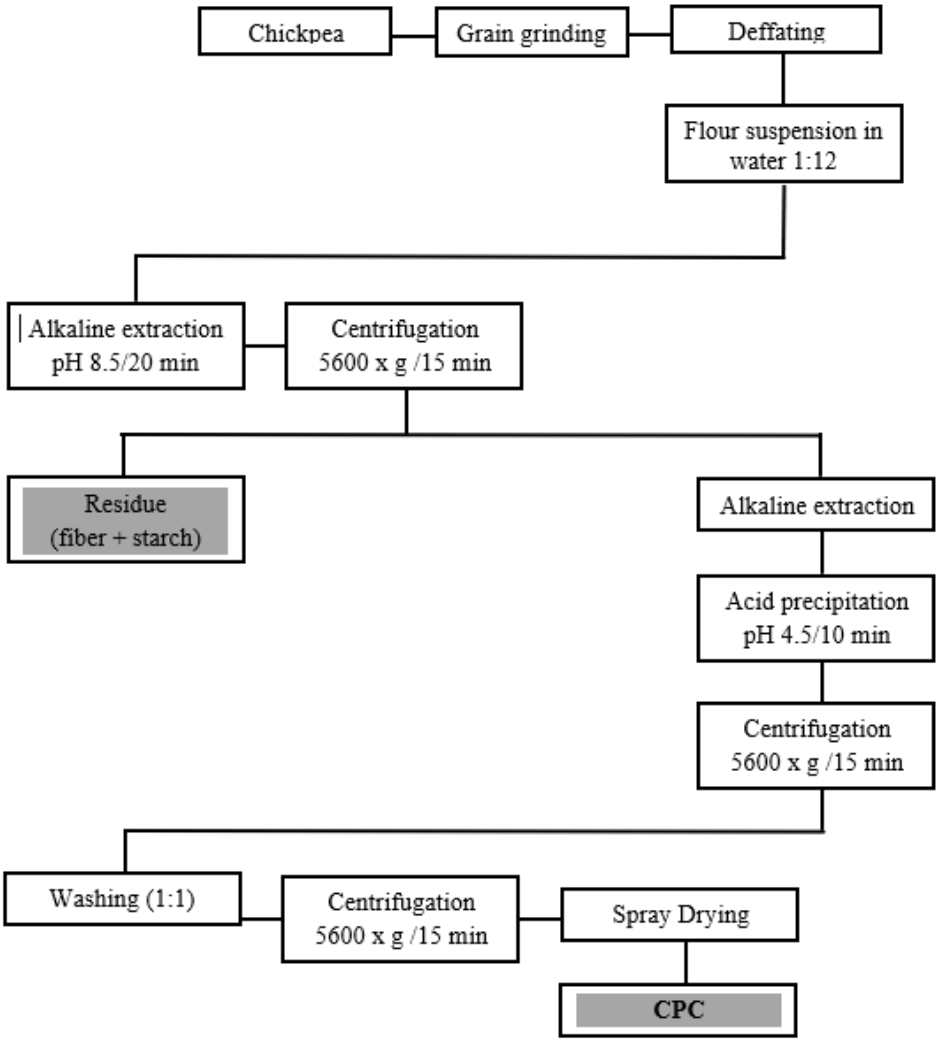

Fig. 3. Flow chart for obtaining chickpea protein concentrate (CPC).

3.4.Morphology

Figure 4 shows the micrograph of chickpea flour (CF) and the protein concentrate (CPC) obtained as described in item 2.2.3. Oval-shaped structures are observed in CF, representing starch granules, with approximate sizes of 20 to 40 μm (length) and 15 to 25 μm (width), typical of chickpeas starch (Kaur & Prasad, 2023; Ruckmangathan et al., 2022). The irregular and small structures on the surface of starch granules are cell wall fragments (CW) and protein bodies (PB), respectively. In CF, the starch granules have a smooth surface and are clearly visible. Similar characteristics were also reported by Kaur & Prasad, (2023) and Ruckmangathan et al., (2022). The main components of the CPC (Fig. 4B) are protein bodies (78% protein), with hollow and wrinkled characteristics and varying sizes of up to 20 μm. Impurities (starch and cell wall material) are also observed, which is consistent with the total dietary fiber (4.26%) content of the CPC. The hollow and wrinkled characteristics observed are common when proteins are subjected to spray-dryer drying process, due to the existence of a high concentration gradient between the drops and the drying medium (hot air), as already reported by other authors Rezvankhah et al., (2021) and Gouvêa et al., (2023).

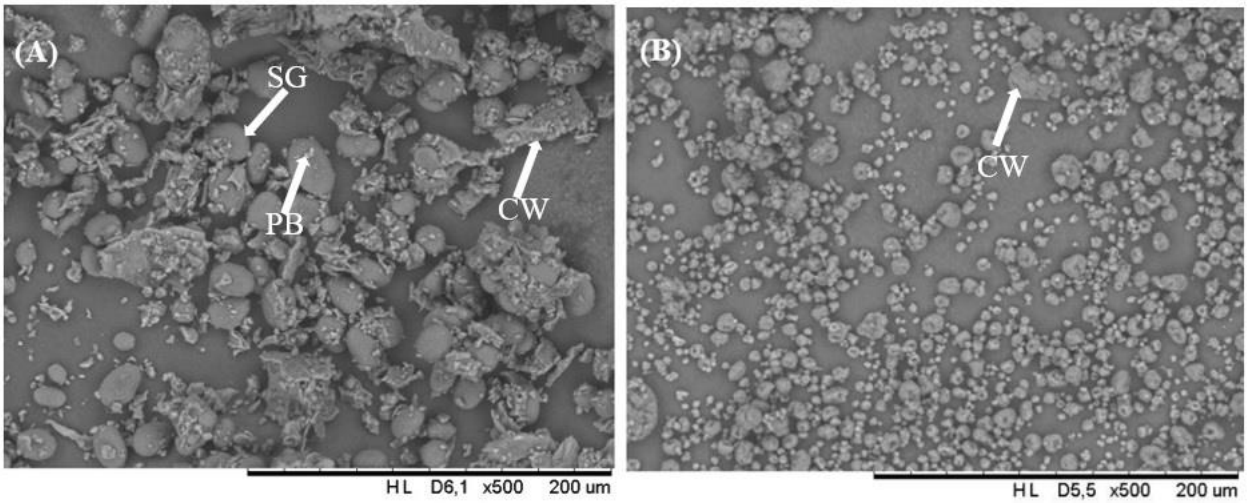

Fig 4. SEM micrographs of (A) chickpea flour and (B) chickpea protein concentrate. SG: starch granule, PB: protein bodies, CW: cell wall fragments.

3.5.Particle size

Particle size is a property that affects the techno-functional characteristics of the materials and, consequently its performance in the final product. Smaller particle sizes are capable of improving the sensory properties of food products due to increased water absorption and foam stability, due to an increase in surface area per volume unit, improving the characteristics of the final product (Özdemir et al., 2022; Ruckmangathan et al., 2022). The mean diameter of the samples [$D_{(4.3)}$] and their percentiles (D_{10}, D_{50}, and D_{90}) are listed in Table 1. The particle size of CPC was approximately

4 times smaller than the CF (26.16 μm and 107.30 μm, respectively), with 90% of the particles smaller than 60.81 μm. This is probably due to the process of obtaining the CPC, as it is known that spray drying conditions (atomizing nozzle, air pressure, etc) have a strong influence on particle size (Shen et al., 2021). Other studies have reported smaller particle sizes for concentrates and isolates when spray dried when compared to other drying techniques (Brishti et al., 2020b; de Paiva Gouvêa et al., 2023; Özdemir et al., 2022; Shen et al., 2021a).

Particle size directly influences the protein content of the flour, protein extraction and techno-functional properties. Higa et al., (2022), in their studies for fine flour (mash 0.5mm) and coarse flour (mash 1.27mm), found particle size in μm for fine chickpea flour D_{10} 8.53, D_{50} 23.23, D_{90} 194.63 and $D_{(4.3)}$ 62.84 and coarse flour of D_{10} 15.97, D_{50} 229.57, D_{90} 769.54 and $D_{(4.3)}$ 312.33 and came to the conclusion that the flour with the smallest particle size increased the extraction yield by 2.80%, protein yield in 10.70% and protein content in 5.80%, when extracted in a solute:solvent ratio of 1:10, using the classic wet route.

The equipment, sieve, variety and peeling or not of the grain used to obtain the flour directly influence the particle size.

Table 1. Particle size of chickpea flour (CF) and chickpea protein concentrate (CPC).

Parameter	**Samples**	
	CF	**CPC**
$D_{(4,3)}$ (μm)	107.30±5.40[a]	26.16±3.08[b]
D_{10} (μm)	8.77±0.60[a]	2.73±0.13[b]
D_{50} (μm)	38.01±2.21[a]	13.06±0.38[b]
D_{90} (μm)	318.00±13.60[a]	60.81±11.17[b]

Different letters in the same line mean significant difference between samples (Tukey test, $p \leq 0.05$).

3.6.Solubility

The solubilities of CF and CPC were studied at pH ranging from 3 to 9 (Fig. 5) The samples presented a U-shaped solubility, showing a lower solubility between pH 4 and 5, which may be due to the isoelectric point of these proteins (Sofi et al., 2020). The highest solubilities of CF and CPC were observed at pH 3 (7.6% and 51.45%, respectively) and pH 9 (83.82% and 90.72%, respectively).

Chang et al., (2022) reported slightly lower values of CPC solubility at pH 3 and pH 9 (47% and 85%, respectively). The lowest solubility was 1% at pH 4-6, similar to the present work. Several studies have shown that cultivars and genotypes have a significant impact on the functional performance of vegetable proteins, in addition to the techniques adopted for drying protein concentrates, which is the most likely cause for the small differences reported (Ma et al., 2022).

The most likely cause for the increased high and low pH solubility of proteins is related to the net positive and negative charges, resulting in an electrostatic repulsive force that helps keep the protein molecules separated, altering the protein's native structure to unfolded form, exposing its hidden functional groups, thus increasing solubility (Ettoumi & Chibane, 2015; Lima et al., 2023; Sofi et al., 2020; Tontul et al., 2018). Close to the isoelectric point, proteins aggregate due to strong intermolecular interactions, resulting in less interaction with water and, therefore, lower protein solubility (Lima et al., 2023).

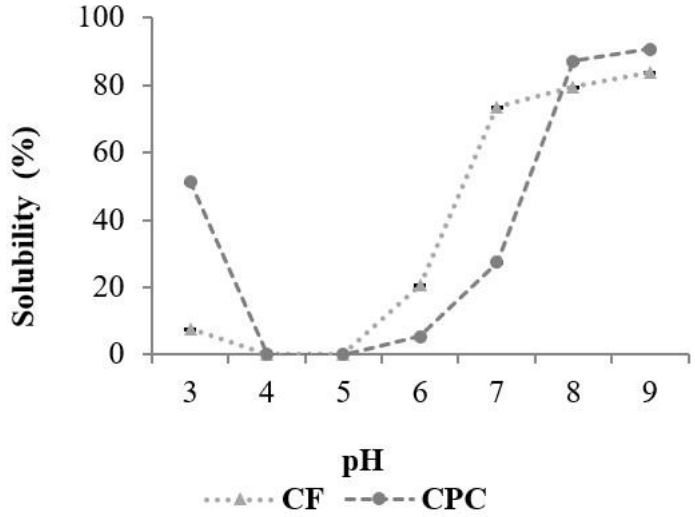

Fig. 5. Solubility of chickpea flour (CF) and chickpea protein concentrate (CPC) at different pH values.

3.7.Water holding capacity (WHC) and oil holding capacity (OHC)

The WHC values of CF and CPC were 2.18 g/g and 1.23 g/g, respectively, while the OHC of CF was 1.69 g/g and of CPC was 1.59 g/g (Fig. 5).Sanjeewa et al., (2010) reported lower values for chickpeas flours from Kabuli and Desi cultivars for both properties: WHC of 0.71 to 0.84 g/g and OHC of 0.81 and 0.88 g/g. Ruckmangathan et al., (2022) found WHC for chikpea flour ranging from 0.78 to 1.20 g/g, OHC from 1.05–1.24 g/g. For CPC, WHC was 2.28 g/g and OHC was between 2.08 and 3.96 g/g. Ghribi et al., (2015), reported WHC for CPC from 2.3 to 5.0 g/g, and OHC from 1.1 to 4.1 g/g. Responses that came closest to our findings for CF were those found by Fernandes et al (2022) who observed a WHC of 2.40 g/g and OHC of 1.37 g/g for the BRS Cristalino chickpea variety. In general, legume protein concentrates and isolates have greater water and fat holding capacity compared to their corresponding flours (Ma et al., 2022; Shevkani et al., 2019), contrary to what was observed in our results. This discrepancy between studies is related to the varieties studied, particle size of both concentrates and flour, type of roller in flour milling, concentrate drying techniques, concentrate processing route and partial denaturation of the protein.

A possible explanation for the flour absorbing more water than the protein concentrate is that spray drying can affect the particles by forming a hydrophobic layer on them, making their interaction with water difficult (Brishti et al., 2020; Gouvêa et al., 2023). Another justification would be that during grain grinding, starch damage can generally occur, which would increase the water retention capacity due to the increase in starch levels exposed to hydration. Furthermore, damaged starch absorbs more water than undamaged starch due to interactions between starch and non-starch components such as proteins and cell wall matrices (Dayakar Rao et al., 2016; Drakos et al., 2017).

Furthermore, although smaller particles have a greater surface area to interact with water and can have high WHC values, very fine particles can have collapsed structures, resulting in the opposite effect (Gouvêa et al., 2023). The lower water absorption of CPC may be related to the production process. Ghribi et al., (2015) reported in their studies that during the spray drying process, a very smooth, thin surface film is formed that is highly resistant to water absorption, in addition to denaturation of the protein. Other conditions that affect the absorption of water and oil are processing conditions (grinding, extraction and drying), varieties used, moisture content, cultural treatments, among others.

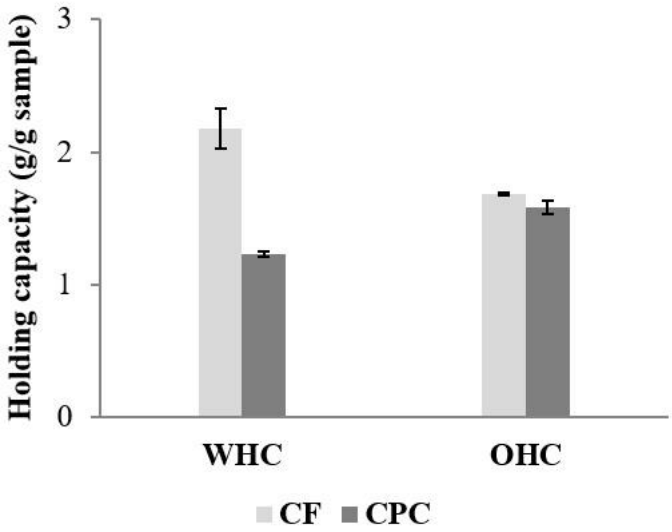

Fig. 6. Water holding capacity (WHC) and oil holding capacity (OHC) of chickpea flour (CF) and chickpea protein concentrate (CPC). Different letters mean significant difference between samples ($p \leq 0.05$).

3.8.Emulsifying capacity (EAI), emulsion stability (ESI), foaming capacity, and foam stability.

Figures (C and D) show the EAI and ESI results obtained for CPC (16.49 m^2/g and 36.64 min, respectively) and CF (18.79m^2/g and 28.69 min, respectively). The ESI of CPC was ~1.5% higher than that of CF. Chickpea flour showed a higher EAI compared to the CPC ~2.25%.

Some similar findings with our studies were reported by Gouvêa et al., (2023), in their studies for legume protein ingredients (isolates and concentrates) EAI ranged from 14.03 m^2/g to 19.39 m^2/g. While Gundogan & Can Karaca, (2020) reported in their studies for isolates from various bean varieties EAI between 15.6 m^2/g to 22.00 m^2/g. While for whole chickpea flour, Ettoumi & Chibane, (2015) obtained EAI of 47.38 m^2/g and ESI of 32.73 min, Kaur & Singh reported ESI of 82.10 min. In protein concentrates obtained from chickpeas and dried by freeze-drying, Karaca et al., (2011), obtained EAI of 47.90 m^2/g and ESI of 82.94 min, while Zhang et al (2023), reported in their findings EAI of 0.66 m^2 /g and ESI of 55.00 min.

Many differences in results for emulsifying capacity and emulsifying stability are detected in different studies, even using the same method, these results depend on the origin and concentration of the protein, in addition to there being differences between the same vegetable protein (Ma et al., 2022). pH is a parameter that influences the emulsifying properties of legume proteins, higher pH values present higher EAI compared to proteins at pH values close to their isoelectric points. Zhang et al., (2009), observed that a chickpea protein isolate obtained by isoelectric precipitation exhibited higher EAI at alkaline pH than at pH close to the isoelectric point of the protein, where the emulsifying capacity of the protein decreased drastically. A similar characteristic with the solubility of proteins, higher pH values, higher EAI and greater solubility, an observation also detected in our studies, where the protein showed high solubility at alkaline pH values.

Chickpea protein concentrate showed excellent emulsifying capacity and emulsion stability, making it possible to apply it in food matrices such as in the preparation of mayonnaise, soups, cakes, sausages and salad dressings.

Figures 7C and 7D show the foaming capacity (FC) and foam stability (FS), respectively, of chickpea flour and protein concentrate. In foam formation, the protein must unfold and be molecularly flexible to form interfacial membranes around the air bubbles (Shen et al., 2021).

The FC and FS values of the CF were, respectively, 82.22% and 92.67%, while for the CPC the values obtained were 77.78% and 93.82%, respectively. CF had a slightly higher FC than CPC (~5%). After 60 min of rest, the CF and CPC foam stability values were very close, less than 1%. The good stability of the CF foam suggests that native proteins soluble in the continuous phase (water) are very surfactant (M. Kaur & Singh, 2005). The high FC value in chickpea flour is due to the conditions in which the most soluble proteins (globulin and albumin) are in their native form, in addition to the flour degreasing stage also helping with this increase, as that fat reduces the ability of proteins to diffuse to the interface (Stone et al., 2019).

According to studies carried out by (Tang et al., 2021), several samples of legume isolates (chickpeas, lentils, beans, and peas) showed foam stability greater than 80% for a resting time of 90

minutes. Maria, (2017) reported for mung bean isolates a FC of 89% and FS of 78%, for a resting time of 30 minutes. Proteins that have high solubility typically have a high foaming capacity, which is due to their high surface charge and excellent balance of hydrophilic-hydrophobic amino acids (Gundogan & Can Karaca, 2020). In our findings, a significant correlation was observed between the foaming capacity and the solubility of CPC. Such studies, when compared with our findings, demonstrate that legume proteins have excellent foaming and stability capabilities, making their application in various food matrices possible.

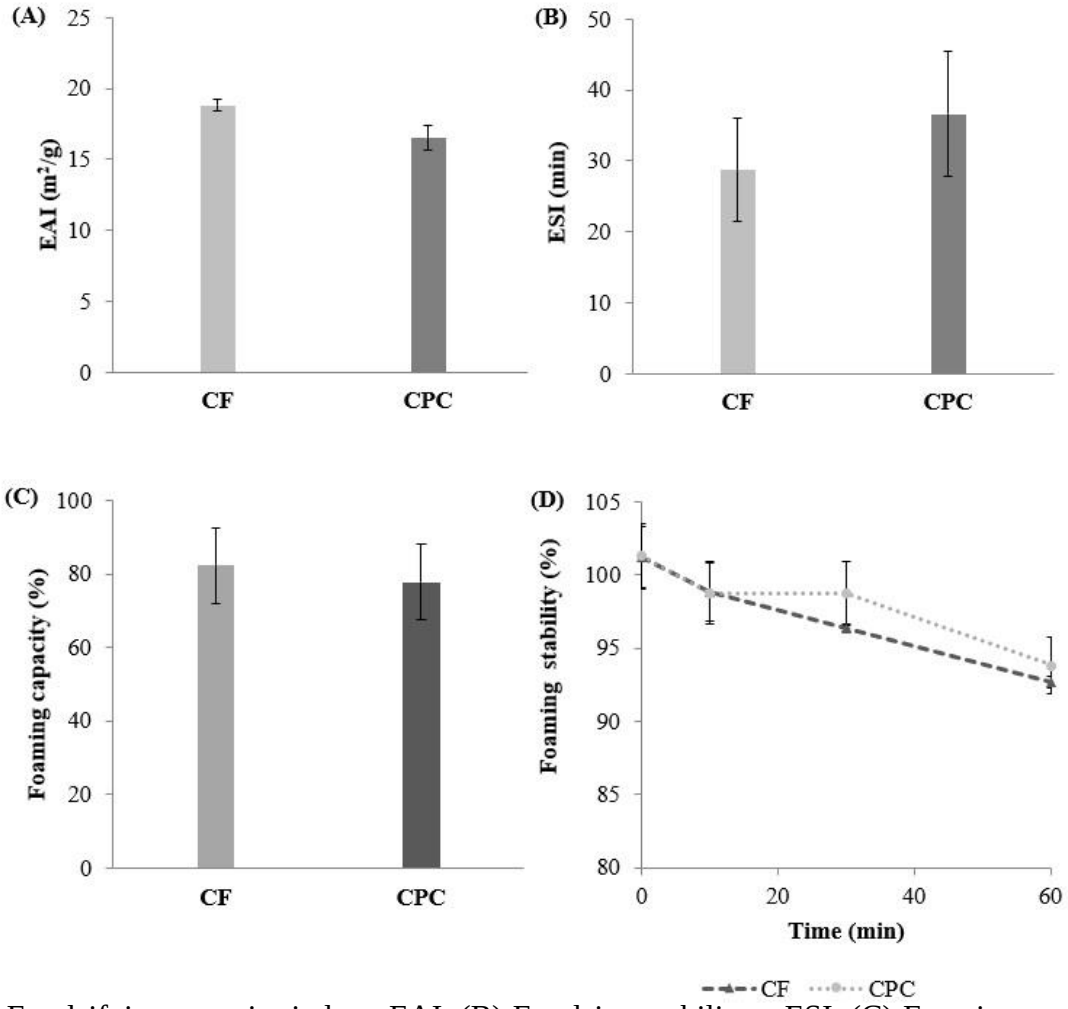

Fig. 7. (A) Emulsifying capacity index– EAI, (B) Emulsion stability – ESI, (C) Foaming capacity, and (D) Foam stability for chickpea flour (CF) and chickpea protein concentrate (CPC).

3.9.Least gelling concentration (LGC)

The LGC results obtained for CF and CPC were 0.10 g/mL and 0.18 g/mL, respectively (Table 2). The lower the concentration in g/mL of the protein, the better its gelling capacity. (Kaur & Singh, 2007). M. Kaur & Singh, (2007) reported LGC values between 0.14 and 0.18 g/mL for chickpea protein isolates and lower gelation concentrations (0.10 to 0.14 g/mL) for their corresponding flours, similar to the data found in our studies. Other studies that used the same wet extraction technique to

obtain CPC reported responses for lower gelation concentrations of 0.115 and 0.18 g/mL (J. Boye et al., 2010c; Kaur & Singh, 2007; Papalamprou et al., 2009b).

The lowest gelation concentration of CF is related to its composition, not only depending on its total protein content, but also on the type, denaturation, state of aggregation of the proteins and the presence of any non-protein substance. Therefore, the gelling capacity of CF is influenced by a physical competition for water between protein gelation and starch gelatinization (M. Kaur & Singh, 2005, 2007; Ma et al., 2022).

Table 2. Least gelling concentration of chickpea flour (CF) and chickpea protein concentrate (CPC).

Sample	Replicates	Concentration (g/mL)									
		0.02	**0.04**	**0.06**	**0.08**	**0.10**	**0.12**	**0.14**	**0.16**	**0.18**	**0.20**
	1	-	-	-	±	+	+	+	+	+	+
CF	2	-	-	-	±	+	+	+	+	+	+
	3	-	-	-	±	+	+	+	+	+	+
	1	-	-	±	±	±	±	±	±	+	+
CPC	2	-	-	±	±	±	±	±	±	+	+
	3	-	-	±	±	±	±	±	±	+	+

"-" Did not form gel; "±" Weak gel; "+" Firm gel.

5.11. Food Application

No differences were observed ($p<0.05$) between fish-like croquettes made with the CPC and the ones with the commercial faba bean concentrate (Table 3) for all the attributes tested. These results indicate that the CPC obtained in the present work presented similar sensory performance to the commercial clean taste ingredient. The means of the hedonic values for sensory acceptance were between the categories “like slightly” and “like moderately”. All scores were among the acceptance range of the scale, which comprises notes between 5 and 9. Therefore, it can be concluded that the fish-like croquettes made from both ingredients were accepted by consumers.

Table 3. Sensory acceptance of fish-like croquettes made with chickpea protein concentrate (CPC) and commercial faba bean concentrate (FBC).

Attribute	CPC	FBC
Overall acceptance	6.50	6.55
Flavor	6.44	6.58
Texture	5.74	6.01

4. Conclusion

Several studies are focused on producing legume protein ingredients, thanks to the techno-functional characteristics presented, and possibility of cultivars adapting to different types of climates Such functional properties of legume proteins, together with the high protein content of isolates and concentrates, provide the opportunity to formulate alternative *plant-based* food products with greater nutritional value compared to many others currently on the market.

The CPC obtained by the classic wet method using the best parameter in alkaline extraction (pH 8.5), stirring time (20 min), solute:solvent ratio (1:12) and acid precipitation (pH 4.5) and stirring time (10 min), had a protein content of ~78% protein (N x 6.25) on a dry basis and a mass yield of approximately 12%. The techno-functional characteristics found were quite interesting, demonstrating that it can be applied to different types of foods. In general, this study provides information on the appropriate parameters for obtaining the protein concentrate, techno-functional characterization, physical properties, and application in food that can be used to optimize the use of CPC by the food industry.

Author statement

The authors declare that the submitted manuscript has not been published elsewhere and it is not under consideration for publication elsewhere.

Ethical Statement

Participants in sensory tests gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this survey" where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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CHAPTER IV

Nutritional composition and *in vitro* digestibility of flours and protein concentrates from kabuli chickpeas and green lentils

Artigo a ser submetido para Food Hydrocolloids

Nutritional composition and *in vitro* digestibility of flours and protein concentrates from kabuli chickpeas and green lentils

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Abstract

Lentils (*Lens culinaris*) and chickpeas (*Cicer arietinum L.)* are nutritious crops, rich in carbohydrates, proteins, dietary fibers and mineral compounds. These grains play an important role in food security, especially among low-income countries. The aim of this study was to compare protein concentrates from lentils, chickpeas and their respective flours in terms of nutritional composition, including macro and micronutrients, antinutritional factors and flatulence-promoting oligosaccharides, and also the protein digestibility of the ingredients The highest protein content was found in lentils, where the flour presented 17% more protein than chickpeas and its protein concentrate was 9% higher on a wet basis. Chickpeas presented a higher content of ash, fat, fiber and carbohydrates in both flour and concentrate. Potassium, phosphorus and magnesium were the minerals with higher concentration in the ingredients, with an increase in sodium in the concentrates compared to the flours. The concentrates also presented high concentrations of iron and essential amino acids. Trypsin inhibitor was increased in lentil concentrate but reduced in chickpea protein concentrate, while phytic acid was reduced in the concentrates and all samples showed low levels of flatulence-promoting oligosaccharides. Protein concentrates presented a higher digestibility when compared to the flours. In terms of nutrition, it was possible to observe that the studied ingredients are great options for the food and beverage industries in terms of replacing protein of animal origin in the development of plant-based products.

Keywords: Pulse proteins, Antinutritional factors, In vitro protein digestibility, Mineral composition.

1. Introduction

The global food security crisis raises the alarm, making the production of alternative proteins more than necessary to meet the growing population of 10 billion inhabitants by 2050. For this, the production of new and diverse *plant-based* protein ingredients have been conquering the market, in which pulses, cereals, algae and mushrooms are important sources of raw materials (L. Chang et al., 2022; Joehnke et al., 2021; Lonnie & Johnstone, 2020).

Among pulses alternatives, peas, beans, chickpeas and lentils are the main cultivars. Pea has already reached maturity in terms of world production and industrial processing for the development of protein ingredients, but there is still a need to better study protein concentrates from lentils (*Lens culinaris*) and chickpeas (*Cicer arietinum L.*), which are legumes from the *Fabaceae* family, defined as dry edible seeds with a low fat content (FAO, 2007), rich in proteins, dietary fibers and micronutrients (Kaur & Prasad, 2021).

In the 2021 harvest, 5.610.104 tons of lentils were produced, with Canada being the largest producer followed by India, Australia, Turkey, Nepal and Bangladesh. Although the three first cited countries represent 70.4% of world production, lentils are cultivated in more than 50 countries and have a full potential to have their production leveraged (FAOSTAT, 2023). Approximately 15.9 million tons of dry chickpea grains were produced in 2021, with the Asian continent responsible for 84.4% of world production, followed by Oceania 5.5%, Africa 5.1%, the Americas 2.9% and Europe 2.1%. India is the largest producer of chickpeas in the world, 11.9 tons in 2021, which represents 74.84% of global production, followed by Australia and Turkey. (FAOSTAT 2023).

Lentils are rich in proteins (~20 to 31%, dry matter) such as globulins (~65%), albumins (~15%), glutelins (~11%) and prolamins (~3%), presenting lysine and leucine as the most abundant essential amino acids. While the protein fraction of chickpeas (~21 to 25%) consists mainly of globulins (~57%), glutelins (~22%), albumins (~10%) and prolamins (~4%), having relatively high levels of free amino acids, particularly glutamic acid, aspartic acid and arginine (Bessada et al.,

2019b; J. Boye et al., 2010d; Joehnke et al., 2021d; Prajapati et al., 2020). Despite the protein presented on the grains, starch (~43%) and dietary fibers (~12%) are also important macronutrients found in both grains, along with high amounts of macrominerals, such as potassium, phosphorus, magnesium and calcium, while the most relevant microminerals are iron and zinc.

Both lentils and chickpeas contain some anti-nutritional factors commonly found in pulses, including lectins, trypsin enzyme inhibitors, phytates, saponins and flatulence-promoting oligosaccharides. These antinutritional factors are known to impair digestive enzymes and sequester essential nutrients, which reduces their bioavailability, making them unavailable for digestion and absorption (Dhull et al., 2023; Kaale et al., 2023). Despite of, as these grains have a great potential to become ingredients for the development plant-based products, knowing the nutritional and antinutritional composition is mandatory to ensure the quality of the ingredients.

In this sense, the aim of this study was to compare protein concentrates from lentils and chickpeas obtained by the classic wet processing route and their respective flours in terms of nutritional composition, antinutritional factors, flatulence-promoting oligosaccharides and protein digestibility.

2. Material and methods

2.1. Material and ingredients processing

Commercial grains of green lentil (Lens culinaris) and kabuli chickpea (Cicer arietinum L.) were purchased from the local market, Rio de Janeiro, Brazil. The whole grains were ground in an LM3100 hammer mill (Perten Instruments AB, Huddinge, Sweden) equipped with a 0.8 mm sieve to obtain the flours.

Both flours (lentil – LF; chickpea – CF) were submitted to protein concentration (lentil – LPC); chickpea – CPC) by the use of alkaline extraction followed by acidic precipitation and were dried by atomization (spray-dried). The chickpea flour was defatted prior the protein extraction.

2.2. Proximate composition

The proximate composition of the ingredients was determined according to official AOAC methods (2010), being: moisture, ash, total fiber, total protein (6.25 x N) and fat with automatic extraction according to (AOCS, 2009). Total carbohydrates were calculated by difference.

2.3. Mineral content

To analyze the mineral content, the adapted AOAC methodology (999.10 and 990.08) was used. The samples were weighed between 0.5-0.6 g of each sample, with tenth of a milligram precision, directly into XPress type PFA® digestion tubes (Spell out, USA) and were added of 6 mL of 69% nitric acid for analysis. The samples were digested in a cavity microwave, model MARS5 (Spell out, USA) with maximum power of 1600 W, heating ramp of 20 min to 180°C and plateau of 180 °C for 20 min. The digest was quantitatively transferred to a 50 mL volumetric flask, completing the volume with ultrapure water.

Quantifications of the elements Na, K, Ca, Mg, Mn, P, Fe, Zn and Cu were carried out on an inductively coupled argon plasma optical emission spectrometer (ICP-OES), model Optima 2100DV (Perkin Elmer, USA), with cyclonic nebulization chamber and concentric nebulizer, with sequential optics and dual-view torch visualization. The conditions of the equipment and method used for quantification followed the following determinations: RF power (W) 1300, nebulizer flow (L min-1) 0.60, plasma flow (L min-1) 15, sample flow (L min-1) 1.50, MEINHARD® Type C Concentric nebulizer and MEINHARD® Cyclonic (glass) nebulization chamber.

2.4. Antinutritional factors and flatulence promoting-oligosaccharides

Trypsin inhibitor was extracted and quantified by the AOCS method (AOCS, 2009). Phytic acid was extracted and quantified by using the AOAC 986.11 (2010) method with some modifications. Phytate quantitation was done with a 2.0 M HCl solution in a 25 mL volumetric flask and direct reading of phosphorus (P) by IPC (inductively coupled plasma). The result was expressed as phytates (mg/g).

The raffinose, stachyose and verbascose were extracted with water under heating and stirring (250 rpm/55 ºC) for 30 min, and then the proteins were precipitated with the addition of acetonitrile and centrifugation for 15 minutes. Then, quantification was carried out using the chromatography method with adaptations (– Mobile phase of 60% acetonitrile in water for raffinose, stachyose and verbascose; 80% acetonitrile in water for sucrose. Column C18, t 40°C, flow 1.4 mL/min) (Macrae, 1998).

2.5. Total amino-acids

The analysis was performed according to AOAC/994.12 (2000), Liu et al., (1995) and AOAC/994.12 (2010). Total amino acid composition was performed on all samples. The method initially performed the protein hydrolysis, according to method 994.12 described in AOAC (2010). Next, three different hydrolysis were carried out: acid hydrolysis (6 M HCl) to determine 18 resistant amino acids, basic hydrolysis (4.2 M NaOH) to quantify tryptophan, and prior oxidation (performic acid) and subsequent acid hydrolysis until quantification of sulfur amino acids. The hydrolysis was carried out in glass ampoules sealed under vacuum and maintained at 110 ºC for 20 h. Tryptophan separation was carried out on a C18 column with fluorometric detection. The sulfur amino acids and those resistant to acid hydrolysis were derivatized with 6-aminoquinolylsuccimidyl-carbamate (AQC), separated by reversed-phase liquid chromatography. Gradient elution was carried out by the use of two solvents. Solvent A: 0.1% trifluoroacetic acid (TFA) in ultrapure water (v/v), and Solvent B: 0.1% TFA in acetonitrile (ACN) (v/v) and detected by fluorescence.

2.6. Soluble proteins and aromatic amino acids

To evaluate the behavior of proteins during digestion, soluble proteins and aromatic amino acids were quantified in the supernatants of the digested samples as well as in the non-digested ones. Soluble proteins were quantified according to Bradford, (1976) by using an albumin standard curve (0.1 to 0.5 mg/mL) and spectrophotometry reading at 595 nm. For measuring the protein

concentration of the samples prior to the in vitro digestion, samples were solubilized in 0.1M KOH instead of using water.

Aromatic amino acids was quantified according to Goodwin (1946). For this, the supernatant of the digested samples as well the undigested ones were mixed with a 10% (w/v) trichloroacetic acid solution, in a 1:1 ratio, and stored under refrigeration, overnight at 4 °C. Subsequently, the mixture was centrifuged at 4,500 ×*g* for 15 min in a refrigerated centrifuge (4 °C). The supernatant was collected and the absorbance at 280 nm was measured with a spectrophotometer AJX-3002PC (Micronal®, São Paulo, Brazil) and a standard curve of tyrosine (0.1 to 0.5 mg/mL) was used.

2.7. In vitro simulated gastrointestinal digestion

Lentil and chickpea flours and their protein concentrates were digested by using the INFOGEST 2.0 static in vitro gastrointestinal digestion protocol (Minekus et al., 2014). Sample preparation was done by pre-hydrating the ingredients to forming a paste as follows. The concentrations for the pastes were (g of sample/g of paste) of: LF 0.2 g/g; LPC 0.25 g/g; CF 0.2 g/g and CPC 0.25 g/g. All samples ended with the same consistency. The digestion was carried out by adding 5 g of each sample into 2 ml of simulated salivary fluid (SSF) containing 75 U/mL of human salivary amylase (Sigma-Aldrich, St. Louis, MO, USA) and then incubated for 2 min at 37 °C. The gastric digestion was immediately proceeded by incubating the oral digested samples with simulated gastric fluid (SGF) (50:50, v/v), containing 2000 U/mL porcine gastric mucosal pepsin (Sigma-Aldrich, St. Louis, MO, USA) at pH 3.0 and 37 °C for 2 h on an orbital shaker. Gastric digestion was stopped on an ice bath to adjust the pH to 7.0 with 1 M NaOH. The intestinal phase was started by adding simulated intestinal fluid (SIF) (50:50, v/v) containing 100 U/mL porcine pancreatin (Sigma-Aldrich , St. Louis, MO, USA) and bovine bile extract (final volume of 10 mM) for 2 hours at 37 °C and pH 7.0. The intestinal phase was interrupted with the use of an ice bath. Samples were the centrifuged at 6.000 x *g*, for 15 min. The supernatants corresponded to the bioaccessible fractions and they were kept frozen until use.

2.8. SDS-PAGE

The SDS-PAGE was done in stacking and running gels prepared with 12% (w/v) polyacrylamide solutions, with sodium dodecyl sulfate (SDS- PAGE) at 100 V for 8 h, as described by Laemmli et al., (1970), 2 mg of each sample of undigested LF, LPC, CF and CPC was used and suspended in sample solubilization buffer (solutions of 1% w/v SDS, 100 mM Tris buffer, glycerol, 2-mercaptoethanol, bromophenol blue and 4N HCl, pH = 9.5). For digested samples, 200 µL of the supernatant was removed after enteric digestion and 100 µL of sample buffer solution was added. Aliquots of 30 µL of the samples were applied to the gel. Bio-Rad molecular weight (kDa) standards were used (phosphorylase B- 104.86 kDa; bovine serum albumin- 82.35 kDa; ovalbumin- 47.49 kDa; carbonic anhydrase- 33.62 kDa; soybean trypsin inhibitor- 27.12 kDa, lysozyme- 17.54 kDa, myosin- 202.44 kDa and β-galactosidase- 116.58 kDa). The gels were stained with Coomassie brilliant blue.

2.9. Statistical analysis

The analyzed parameters were submitted to ANOVA. All analyzes were performed in triplicate, unless otherwise specified, and means and standard deviations were calculated, and when F values were significant ($p < 0.05$), Tukey's test was applied at the significance level of 0.05 for comparison of mean values using STATISTICA software, version 7.0 (StatSoft Inc., Tulsa, OK, USA).

3. Results and discussion

3.1. Proximate composition of chickpea and lentil flours and protein concentrates

The proximate composition of LF, LPC, CF and CPC is presented in Table 1. In general, the composition of the flours and concentrates of both pulses were quite similar, with some minor differences among them. Lentil flour had a lower ash and fat content than chickpea flour, drawing attention to the initial protein content on the flours, which was ~17% higher in lentils (23.50%) than in chickpea (19.56%). In terms of fat, even after defatting the chickpea flour, it still had a higher fat content (2.29%) than chickpea.

The fat content of whole chickpea flour was 6.08%, and the extracted oil showed 62.34% yield when obtained with petroleum ether in a Soxlet system, for 48 hours. The oil composition highlights the presence of linoleic (54.59%), oleic (24.61%) and palmitic acid (9.09%), comprising 88% of the total oil composition, which is in accordance to the literature (Jukanti et al., 2012; Lou et al., 2010; Zia-Ul-Haq et al., 2007).

Table 1. Proximate composition of lentil and chickpea flours and protein concentrates.

Composition	**Samples**			
	LF	**LPC**	**CF**	**CPC**
Moisture (g/100g)	9.01±0.02	5.18±0.03	3.53±0.02	5.60±0.06
Ash (g/100g)	2.54±0.04	2.23±0.03	3.46±0.03	3.15±0.29
Protein (g/100g) (N x 6.25)	23.50±0.02	80.56±0.13	19.56±0.05	73.19±0.32
Fat (g/100g)	1.12±0.07	3.13±0.17	2.29±0.05	4.21±0.30
Dietary Fiber (g/100g)	12.29±0.24	3.05±0.20	12.87±0.83	4.26±0.72
Carbohydrate (g/100g)	51.54	5.85	58.29	9.59
Energy (kcal/100g)	235.74	373.81	344.16	367.33

Each value is the mean ± SD of three determinations.
LF- lentil flour; LPC- lentil protein concentrate; CF- defatted chickpea flour; CPC- defatted chickpea protein concentrate.

When comparing the protein concentrates, a high similarity was also found among the grains. CPC presented a slightly increased amount of ash, fat and dietary fiber content when compared to LPC. However, LPC showed a 9% higher protein content on a wet basis (80.56%) when compared to CPC (73.19%) and that is probably related to the initial protein content of the flours, as the protein concentration factor was of 3.7x for lentil flour and 3.4x for the chickpea one, showing that the extraction processes were efficient for both flours.

Naturally, when pulses protein concentrates are obtained from raw flours, carbohydrate and dietary fibers contents decrease in the final products (Du et al., 2014). A carbohydrate reduction rate of 8.8x was observed for lentil and 6.1x, for chickpea concentrates. Considering the dietary fibers, the reduction rates were of 4.0x and 3.0x for lentil and chickpeas, respectively. These data are in accordance to previous literature findings (Ruckmangathan et al., 2022; Sánchez-Vioque et al.; Toews & Wang, 2013).

At last, the composition of the concentrates from lentil and chickpeas were very high in protein when comparing to other commercial proteins from pulses, reaching values to be considered as protein isolates, according to available products on the market (Gouvêa et al., 2023).

3.2. Mineral content

Table 2 presents the macro- and micro minerals found in lentil and chickpea flours and their protein concentrates. A higher concentration of sodium and phosphorous was observed in both protein concentrates, together with a decrease of potassium, magnesium and calcium.

Table 2. Mineral composition of lentil and chickpea flours and protein concentrates.

	Minerals	**Samples**			
	(mg/Kg)	LF	LPC	CF	CPC
Macro-	Sodium	ND	1343.77±27.93	179.30±5.91	1551.77±28.49
	Potassium	10040.85±16.40	1895.97±42.78	12247.63±40.41	709.73±6.66
	Magnesium	1087.29±10.31	620.77±8.55	1486.51±22.12	350.28±2.12
	Calcium	522.31±2.81	890.47±24.79	1449.54±36.63	1206.52±15.70
	Phosphor	3915.78±44.90	7617.67±31.99	3657.42±85.68	8371.38±63.25
Micro-	Manganese	11.19±0.08	24.15±0.12	37.76±0.62	21.12±0.57
	Iron	74.19±0.56	400.93±40.45	56.14±0.66	307.78±1.66
	Zinc	36.00±0.19	115.27±1.68	35.09±0.25	35.30±1,67
	Copper	8.15±0.05	19.20±0.13	8.69±0.04	14.21±0,50

Each value is the mean ± SD of three determinations.
LF- lentil flour; LPC- lentil protein concentrate; CF- defatted chickpea flour; CPC- defatted chickpea protein concentrate. ND - Not detected.

The increase in sodium in the protein concentrates is expected and it is due to the obtaining process, during the acid precipitation of the alkaline extract, where NaCl is formed (Tang et al., 2021).

Phosphorus was substantially increased (~2x) in the concentrates, and it is an abundant mineral in the human body, being an important constituent of DNA and RNA, and crucial in many metabolic processes, such as those involving buffers in body fluids that helps the maintenance of acid-base balance.

Potassium was abundant in the lentil (10040.85±16.40 mg/kg) and chickpea (12247.63±40.41 mg/kg) flour samples with a reduction of ~81% and 94% in protein concentrates, respectively. Wang

& Daun (2006), in their studies, reported a higher concentration of potassium in raw lentil samples. Potassium performs several important functions in the body, acting on acid-base balance, conducting nerve impulses, muscle contraction, regulating osmotic pressure, especially in the heart muscle and cell membrane function (Chongtham et al., 2021).

As for micro minerals, the highest concentrations were found in the protein concentrates with emphasis on iron for chickpeas (400.93±40.45 mg/kg) and lentils (307.78±1.66 mg/kg) and zinc for lentils (115.27±1.68 mg/kg), which are extremely important micro minerals for human health, (Campos-Vega et al., 2010).

Comparing the increase of micro minerals from the flours to the concentrates, iron (~+ 5.5x) and cupper (~+ 2x) stood out and is probably due to their interaction with the hydrophobic part of the protein. Iron, in special, is essential for almost all living organisms, participating in a wide variety of metabolic processes. In humans, its deficiency can cause anemia and affect brain function (Lorinczova et al., 2020).

The differences found between the studies by Kaur et al., 2019, Wang & Guo, 2021; Zia-Ul-Haq et al., 2007 with our findings are justified by Vandemark et al., (2018), according to them, the genotypic effects, place of cultivation, year planted and their interaction effects significantly influenced the mineral composition of lentils and chickpeas. Protein concentrate could be effective in combating hidden hunger, due to their richness in some essential minerals in the human diet.

3.3. Amino acid profile

The amino acid profile of lentil and chickpea flours and their respective concentrates are shown in table 3 and compared with the FAO recommendations (2007).

Amino acid composition generally indicates the nutritional quality of a protein source (Zia-Ul-Haq et al., 2007). In our study, the amounts mentioned refers to ingredients, which will be part of a food or beverage formulation, so it is worthed to enphasize that the values will be adjusted to the concentration of the ingredient into the final product.

Table 3. Amino acid profile of lentil and chickpea flours and protein concentrates.

Amino acids (mg/g)	**LF**	**LPC**	**CF**	**CPC**	**FAO Standards***
Aspartic acid	107.78±0.09	102.75±0.34	123.76±0.05	105.38±0.17	
Serina	62.46±0.05	67.27±0.17	47.85±0.06	63.44±0.11	
Glutamic	165.34±0,13	163.19±0.53	167.49±0.02	162.56±0.28	
Glycine	47.15±0.01	47.68±0.09	35.48±0.03	45.49±0.10	
Histidine	**25.11**±0.01	**23.47**±0.04	**23.93**±0.03	**24.83**±0.03	**15**
Arginine	96.14±0.05	94.07±0.16	82.51±0.10	95.37±0.04	
Threonine	**41.64±0.03**	**38.99±0.07**	**37.95±0.02**	**33.60±0.03**	**23**
Alanine	40.42±0.03	36.04±0.09	42.08±0.03	36.10±0.04	
Proline	41.64±0.01	41.40±0.13	47.03±0.03	42.78±0.09	
Tyrosine	34.29±0.01	38.99±0.10	35.48±0.03	32.76±0.14	
Valina	**46.54**±0.02	**46.39**±0.13	**44.55**±0.03	**40.28**±0.09	**39**
Lysine	**74.10**±0.08	**71.89**±0.19	**44.55**±0.03	**66.15**±0.12	**45**
Isoleucine	**40.42**±0.02	**41.77**±0.09	**42.90**±0.01	**39.44**±0.06	**30**
Leucine	**72.26**±0.03	**75.03**±0.20	**80.03**±0.01	**73.66**±0.08	**59**
Phenylalanine	54.50±0.02	60.80±0.12	68.48±0.05	67.40±0.12	
Tryptophan	**29.39**±0.01	**33.45**±0.03	**14.03**±0.01	**37.98**±0.03	**6**
Cysteine	12.25±0.00	7.58±0.01	34.65±0.01	16.69±0.04	
Methionine	**8.57±0.01**	**9.24±0.02**	**27.23±0.01**	**16.07±0.01**	**16**
Phe + Tyr	**88.89**	**99.79**	**103.96**	**100.16**	**38**
Met + Cys	**20.82**	**16.82**	**61.88**	**32.76**	**22**

LF- lentil flour; LPC- lentil protein concentrate; CF- defatted chickpea flour; CPC- defatted chickpea protein concentrate. *Amino acid score calculated with EAA requirements for adults according to WHO and FAO (2007). Phe = phenylalanine; Tyr = tyrosine; Met = methionine; Cys = cysteine.

Lysine presented a concentration of 74.10 mg/g in LF and 71.89 mg/g in LPC, with a reduction in concentrate of ~3%, while CF had 44.55 mg/g and CPC 66.15 mg/g, with an increase of ~48.50%. Another essential amino acid that obtained a high concentration compared to the other essential amino acids was leucine, LF presented 72.26 mg/g and LPC 75.03 mg/g, there was an increase of ~3.80%, CF presented 80.03 mg/g and CPC 73.66%, reducing its concentration by ~8.00%. The amino acids that stood out in highest concentrations in our studies were aspartic acid and glutamic acid in all samples, both in flours and concentrates, being a common characteristic for all grain legumes, which are rich in endogenous amino acids (Khazaei et al., 2019). Our findings in flours had little difference from studies carried out by Khazaei et al., (2019) on lentil grains and raw chickpeas, this small difference is related to the grain genotype, time and place of planting.

3.4. Antinutritional factors and flatulence-promoting oligosacharides

Table 4 presents the results found for the antinutritional factors of lentil and chickpea flours and protein concentrates.

One of the antinutritional factors present in legumes are trypsin inhibitors, which are capable of binding to trypsin, inhibiting its activity and interfering with protein digestion (Mondor et al., 2009). Trypsin inhibitors in LF was 7.60 TIU/mg and in LPC 8.50 TIU/mg, there was an increase of ~12% in concentration. According to Avilés-Gaxiola et al (2018), this increase is related to the initial protein content of the flour, the higher this concentration, the more likely it is to increase the content of trypsin inhibitors in the concentrates, as proteinase inhibitors are found within of protein bodies, which makes it difficult to reduce, in addition to also depending on the type of legume and the process of obtaining the protein. In chickpeas there was a reduction of ~30%, CF flour presented 12.91 TIU/mg and CPC was 8.99 TIU/mg. The results for chickpeas indicate that an important proportion of trypsin inhibitors was removed from CF during the pilot-scale protein extraction and processing procedure applied for the preparation of CPC. Mondor et al., (2009), found values much higher than our findings, for Kabuli chickpeas, the defatted flour presented 20.60 TIU/mg and the protein concentrate was 21.00 TIU/mg, despite having an increase in their findings in the concentrate, there were no significant differences, isoelectric precipitation was used to obtain the concentrate, but under different conditions.

Flours (6.78 mg/g and 7.54 mg/g) had lower levels of phytic acid compared to protein concentrates (10.38 mg/g and 15.78 mg/g). Phytic acid (myo-inositol-hexakiphosphate IP6) is the main storage form of phosphorus (P) in cereal and legume seeds that are staple foods around the world, especially in developing countries. In legumes, according to Boeck et al., (2021), the levels vary from 0.27% to 2.90%, therefore, in our studies, both flours and concentrates were between the values reported by Boeck et al (2021). Phytic acid is considered an antinutrient, as it binds essential

microminerals (Fe and Zn) in seeds, in addition to forming complexes with minerals (Fe, Ca, Mg and Zn) from other foods during intestinal digestion, thus interfering with bioavailability. of these minerals, which can cause serious diseases due to a lack of some minerals (Antoine et al., 2022; Thavarajah et al., 2010; Zhang et al., 2018).

The digestion of phytic acid in humans, and in monogastric (non-ruminant) animals, does not occur, due to the absence of endogenous enzymes, such as phytase, which can catalyze the hydrolysis of phytic acid into its component (Ojo, 2020). The extraction process used did not reduce the phytic acid content in the concentrates, probably the conditions used were not capable of breaking the ternary complex, which binds phytic acid to the protein and, therefore, increased the content in the concentrates (Arntfield et al.; 1985). According to Ojo, (2020), there is a lack of information about the interaction of phytic acid with legume proteins, which is why it is not very well understood.

Verbacose, raffinose and stachyose were the main oligosaccharides found in lentil flour, verbacose and raffinose were not detected in lentil concentrates after the wet extraction process, while stachyose had a reduction of ~79%, therefore, the process proved be effective, due to the low quantification of flatulence-promoting oligosaccharides, since this is a recurring complaint from consumers when ingesting grains and products made from legume grains. In chickpeas, only stachyose was detected in the flour (2.28 g/100g), verbacose and raffinose were not detected, it was probably eliminated in the flour degreasing process. None of the oligosaccharides were detected in the CPC. The low quantification of oligosaccharides in the concentrates is due to the obtaining process, most of these sugars are soluble in water and probably left in the supernatant of the acid precipitation and washing steps (Gu et al., 2023). De Angelis et al., (2021), reported in their studies for red lentil and Kabuli chickpea flour before and after separation by dry fractionation into a fine fraction of the flour the following results for oligosaccharides (verbacose, raffinose and stachyose): Flour fine red lentil flour (n.d., 18.39 mg/g-1,45.74 mg/g-1), fine chickpea flour (n.d., 41.9 mg/g-1, 30.1 mg/g-1). Comparing with our findings, chickpea flour was similar for verbacose, but there was

a difference for the two flours in other oligosaccharides, this may have occurred due to the flour production process, variety, growing season, grain genotype.

Table 4. Antinutritional factors and flatulence-promoting oligosaccharides of lentil and chickpea flours and protein concentrates.

	Sample			
	LF	LPC	CF	CPC
	Antinutritional factors			
Trypsin Inhibitor (TIU/mg)	7.60±0.00	8.50±22.91	12.91±0.00	8.99±28.07
Phytic acid (mg/g)[2]	6.78±0.02	10.38±0.28	7.54±0.38	15.78±0.72
	Oligosaccharides			
Verbascose (g/100g)	0.75±0.06	ND*	ND*	ND*
Raffinose (g/100g)	0.04±0.00	ND*	ND*	ND*
Stachyosis (g/100g)	1.52±0.30	0.32±0.01	2.28±0.05	ND*

* Not Detected; LF- lentil flour; LPC- lentil protein concentrate; CF- defatted chickpea flour; CPC- defatted chickpea protein concentrate.

3.5. In vitro simulated gastrointestinal digestion

The digestibility power of animal proteins is greater than that of vegetable proteins, the main cause is the composition of vegetable proteins, as they contain industrial fibers and antinutritional compounds that make digestion difficult (Chamone et al., 2023; Mulla et al., 2022). Both flour samples (LF and CF) and concentrates (LPC and CPC) had good digestibility of soluble proteins. LF and CF showed digestibility (86.56% and 80.15%), while LPC and CPC showed digestibility (90.04% and 85.48%) Figure 1.

Protein concentrates had greater digestibility than flours. This may occur because the access of digestive enzymes to labile peptide bonds will be more limited in flours than in concentrates. Furthermore, the denaturation processes that occur during protein extraction can increase the accessibility of proteins to digestive enzymes and thus improve hydrolysis. Flours contain other components, such as phytic acid and tannins, which can interact with different proteins, reducing their digestibility (Barbana & Boye, 2013; Córdova-Ramos et al., 2020). Barbana & Boye, (2013), reported in their studies the digestibility for green lentil flour of 75.90% and for the protein concentrate of 82.80%. Monsoor & Yusuf, (2002), reported digestibility for lentil concentrate of 95.15% and chickpea concentrate of (89.01%), more recent studies reported high digestibility also for lentil and

chickpea concentrates. Such differences, compared to our studies, are related to differences in the protein extraction process or even the genotype of the grains.

This digestibility is confirmed with an increase in the content of aromatic amino acids after digestion (Fig. 1), showing that the proteins were broken down into peptides and free amino acids.

Even with little difference, the digestibility of the lentil protein concentrate (90.04%) was greater than that of chickpeas (85.48%), this greater digestibility correlates with the lower content of trypsin inhibitors and phytic acid found in the concentrate of lentils, which could increase their digestibility.

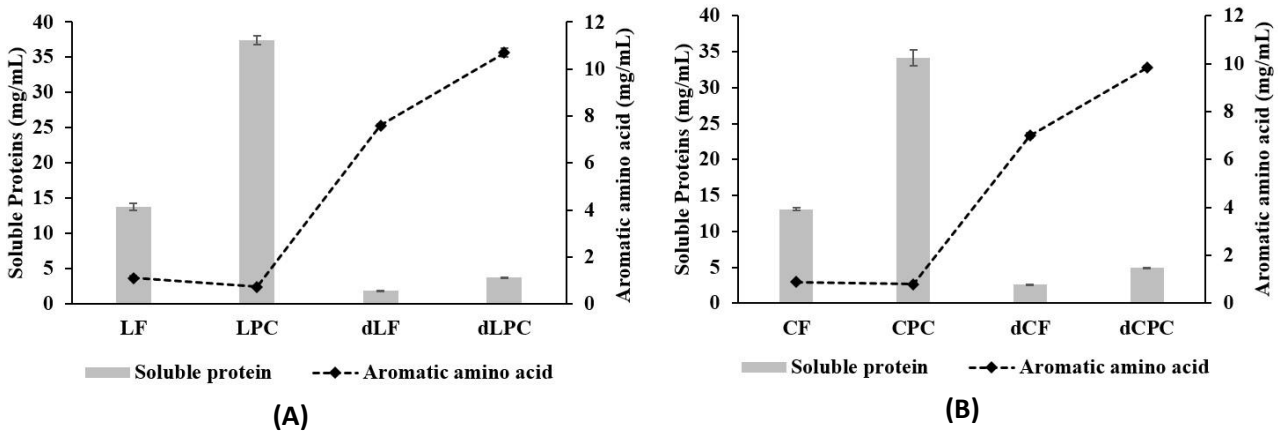

Figure 1. Soluble protein and aromatic amino acid contents from (A) lentil and (B) chickpea flours and protein concentrates prior and after simulated in vitro digestion. LF-lentil flour; LPC-lentil protein concentrate; CF-defatted chickpea flour; CPC-defatted chickpea protein concentrate. “d” before the sample name refers to the digested samples.

3.6. SDS-PAGE

SDS-PAGE is a technique that aims to verify the ability of digestive enzymes to digest proteins present in flours and concentrates after in vitro digestion and compare the protein fractions of digested and undigested samples (Chamone et al., 2023).

The majority of proteins found in legumes are globulins (~70-78%), followed by albumins (10-20%) (Vogelsang-O'Dwyer et al., 2020). Chickpeas are largely composed of globulins (53-60%), glutelins (19-25%), albumins (8-12%), and prolamins (3-7%), while lentils are composed of legumin (~45%), albumins (~17%), glutelins (~11%), vicilin (~4%) and prolamins (~3) (Bessada et al., 2019).

In general, a greater abundance of protein bands is observed in protein concentrates when compared with lentil and chickpea flours, as expected, since the proteins were concentrated in these latter fractions (Figure 2). The proteins found in chickpea flour, the main protein bands were obtained around 20 kDa and between 30 and 40 kDa, while the proteins in lentil flour gave dense bands between 15 and 20 kDa, 30 and 40 kDa and 40 and 70 kDa. Similar findings were reported by Aydemir & Yemenicioĝlu, (2013) for crude protein from lentils and raw chickpeas.

For both lentils and chickpeas, an intense digestibility of the main proteins of these grains is observed, shown by bands between 15-20, 25-30, 40-50, and 55-70 kDa, referring to legumin proteins, acidic and basic subunit, vicilin, covacilin, respectively. At the end of the digestion process, after 4 hours between the gastric and intestinal phases, only the bands referring to the digestive enzymes used in simulating the digestive process are observed.

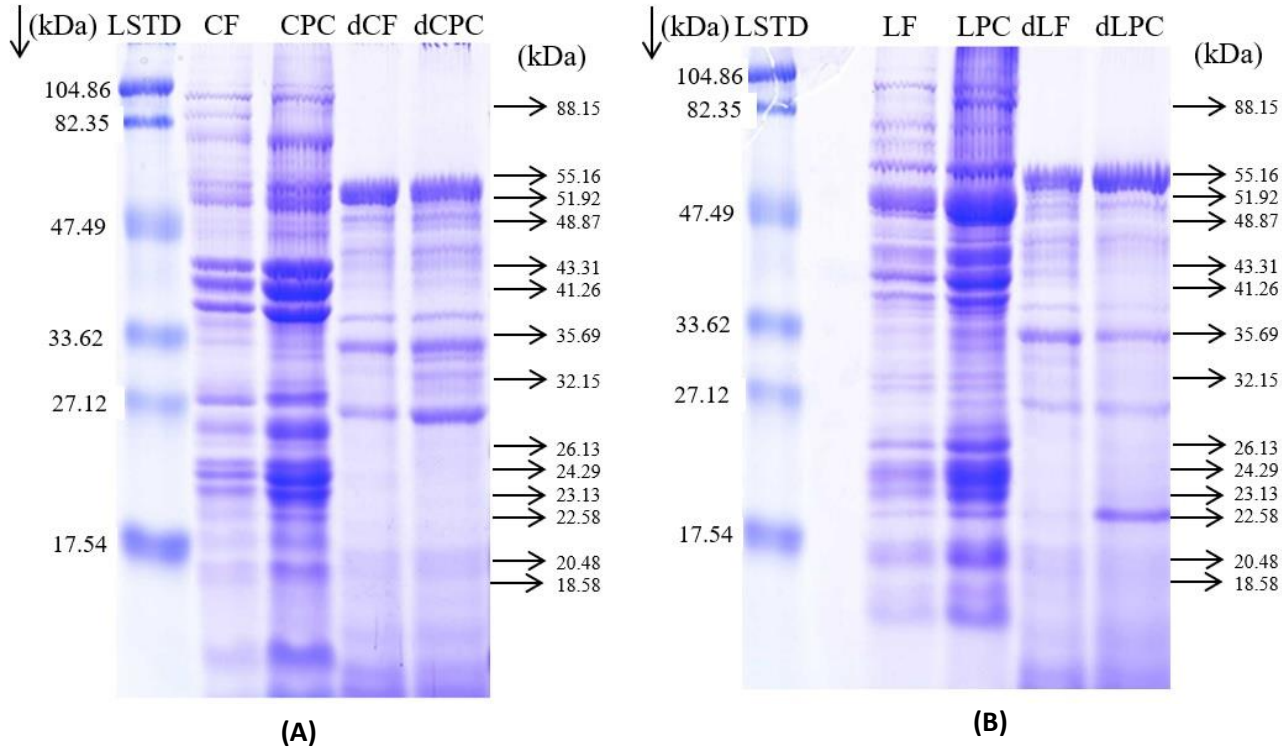

Figure 2. SDS-PAGE of flours and protein concentrates from (A) lentils and (B) chickpeas. kDa-molar mass, LSTD-low molecular protein standard solution, LF- lentil flour; LPC- lentil protein concentrate; CF- defatted chickpea flour, CPC- defatted chickpea protein concentrate. "d" before the sample name refers to the digested samples.

4 Conclusions

In this study, it was possible to observe that obtaining lentil and chickpea protein concentrates by wet means under determined conditions directly impacted their proximate composition, mineral content and antinutritional factors in the flours to different degrees. In the analysis of amino acids, it was detected that the essential ones are in concentrations higher than the FAO recommendations (2007) and that some macro and microminerals are found in satisfactory quantities in the concentrates. It was possible to notice that the lentil and chickpea proteins in the concentrates showed good digestibility, both by the Bradford soluble protein detection method, analysis of aromatic amino acids, through tyrosine and by SDS-PAGE. The reduction of flatulence-causing compounds (oligosaccharides) in concentrates occurred almost completely, thus improving the quality of the ingredient.

This study offers valuable information about the nutritional properties of lentil and chickpea protein concentrates, which can be used to optimize their direct application in the food industry.

Author statement

The authors declare that the submitted manuscript has not been published elsewhere and it is not under consideration for publication elsewhere.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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4 CONCLUSÃO GERAL

Com a realização deste estudo, foi possível obter farinhas e concentrados proteicos de grão-de-bico e lentilha que apresentaram alto valor nutricional, características tecno-funcionais que atendem o mercado com atributos similares a outros ingredientes atualmente disponíveis no mercado, contribuindo com informações que poderão subsidiar a produção nacional de ingredientes proteicos para o mercado *plant-based*, podendo vir a suprir com a demanda da indústria de alimentos por ingredientes de base nacional.

6 APÊNDICE

Escrita Científica durante o doutorado

DE PAIVA GOUVÊA, L., CALDEIRA, R. F., DE LIMA AZEVEDO, T., GALDEANO, M. C., FELBERG, I., LIMA, J. R., & MELLINGER, C. G. (2023). Physical and techno-functional properties of a common bean protein concentrate compared to commercial legume ingredients for the plant-based market. **Food Hydrocolloids**, 137, 108351.

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