



**UNIVERSIDADE ESTADUAL DE CAMPINAS  
FACULDADE DE ENGENHARIA DE ALIMENTOS**

**KATHERINE KELLY NAVARRO VALDEZ**

**QUALITY CHARACTERISTICS OF CORN PASTA CONTAINING MIXTURES OF ANDEAN  
LUPIN (*Lupinus mutabilis* Sweet) AND ANDEAN POTATO (*Solanum tuberosum*) FLOURS**

**CARACTERÍSTICAS DE QUALIDADE DE MASSAS ALIMENTÍCIAS DE MILHO  
CONTENDO MISTURAS DE FARINHAS DE TREMOÇO (*Lupinus mutabilis* Sweet) E  
BATATA (*Solanum tuberosum*) ANDINOS**

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Dissertation presented to the Faculty of Food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of Master in Food Technology

Supervisor/Orientador: Profa. Dra. Maria Teresa  
Pedrosa Silva Clerici.

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To my future children, as a testimony  
of God's powerful hand upon their  
parents in foreign lands.

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To God, who renews my strength every day. “All that I am belongs to You, God; every achievement will always be to glorify Your holy name”.

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## RESUMO

Os cultivos andinos são reconhecidos por seu valor nutricional e potencial no desenvolvimento de produtos sem glúten, incluindo massas alimentícias. Este estudo avaliou o potencial da batata andina e do tremoço andino na qualidade de massas à base de milho. O primeiro artigo abordou as propriedades físico-químicas, tecno-funcionais e reológicas das farinhas de milho amarelo duro, batata andina e tremoço andino. O segundo artigo desenvolveu doze formulações de massa alimentícia do tipo parafuso utilizando essas farinhas, e otimizou suas características físicas e de cozimento para gerar duas formulações ótimas, que foram avaliadas quanto às características físicas, de cozimento, nutricionais e sensoriais. A farinha de tremoço (FT) foi rica em gordura, proteína, fibra alimentar e cálcio, mas pobre em cinzas e carboidratos digeríveis, supridos respectivamente pela farinha de batata (FB) e farinha de milho (FM). FB e FT foram ricas em lisina, mas pobres em leucina, enquanto a FM apresentou o perfil oposto. Nenhuma das farinhas foi rica em metionina ou cisteína. As farinhas diferiram nos níveis de minerais (fósforo, potássio e magnésio) e em minerais exclusivos (cálcio no tremoço; ferro e zinco no milho e tremoço). A FB exibiu a coloração amarela mais intensa e grânulos de amido retrogradado atribuído ao seu pré-tratamento (branqueamento), enquanto a FM apresentou amido nativo semiduro, e a FT teve uma matriz a base de fibra, proteína e lipídios. A FT demonstrou as maiores propriedades emulsificantes e, junto com a FM, apresentou a maior estabilidade de espuma. A FB foi superior em todas as outras propriedades tecnológicas. FM e FB mostraram tempo de pico e viscosidade máxima semelhantes, no entanto a FM formou um gel mais duro devido à maior retrogradação, enquanto a FT não formou pasta. As melhores características relacionadas à forma (distância espiral intermediária e mínima deformação) nas doze massas foram atribuídas às propriedades estruturais dos amidos da FM e FB, enquanto os baixos níveis de manchas foram decorrentes do baixo teor de cinzas. Tempos de cozimento reduzidos e menor absorção de água foram associados ao baixo teor de amido e propriedades emulsificantes da FT. A otimização de resposta múltipla produziu duas massas otimizadas (MO<sub>1</sub> e MO<sub>2</sub>), cujas características de qualidade foram influenciadas pelas composições das farinhas. A MO<sub>2</sub> apresentou menor deformação física, tempo de cozimento mais curto, tonalidade amarela mais intensa e maior contribuição de energia e lisina, enquanto a MO<sub>1</sub> apresentou menor perda de cozimento, melhor absorção de água e perfil superior de aminoácidos essenciais. Ambas massas apresentaram composições minerais semelhantes, exceto por maior fósforo na MO<sub>1</sub> e maior enxofre e cloro na MO<sub>2</sub>, microestruturas semelhantes (amido e proteína distinguíveis em amostras secas, mas não nas cozidas), e ambas atingiram boa aceitabilidade sensorial, com a MO<sub>2</sub> seca exibindo tonalidade amarela mais atraente e a MO<sub>1</sub> cozida exibindo cheiro mais familiar de milho, enquanto a textura foi o atributo mais desafiador para aceitação. Assim, a batata andina e o tremoço andino apresentaram propriedades interessantes para o desenvolvimento de massas alimentícias com características de qualidade aceitáveis.

**Palavras-chave:** milho; sem glúten; massas alimentícias; otimização.

## ABSTRACT

Andean crops are renowned for their nutritional value and their potential in the development of gluten-free products, including pasta. This study evaluated the potential of Andean potato and Andean lupin on the quality of gluten-free corn-based pasta. The first article analyzed the physico-chemical, techno-functional, and rheological properties of hard yellow corn, Andean potato, and Andean lupin. The second article developed twelve fusilli pasta formulations using these flours, and optimized their physical and cooking characteristics to generate two optimum formulations, which were further assessed for physical, cooking, nutritional, and sensory attributes. Lupin flour (LF) was rich in fat, protein, dietary fiber, and calcium, but lacked ash and digestible carbohydrates, which were supplied by potato flour (PF) and corn flour (CF), respectively. PF and LF were high in lysine but low in leucine, while CF had the opposite profile. None of the flours were rich in methionine or cysteine. The flours differed in mineral levels (phosphorus, potassium and magnesium) and mineral exclusivity (calcium in lupin, and iron and zinc in corn and lupin). PF exhibited the most intense yellow hue and retrograded starch granules attributed to its pretreatment (blanching), while CF had semi-hard native starch, and LF contained a protein-lipid-fiber matrix. LF had the highest emulsifying properties, and along with CF, exhibited the highest foam stability. PF was superior in all other technological properties. CF and PF had similar peak times and peak viscosities; however, CF formed a harder gel due to greater retrogradation, while LF did not form paste. The best shape-related characteristics (intermediate spiral distance and minimal deformation) in the twelve pastas were due to the structural properties of CF and PF starches, while low speck levels resulted from the low ash content, and shorter cooking times and reduced water absorption were linked to the negligible starch content and emulsifying properties in LF. The multi-response optimization produced two optimum pastas (OP<sub>1</sub> and OP<sub>2</sub>), whose quality attributes were influenced by their flour compositions. OP<sub>2</sub> exhibited less physical deformation, shorter cooking time, a more intense yellow hue, and higher contributions of energy and lysine, while OP<sub>1</sub> showed lower cooking loss, better water absorption, and a superior essential amino acid profile. Both pastas had similar mineral compositions, except for higher phosphorus in OP<sub>1</sub> and higher sulfur and chlorine in OP<sub>2</sub>, similar microstructures (starch and protein distinguishable in dry samples but not in cooked ones), and achieved good sensory acceptability, with dry OP<sub>2</sub> exhibiting a more appealing yellow hue and cooked OP<sub>1</sub> displaying a more familiar corn-based odor, while texture was the most challenging attribute for acceptance. Therefore, Andean potato and Andean lupin presented interesting properties to develop pasta with acceptable quality characteristics.

**Keywords:** *corn; gluten-free; pasta; optimization.*



## RESUMEN

Los cultivos andinos son reconocidos por su valor nutricional y su potencial en el desarrollo de productos sin gluten, incluyendo los fideos. Este estudio evaluó el potencial de la papa andina y lupino andino en la calidad del fideo sin gluten a base de maíz. El primer artículo analizó las propiedades fisicoquímicas, tecno-funcionales y reológicas del maíz amarillo duro, la papa andina y el lupino andino. El segundo artículo desarrolló doce formulaciones de fideos ‘tornillo’ usando esas harinas y optimizó las características físicas y de cocción para generar dos formulaciones óptimas, cuyos atributos adicionales físicos, de cocción, nutricionales y sensoriales fueron evaluados. Los resultados destacaron los efectos complementarios de las harinas. La harina de lupino (HL) fue rica en grasa, proteína, fibra dietética y calcio, pero careció de cenizas y carbohidratos digeribles, los cuales eran proporcionados por la harina de papa (HP) y la harina de maíz (HM), respectivamente. HP y HL fueron ricas en lisina, pero bajas en leucina, mientras que HM mostró el perfil opuesto. Ninguna de las harinas fue rica en metionina o cisteína. Las harinas difirieron en los niveles de minerales (fósforo, potasio y magnesio) y exclusividad de minerales (calcio en lupino; hierro y zinc en maíz y lupino). HP mostró el tono amarillo más intenso y gránulos de almidón retrogradado atribuido a su pretratamiento (escaldado), mientras que HM presentó almidón nativo semiduro y HL presentó una matriz de proteína-lípido-fibra. HL demostró las mejores propiedades emulsionantes y, junto con HM, la mayor estabilidad de espuma. HP fue superior en todas las demás propiedades tecnológicas. HM y HP tuvieron tiempos y viscosidades de pico similares; sin embargo, HM formó un gel más duro debido a una mayor retrogradación, mientras que HL no formó pasta. Las mejores características de forma (distancia intermedia de las espirales y mínima deformación) en las doce pastas se debieron a las propiedades estructurales de los almidones de HM y HP, mientras que los bajos niveles de manchas resultaron del bajo contenido de cenizas, y los tiempos de cocción más cortos y la menor absorción de agua estuvieron relacionados con el contenido insignificante de almidón y las propiedades emulsionantes de HL. La optimización multirrespuesta produjo dos formulaciones optimizadas de fideo (FO<sub>1</sub> y FO<sub>2</sub>), cuyos atributos de calidad fueron influenciados por las composiciones de las harinas. FO<sub>2</sub> mostró menos deformación física, menor tiempo de cocción, un tono amarillo más intenso y mayores contribuciones de energía y lisina, mientras que FO<sub>1</sub> presentó menor pérdida de cocción, mejor absorción de agua y un perfil superior de aminoácidos esenciales. Ambos fideos tuvieron composición mineral similar, excepto por un mayor contenido de fósforo en FO<sub>1</sub> y mayores contenidos de azufre y cloro en FO<sub>2</sub>, microestructura similar (almidón y proteínas distinguibles en las muestras secas, pero no en las cocidas), y lograron buena aceptación sensorial. FO<sub>2</sub> seco mostró un tono amarillo más atractivo, mientras que FO<sub>1</sub> cocido exhibió un olor más familiar a maíz, mientras que la textura fue el atributo más desafiante para la aceptación. Por lo tanto, la papa andina y el lupino andino mostraron características interesantes para desarrollar fideos de calidad aceptable.

**Palabras clave:** maíz, sin gluten; fideo; optimización.

## SUMMARY

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**CHAPTER 1 – General introduction, objectives and structure of the dissertation**

## 1.1. General introduction

Wheat, one of the most significant staple cereals globally in terms of production, harvested area and trade, primarily provides carbohydrate-based energy, offering a substantial caloric contribution to the human diet (OECD/FAO 2022; FAO, 2023). Around the world, wheat flour is typically used for bakery goods and pasta products, with common wheat (*Triticum aestivum*) typically preferred for the former and durum wheat (*Triticum durum*) used primarily for the latter. The selection of a specific class and cultivar of wheat depends on the elasticity and extensibility needed to elaborate the final product, which influences dough strength (EDWARDS et al., 2001). Gluten, the primary structural protein in wheat flour, is the responsible for providing high-quality characteristics to wheat-based products by forming a network of intermolecular disulfide bonds in the dough, mainly attributed to its storage proteins, gliadin and glutenin (SUSANNA; PRABHASANKAR, 2012).

However, wheat consumption has raised concerns, mainly due to the ingestion of gluten, which can lead to health issues in some individuals. Currently, up to 1 % of the population suffers from celiac disease (CD), an inappropriate immune response to dietary wheat gluten or similar proteins found in barley or rye, while approximately 1–3 % is affected by other gluten-related disorders such as wheat allergy (WA) and non-celiac gluten sensitivity (NCGS) (SINGLA et al., 2024). A lifelong restriction of gluten (for CD and NCGS) or wheat (for WA) in the diet is the recommended treatment for these conditions (JNAWALI et al., 2016). Another concern related to wheat consumption is food security. Due to limited wheat production potential of developing countries—especially of durum wheat—they rely on wheat imports (USDA, 2024), being vulnerable to fluctuating prices of this crop.

The global gluten-free market, with an expected compound annual growth rate of 9.2% from 2022 to 2030 (GRAND VIEW RESEARCH INC., 2022), has focused on introducing alternatives to gluten-containing products, which must contain a gluten level not exceeding 20 ppm according to Codex and FDA guidelines (JNAWALI et al., 2016). Pasta is one of the food products most impacted by the gluten-free market, as its primary ingredient is wheat flour. Gluten-free cereal pasta is the most widely researched and conventionally consumed, so cereals can serve as a primary ingredient in pasta, combined with novel ingredients that offer functional and nutraceutical properties to help prevent nutrition-related diseases and enhance well-being (MASTROMATTEO et al., 2011). However, the level of ingredient substitution in pasta making is crucial in determining whether the properties are desirable or undesirable, especially those related to technological aspects (BUSTOS; PEREZ; LEON, 2015).

Crops from the Andean Region, comprising Peru, Bolivia, Colombia, Venezuela and Ecuador, are sustainable alternatives that have been largely overlooked in the global food market for centuries, but in recent decades, they have gained significant interest for their nutritional benefits. However, their composition can vary depending on intrinsic characteristics of the crop, added to geographical and climatic conditions (SALAZAR et al., 2021). Most studies on the inclusion of Andean crop flours focus on bread, while their addition to pasta formulations remains limited. Challenges such as taste and palatability of pasta are the main considerations in the addition of Andean crop flours (BUSTOS; PEREZ; LEON, 2015). Therefore, it is essential to gain knowledge about the suitability of Andean crop properties and explore their potential to substitute or complement traditional flours in various food applications.

Andean potato is an important carbohydrates source of low energy density (Burgos et al., 2020), while Andean lupin is a notable source of unique macronutrients, especially proteins and lipids (GUTIERREZ-CASTILLO et al., 2022). Both have important micronutrients that boost human health. These crops are essential for feeding highland farming families. They are not globally traded commodities, so their prices are generally influenced by local production costs, making them highly valuable for local food security. In Peru, government initiatives to promote the consumption of these Andean crops include efforts to partially reduce wheat imports by encouraging the use of potato flour in bread (FAO, 2009) and incorporating *tarwi* flour into school breakfasts (MIDAGRI, 2021). Culinary fairs featuring innovative and traditional dishes made with these crops have also gained popularity.

The use of Andean potato and Andean lupin flours in gluten-free cereal pasta is a way for widely cultivated and versatile crops like cereals to help raise awareness of Andean crops, since there is scarce information about the use of their flours in the development of gluten-free pasta. Therefore, the objective of this work is to evaluate the potential of potato and lupin flours from Peru in the development of gluten-free pastas made of hard yellow corn flour from Brazil. This approach highlights the collaborative work between Brazil and Peru, and seeks to support the production and industrialization of these Andean crops, promoting cultural diversity, food security, and sustainability, and benefiting the livelihood of local communities, as well as offering an innovative option of gluten-free pasta to consumers.

## 1.2. Objective

### *General objective*

To evaluate the potential of the Andean potato, Andean lupin, and hard yellow corn flours on the quality characteristics of gluten-free pasta formulations.

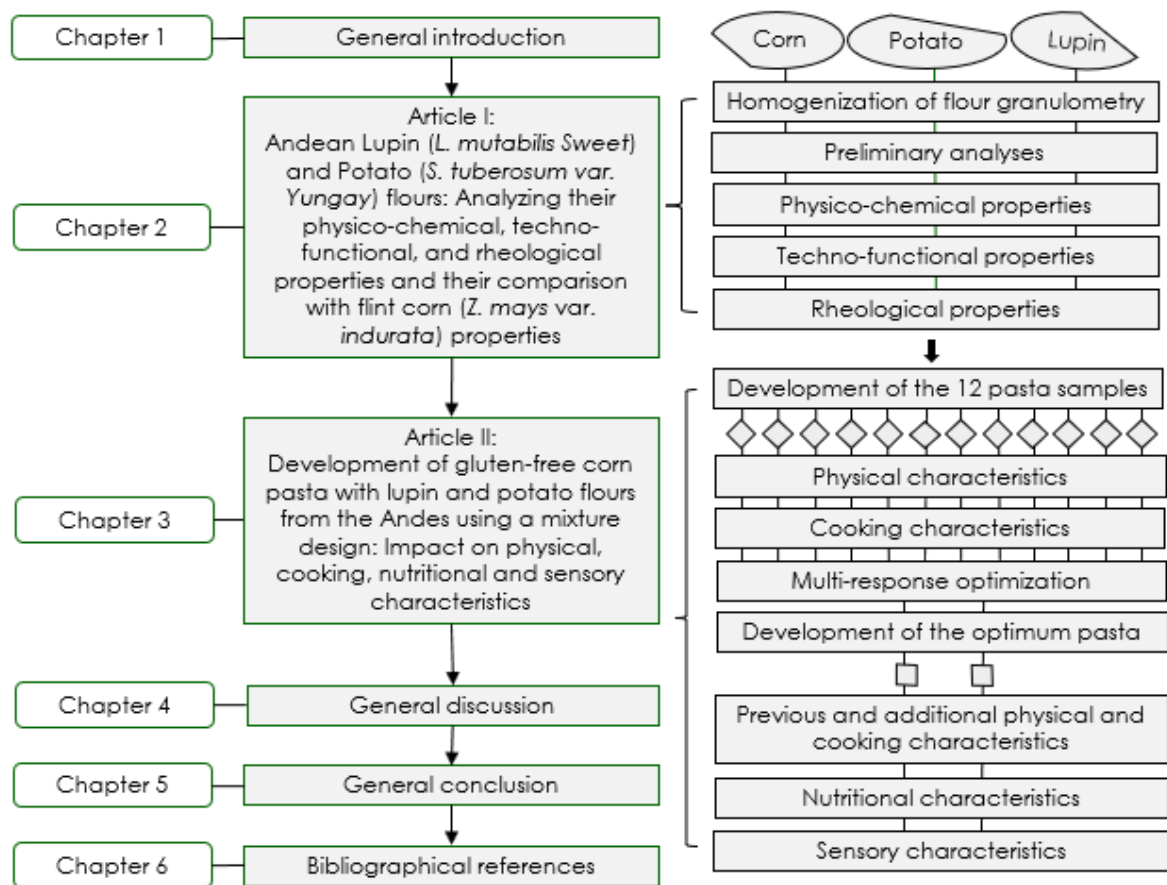
### *Specific objectives*

- To analyze the physico-chemical, techno-functional, and rheological properties of the lupin, potato and corn flours separately.
- To generate pasta formulations containing lupin, potato, and corn flours using the Mixture Design methodology and develop them experimentally.
- To analyze the physical and cooking characteristics of the pasta formulations.
- To perform the multi-response optimization from the selected physical and cooking characteristics for obtaining optimum pasta formulations.
- To develop and validate the optimum pastas, and analyze their physical, cooking, nutritional and sensory characteristics.

### 1.3. Structure of dissertation

This dissertation is divided into 6 chapters. The articles presented correspond to articles not yet published in international scientific journals. The structure of the dissertation is illustrated in Fig. 1.

**Fig. 1.** Flowchart of the dissertation structure



The first chapter covers the general introduction, outlining key information about the dissertation topic. It includes an overview of the problem, as well as the purpose, hypothesis, and objectives of the study.

The second chapter includes the article **“Andean Lupin (*L. mutabilis* Sweet) and Potato (*S. tuberosum* var. *Yungay*) flours: Analyzing their physico-chemical, techno-functional, and rheological properties and their comparison with flint corn (*Z. mays* var. *indurata*) properties”**. This study explored and compared the intrinsic properties of corn, potato and lupin flours among themselves and with those of their respective species reported in the literature.

The third chapter presents the article **“Development of gluten-free corn pasta with lupin and potato flours from the Andes using a mixture design: Impact on physical, cooking, nutritional and sensory characteristics”**. This study evaluates the effect of key pasta quality characteristics based on twelve pasta formulations developed using mixture design, and performs a multi-response optimization to identify two optimum pasta formulations, which are then analyzed and compared in greater detail.

The fourth chapter provides a general discussion of the dissertation connecting the results of both articles to establish cause-and-effect relationships between the first and second articles, while also offering suggestions for future research.

The fifth chapter summarizes the main conclusions of the dissertation.

The sixth chapter contains the references used in the development of this dissertation.



**CHAPTER 2 – Andean Lupin (*L. mutabilis* Sweet) and Potato (*S. tuberosum* var. *Yungay*) flours: Analyzing their physico-chemical, techno-functional, and rheological properties and their comparison with flint corn (*Z. mays* var. *indurata*) properties.**

## ARTICLE I

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**Andean Lupin (*L. mutabilis* Sweet) and Potato (*S. tuberosum* var. *Yungay*) flours: Analyzing their physico-chemical, techno-functional, and rheological properties and their comparison with flint corn (*Z. mays* var. *indurata*) properties.**

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## Abstract

Andean potato and Andean lupin are currently local foods for specific rural communities in South America, whereas hard yellow corn is widely consumed around the world. These flours were studied in terms of physico-chemical, techno-functional and rheological properties to explore their similarities and differences for potential combined use. The preliminary tests proved that the flours were non-alkaloid toxic and well preserved. Lupin had the highest content of crude fat (24.05%), crude protein (44.93%), dietary fiber (27.02%), while ash content (2.91%) was predominant in potato. Corn had the highest digestible carbohydrates (87.35%) and better essential amino acid composition (EAA/NEAA: 0.79). Potato and lupin were rich in lysine but limited in leucine (EAA score: 86.86%) and sulfur-containing amino acids (EAA score: 85.46%), respectively, and corn was limited in lysine (EAA score: 55.27%) but rich in leucine. Potassium was mainly found in potato, phosphorus in lupin, and magnesium in corn, while iron and zinc were present only in corn and lupin, and calcium exclusively in lupin. Physically, all flours had high luminosity ( $L^* > 80$ ) with varying yellow hues. Corn morphology revealed relatively hard native starch, while modified starch was evident in potato, and a spongy protein-lipid-fiber matrix was shown in lupin, with homogeneous particle size distribution for lupin (1.35–12.09  $\mu\text{m}$ ) and corn (4.95–24.90  $\mu\text{m}$ ) but wide dispersion in potato (12.01–158.97  $\mu\text{m}$ ). Water absorption, solubility, swelling, and foam capacity were higher for potato; while lupin demonstrated superior emulsifying properties, and along with corn, had better foam stability. Corn and potato formed pastes under RVA conditions with similar peak times and peak viscosities but with higher breakdown, setback and final viscosities for corn, resulting in a harder gel due to its higher tendency to retrograde. These findings highlight the distinct and complementary properties of corn, potato, and lupin flours to enhance food nutrition and security.

**Keywords:** *Latin American crop, flour characterization, gluten-free, sustainability, food ingredient.*

## 1. Introduction

Potato is one of the major global crops recommended for contributing to food security, with a global production of approximately 366 million tons (Buzera et al., 2023), expected to reach 750 million tons by 2030 (FAO, 2022). This tuber provides nutrients such as proteins, carbohydrates, minerals, and antioxidants (Buzera et al., 2022). In Peru, potatoes are especially diverse and classified into native and improved varieties. Improved Andean varieties, like *Yungay*, offer farmers crucial resilience against climate and pests challenges, while enhancing yields. Improved varieties account for around 60% of potato-farming area in Peru and have a ready market due to their favorable characteristics (CIP, 2021) and relatively lower retail prices compared to native varieties. The *Yungay* potato is commonly consumed as a garnish in various Peruvian dishes, featuring pale-yellow flesh, a mild flavor, and a relatively sticky, non-floury consistency.

Lupin is a legume including four common species (*L. angustifolius*, *L. albus*, *L. luteus*, and *L. mutabilis*) (Mazumder et al., 2021) and is considered an important protein source, attributed with functional and nutraceutical properties that offer health benefits (Czubinski et al., 2021). It can be easily grown in cool-temperatures and can thrive with minimal use of nitrogen fertilizer (Arzami et al., 2022). Unlike the others, *L. mutabilis*, (Andean lupin, pear lupin or tarwi) is cultivated in the South American Andes, being the only domesticated lupin species with protein and oil content comparable to soybeans. The seeds, oval and opaque-white, are consumed after alkaloids are removed through a debittering process (Carvajal-Larenas et al., 2016), showing potential of a modern, sustainable food source for a rapidly growing population, as well as combating child malnutrition.

Andean lupin and Andean potato crops have economically sustained highland households in the South American Andes. They are traditionally sold as fresh food in Peru, but efforts are being made to incorporate them into processed food products. Processing these crops into flour enhances their industrial applications and reduces their bulkiness, perishability, and costs associated with storage and transportation. On the other hand, corn is one of the most widely cultivated crops worldwide, used both for direct human consumption and as poultry feed. Among corn varieties, yellow hard corn, considered a type of flint corn, is the main input for poultry feeding in Peru. In Brazil, the use of this type of corn is more diversified, including the production of *canjica amarela* and its flour, *fubá*, which is primarily used for making cakes, extruded snacks, and pasta (Manfroí, 2022).

The possibility of combining corn flour with emerging regional flours, such as Andean potato and Andean lupin, could address some of corn's nutritional gaps, enhance food sovereignty, promote sustainable agriculture, and improve the economic conditions of family farmers, since they can be an

alternative option to substitute or complement traditional flours in the industry. However, it is crucial to first thoroughly understand the properties of the Andean potato and lupin flours individually to determine their suitability for food applications containing corn flour, including those for people with celiac disease or other gluten-related disorders. Therefore, this research aims to analyze the physicochemical, techno-functional, and rheological properties of Andean lupin and Andean potato flours and compare them with those of corn flour.

## **2. Material and Methods**

### **2.1. Material**

Corn (*Zea mays* var. *indurata*) flour was purchased from Douradinha da Fazenda S.A.C. (São Paulo, Brazil). Potato (*Solanum tuberosum* var. *Yungay*) and Andean lupin (*Lupinus mutabilis* Sweet) flours were purchased from Agro Mi Perú Foods S.A.C (Lima, Peru), and Nutri Mix S.A.C. (Lima, Peru), respectively, and then sent to Brazil. According to the suppliers' specifications, the corn was cultivated in Anápolis (Goiás, Brazil), while the potato in Huamanga (Ayacucho, Peru) and the lupin in Huaylas (Ancash, Peru).

The flours were studied as provided by the suppliers, with prior processing steps beyond our control. Corn flour was produced from degermed raw corn grains (known as *fubá* in Brazil) to prevent high lipid content in the germ from reducing the flour's shelf life. Potato flour was made from peeled tubers using an artisanal thermal process to prevent enzymatic browning. Lupin flour was made from debittered raw lupin seeds using an artisanal aqueous debittering process to remove alkaloids.

### **2.2. Homogenization of flour granulometry**

Each flour was sieved through a 250 µm mesh, packaged in resealable Doy-pack metallic bags, and stored in a dry, ventilated place until use. The particles of higher granulometry were reduced in a blender (PRD-0450, Metvisa, Brasil) and passed again through a 250 µm mesh sieve.

### **2.3. Preliminary analyses**

#### **Qualitative test for alkaloids**

The alkaloid analysis was performed following the procedure described by Rajkumar et al. (2022) with slight modification. Each flour (2 g) was mixed with ethanol 70% (20mL) in a flask at 200

rpm for 150 min on a magnetic stirrer (LGI-MSH-20D, LGI Scientific, Brazil), filtered through Whatman N°1 paper and concentrated to 10 mL in a hotplate at 60°C. The extracts were evaluated to determine the presence (+) or absence (-) of alkaloids. Each extract (2 mL) was dissolved in hydrochloric acid 10% (5 mL), filtered and treated with 2- 3 drops of the following reagents: Dragendorff (potassium bismuth iodide), Mayer (potassium mercuric iodide), Bertrand (silico-tungstic acid) and Sonnenschein (phosphomolybdic acid in 10% HNO<sub>3</sub>) forming orangish-red, creamy-white, creamy-white and blue-green precipitates, respectively, if positive reactions occur.

#### **pH and total titratable acidity**

pH was determined according to AOAC methodology 943.02 (AOAC, 2023) with slight modification. Each sample (5 g) was mixed with distilled water (50 mL) in a beaker at 25°C for 5 min using an electric agitator (Dremel 3000, Bosch, Brazil). The measurement was made with a digital pH-meter (HI2221, Hanna Instruments, USA), previously calibrated with buffer solutions at pH 4 and 7.

Total titratable acidity (TTA) was determined according to the method of the Adolfo Lutz Institute (IAL, 2008). Each flour (2.5 g) was mixed with ethanol 50% (50 mL) on a magnetic stirrer at 1,000 rpm for 5 min and centrifuged at 5,000 rpm for 10 min. The supernatant, with 2-3 drops of phenolphthalein added, was titrated with 0.1 N NaOH until the endpoint (pH:  $8.3 \pm 0.1$ ) was reached, using a pH meter (AK90, ASKO, Brazil). A blank (containing only ethanol 50%) was prepared to eliminate potential interferences. The volume of NaOH spent during titration of the sample and the blank were recorded. The results were expressed as mL eq NaOH/100 g of dry sample.

## **2.4. Physico-chemical properties**

### **Proximate composition**

The proximate composition was determined using AOAC methodology (AOAC, 2023) for moisture (925.10, air-oven method), crude protein (960.52, micro-Kjeldahl method), crude fat (920.85, Soxhlet method), ash (923.03, direct method) and dietary fiber content (985.29, enzymatic-gravimetric method). For protein determination, the factor *N* was 6.25 and 5.71 for corn and lupin flours, respectively (Jones, 1931), and 6.24 for potato flour (Van Gelder, 1981). Digestible carbohydrate (starch + sugars) content was calculated by difference, subtracting crude protein, crude fat, ash and dietary fiber from the flour's initial dry weight. The results were expressed as a percentage of the initial dry weight.

### Amino acid profile

#### - Amino acid composition

The amino acids, except for tryptophan, were detected by reversed-phase HPLC equipment (Alliance 2695, Waters, USA) at 254 nm, after treating the flours with acid hydrolysis, derivatization and buffer dilution (Hagen et al., 1989; White et al., 1986). Tryptophan was detected by UV/Vis spectrophotometer (Genesys 50, Thermo Fisher, USA) at 590 nm after treating the flours with enzymatic hydrolysis (Lucas & Sotelo, 1980). The results were expressed as g of amino acid/ 16 g N.

#### - Amino acid quality

The essential amino acids (EAAs) scores were determined using Eq. (1) (Mitchell & Block, 1946), based on each flour's tested protein (TP) relative to a reference protein (RP) for adults, as recommended by the Food and Agriculture Organization (FAO, 2013). The limiting amino acid (LAA) for each TP was the EAA score lower than the EAA of the RP. The essential to non-essential amino acid ratio (EAA/NEAA) was used to evaluate the distribution of those amino acids in the TPs.

$$EAA \text{ score } (\%) = \frac{mg \text{ EAA in } 1 \text{ g of tested protein}}{mg \text{ EAA in } 1 \text{ g of reference protein}} \times 100 \quad (1)$$

### Mineral characterization

The elements in the flours were qualitatively detected using Energy Dispersive X-ray Spectroscopy (EDS) with a detector (6070, Oxford Instruments, England) coupled to a Scanning Electron Microscope (Leo 440i, Leo Electron Microscopy, England). The samples consisted of flour ashes, which were spread on slides, fixed to specimen stubs with double-sided carbon tape, and placed in the EDS-SEM system. Three areas of the images were visualized at 500x to identify the elements and their corresponding percentages based on their relative peak heights.

### Color

Color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) were determined in the CIELAB system using a colorimeter (MiniScan XE 3500, HunterLab, USA) previously calibrated in white and black plates. Additionally, the visual color of the flours was described.

### Morphology

The morphology of the flours was observed using Optical Microscopy (OM) and Scanning Electron Microscopy (SEM). For OM, a small portion of the flours were placed on glass microscope slides and observed using a high-resolution light microscope (BX51, Olympus, USA) at 40x and 100x. For SEM, a small portion of the flours was fixed to the specimen stub with double-sided carbon tape, coated with a thin gold layer using a sputter coater (K450, Emitech, UK), and observed at 500x and

1000x using a scanning electron microscope (TM-4000 Plus, Hitachi High-Tech, Japan) at 5 kV acceleration voltage, 6.3 mm working distance and 55  $\mu$ A beam current, with a mixed image signal. From SEM images, the vertical and horizontal dimensions of 100 granules from each flour were measured using ImageJ software (v. 1.54i, Fiji, USA) to calculate particle size and particle distribution, and the data were processed using OriginPro software (v. 10.1, OriginLab, USA).

## 2.5. Techno-functional properties

### Emulsifying activity (EA) and emulsion stability (ES)

Emulsifying properties were determined according to the methodology of Stone et al. (2019). Each sample (1 g) was mixed at high speed (8,000 rpm) with distilled water (15mL) for 30 s and soybean oil (15mL) for another 60 s using a digital Ultra-Turrax homogenizer (T-25, IKA, Germany). The total emulsion height (TEH) of the tubes was measured. The emulsions were centrifuged at 1,300 x g for 15 min. The emulsified layer height (ELH), located in the middle layer, was also measured. EA was calculated in Eq. (2).

$$EA (\%) = \frac{ELH}{TEH} \times 100 \quad (2)$$

The emulsions prepared for EA were heated in a water bath at 80 °C for 30 min, cooled with tap water for 15 min, and centrifuged at 1,300 x g for 15 min. The remaining emulsified layer height (RELH) was measured. ES was calculated as the ratio between the EA after heating and that of the initial emulsion, with the result expressed as a percentage.

### Water solubility index (WSI), water absorption index (WAI), oil absorption index (OAI)

WSI and WAI were determined using the method described by Anderson et al. (1970) with slight modification. Each sample (0.5 g) was mixed with distilled water (25 mL) at high speed (5,000 rpm) for 1 min with the Ultra-Turrax homogenizer, left at room temperature for 30 min and centrifuged at 5,000 rpm for 20 min. The supernatant was drained for 30 sec, allowing the precipitate to be weighed. The supernatant from WAI was evaporated in a Petri plate using a hot air oven (TE-394/2, Tecnal, Brazil) at  $105 \pm 1$  °C and the recovered dry residue (soluble solids) was weighed. WSI was determined in Eq. (3) corrected for the total volume.

$$WSI (\%) = \frac{\text{dried residue (g)}}{\text{sample (g)}} \times 100 \quad (3)$$

OAI was determined using the same methodology for WAI with some adaptations. Soybean oil was used instead of water, mixing was carried out at 8,000 rpm and the supernatant after centrifugation



was drained for 3 min. WAI, expressed as g of water retained per g of sample, and OAI, expressed as g of oil retained per g of sample, were calculated using Eq. (4).

$$OAI, WAI (g/g) = \frac{\text{precipitate (g)} - \text{sample (g)}}{\text{sample (g)}} \quad (4)$$

### Swelling capacity (SC)

SC was determined according to Nawaz et al. (2015). Each sample (0.5 g) was mixed with distilled water (25 mL) and centrifuged at 2,500 rpm for 1 min, heated at 60 °C for 20 min in a water bath, cooled in ice bath to room temperature, and centrifuged at 5,000 x g for 15 min. The supernatant was drained for 30 s before weighing the precipitate. SC was calculated in Eq. (5).

$$SC = \frac{\text{precipitate (g)}}{\text{sample (g)}} \quad (5)$$

### Foaming capacity and foam stability

Foaming capacity (FC) was determined by the method of Kaur & Singh (2005). Each sample (1 g) was mixed manually and gently with distilled water (40 mL) and whipped with an electric agitator at high speed (N° 8) for 2 min. The volume of the suspensions was measured before and immediately after whipping. FC was calculated in Eq. (6) as the percentage increase in volume.

$$FC = \frac{\text{vol.after whipping} - \text{vol.before whipping}}{\text{vol.before whipping}} \times 100 \quad (6)$$

Then, the suspensions were allowed to rest for 60 min, and the volume was measured again. FS was calculated in Eq. (7) as the percentage decrease in foam volume.

$$FS = \frac{\text{vol.after whipping} - \text{vol.after 60 min}}{\text{vol.after whipping}} \times 100 \quad (7)$$

## 2.6. Rheological properties

### Pasting properties

A Rapid Visco Analyzer (RVA) device (4500 series, Perten Instruments, Australia) with the Thermocline for Windows© software (v. 3.15) were used to determine the viscosity of the flours, following the method 76–21.01 by AACC (2010). Depending on the sample's moisture, about 3 g of flour (at 14% moisture basis) was mixed with about 25 mL of water in an aluminum canister, and dispersed using the device's own acrylic paddle. The canister/paddle assembly was fixed into the device and the Standard 1 configuration was chosen. After calibration, the dispersions were loaded (stirring at 960 rpm for 10 s, followed by 160 rpm until the end of the assay). Parameters such as pasting temperature

(°C), peak time (min), peak viscosity (cP), trough (cP), breakdown (cP), setback (cP), and final viscosity (cP) were calculated from the viscograms.

### **Texture profile**

The pastes obtained in the RVA analysis were kept in the aluminum canisters, refrigerated ( $5 \pm 2^\circ \text{C}$ ) overnight, and left for two hours at room temperature to reach thermal equilibrium. The textural properties of the RVA pastes were evaluated using a texture analyzer (TA-XT2, Stable Micro Systems, UK) with a texture profile analysis (TPA) mode. Each canister was placed upright on the metal plate of the equipment and the paste was compressed with a 20-mm cylindrical acrylic probe at a speed of 0.5 mm/s to a penetration distance of 10 mm (Bento et al., 2021). The samples were compressed twice to create a force-time curve in which hardness (height of first compression), and adhesiveness (negative area between the two cycles during retraction of the probe) were determined, using the Exponent Lite software (v. 5, Stable Micro Systems, UK) to process the data.

## **3. Statistical analysis**

Data were analyzed using analysis of variance (ANOVA) with means compared by Tukey's test ( $p < 0.05$ ) for three samples, or by Student's t-test for two samples, using the statistical software Minitab (v. 13, Minitab Inc., USA). The results were reported as the mean  $\pm$  standard deviation (SD). Triplicate observations were used for physico-chemical, techno-functional and rheological analysis. The principal component analysis (PCA) reduced the extensive data set and projected it into a two-dimensional space in order to observe the main comparison among the flours.

## **4. Results**

### **4.1. Preliminary analyses**

#### **Qualitative test for alkaloid**

Some studies have reported the presence of alkaloids in corn grain due to contamination from inadequate agricultural practices such as co-harvesting with other plants (Torović et al., 2023). The absence of alkaloids in the potato extract could have been favored by the elimination of the tuber peel before grinding. Unlike potato leaves, the tuber usually accumulates glycoalkaloids in smaller quantities, being significantly reduced by peeling, since the peel contains the major part of glycoalkaloids present in the tuber (Zarins & Krūma, 2017). The absence of alkaloids in lupin could indicate an effective prior

aqueous debittering process, which may have reduced quinolizidine alkaloids to values below the safe limit for human consumption (200 mg/kg of lupin seed) (Cortés-Avendaño et al., 2020).

In the present study, the Dragendorff, Mayer, Bertrand, and Sonnenschein analyses of corn, potato, and lupin extracts consistently revealed the absence ('-') of alkaloids. This finding is crucial for the continuity of the present study as it helps demonstrate the unlikelihood of these flours causing alkaloid intoxication.

#### **pH and total titratable acidity (TTA)**

The pH measures the concentration of free hydrogen ions, while the titratable acidity includes both bound and free hydrogen ions, expressed as a specific acid (USDA, 2015). The pH ranged from 5.89 to 6.10, indicating a slightly acidic nature of the flours, while the TTA ranged from 1.04 to 2.25 mL eq NaOH/ 100g dry sample (Table 1). Similar pH results have been reported for corn by Tambo Tene et al. (2019), for potato by Osei Tutu et al. (2024), and for lupin by Maray (2023). Moreover, similar TTA results have been reported for other flours derived from roots and cereals (Tambo Tene et al., 2019).

The primary concern in flours is storage, such as inadequate moisture and temperature, leading to hydrolysis of compounds and impacting the acidity and pH of the flour. A lower pH and higher titratable acidity than typical values for a specific flour may be indicators of deterioration, affecting the final product quality (Sruthi & Rao, 2021). Thus, the pH and TTA of the flours in this study indicate that they are well preserved.

### **4.2. Physico-chemical properties**

#### **Proximate composition**

The proximate composition was significantly different ( $p < 0.05$ ) among the flours (Table 1). Lupin flour had the highest crude protein (44.93%,  $N = 5.71$ ), crude fat (22.20%), and dietary fiber (29.01%). Higher protein levels (54.44–57.5%,  $N = 6.25$ ) and similar fat content (16.60–24.81%) were reported for whole debittered lupin flour in other studies (Córdova-Ramos et al., 2020; Carvajal-Larenas et al., 2016). Variations in lupin protein content are primarily due to different conversion factors used, with debittering techniques also potentially affecting the results. Processing time and changes of water per day can influence the protein content of lupin (Carvajal-Larenas et al., 2016). The protein contents of corn ( $N = 6.25$ ) and potato flours ( $N = 6.24$ ) were below 10%, similar to previous reports (7.10% and 8.30–9.68%, respectively) (Gwirtz & García-Casal, 2013; Wang et al., 2020). In general, legumes are richer in protein than other crops, since nitrogen-fixing bacteria live in the nodules of legume roots (Çakir et al., 2019). Both potato and corn flours had low fat content ( $< 1.00\%$ ), similar to results reported

by Leivas et al. (2013) for potato flour (0.31–0.34%), and by Prasanthi et al. (2017) for corn flour (1.25%).

Dietary fiber in lupin flour was slightly lower than that reported for the other lupin species (32.62–42.96%) (Mazumder et al., 2021). *L. mutabilis* usually has higher protein and fat content compared to *L. albus*, *L. luteus*, and *L. angustifolius* (Czubinski et al., 2021), which results in lower fiber content. The dietary fiber in the potato flour was closer to the result for whole potatoes (8.50%) than for peeled potatoes (7.50%) reported by Mullin & Smith (1991). Approximately 50% of potato skin is dietary fiber (Susarla et al., 2019), and the irregular form of Andean potatoes can make thorough peeling difficult, leaving some skin stuck in the dimples of the tuber flesh. The result of dietary fiber for the corn flour was consistent with an earlier report by Prasanthi et al. (2017). Potato flour had a higher ash content (2.91%) compared to the other flours and was also higher than the value reported by Wibowo et al. (2019) for high-temperature blanched potato flour (1.33%). This may be attributed to lower refinement and higher mineral content, making the potato flour appear coarser and less uniform than the others, with some dark specks probably from peel residues in the flesh. The ash content in flours could increase due to the presence of the non-endosperm parts, indicating lower technological quality but higher nutritional value. Similar results of ash content were found in literature for corn flour (Gwirtz & García-Casal, 2013) and lupin flour (Córdova-Ramos et al., 2020).

Digestible carbohydrates content in lupin flour was very low (2.16%). Lupin carbohydrates primarily consist of non-starch polysaccharides (dietary fiber), with a small amount of sugar and negligible starch. As a non-starch legume, the oligosaccharides constitute almost all the carbohydrates other than fiber (Czubinski et al., 2021). On the other hand, the digestible carbohydrates in potato and corn flours were higher than the other nutrients of their composition. Approximately 65-75% of a potato tuber is starch, while corn grain contains around 64-78% starch (Leivas et al., 2013; Eckhoff & Watson, 2009). The digestible carbohydrates content of the flours was in agreement with the results of other researchers for lupin (4.1%), potato (80.7%) and corn (88.5%) (Czubinski et al., 2021; Nascimento & Canteri, 2018; Prasanthi et al., 2017).

The results for chemical composition were calculated without considering the moisture content of the flours, which was below 14%, the maximum level commonly recommended in technical specifications to ensure stability of flours during storage.

**Table 1.** pH, total titratable acidity and proximate composition of the flours

Analysis	Corn flour	Potato flour	Lupin flour
pH	6.10 ± 0.05 <sup>a</sup>	6.00 ± 0.06 <sup>a,b</sup>	5.89 ± 0.05 <sup>b</sup>
TTA <sup>1</sup>	1.04 ± 0.13 <sup>c</sup>	2.25 ± 0.13 <sup>a</sup>	1.51 ± 0.12 <sup>b</sup>
Crude protein <sup>2</sup>	7.08 ± 0.06 <sup>c</sup>	8.75 ± 0.11 <sup>b</sup>	44.93 ± 0.05 <sup>a</sup>
Ash <sup>2</sup>	0.45 ± 0.02 <sup>c</sup>	2.91 ± 0.01 <sup>a</sup>	1.71 ± 0.00 <sup>b</sup>
Crude fat <sup>2</sup>	0.90 ± 0.04 <sup>b</sup>	0.28 ± 0.02 <sup>c</sup>	22.20 ± 0.10 <sup>a</sup>
Dietary fiber <sup>2</sup>	4.21 ± 0.00 <sup>c</sup>	8.22 ± 0.00 <sup>b</sup>	29.01 ± 0.00 <sup>a</sup>
Digestible carbohydrates <sup>2,3</sup>	87.35	79.83	2.16

Results are presented as mean ± SD (n = 3), except for digestible carbohydrates. Means not sharing the same letter within a row are significantly different by Tukey test (p < 0.05).

<sup>1</sup> Total titratable acidity, results are expressed as mL eq NaOH/100 g dry matter.

<sup>2</sup> Results are expressed on a dry basis, considering the experimental moisture of the flours (%): corn (11.64 ± 0.04), potato (10.09 ± 0.01), and lupin (5.00 ± 0.03).

<sup>3</sup> Results are calculated by difference.

### Amino acid profile

The amino acid composition and quality of the flours are shown in Table 2. Lupin flour had the highest amino acid content (47.66%), while corn flour had the lowest (5.55%). The amino acid analysis, recommended method to determine true protein (FAO, 2003), revealed that the Kjeldahl method overestimated the protein content in corn and potato flours, while underestimated it in lupin flour. Non-protein nitrogen compounds and a high degree of protein amidation are nitrogen sources that can influence the protein multiplier (Czubinski et al., 2021).

Among the essential amino acids, leucine was predominant in corn and lupin flours, while lysine stood out in potato and lupin flours, whereas the lowest value was for cysteine in all flours. Among the nonessential amino acids, glutamic and aspartic acids were the most abundant, while the lowest concentrations were observed for glycine and alanine. Compared to the protein reference from the FAO (2013), LAA of the tested proteins was lysine in corn flour, leucine in potato flour, and methionine + cysteine in lupin flour, with EAA scores ranging from 55.27%, to 86.86% (scores greater than 100% were recorded for the remaining essential amino acids in the tested proteins). Similar amino acid

composition and the same LAA, with small variations in scores, was found by other authors in corn flour (Li et al., 2022), potato flour (Bártová et al., 2015), and lupin flour (Grela et al., 2017). Lysine is typically low in cereal-based diets but is found in higher quantities in legumes and most tubers, whereas tubers and legumes are limited in sulfur-containing amino acids (methionine and cysteine) (Chandrasekara, 2018; Stone et al., 2019). Since each tested protein is limited by a different essential amino acid, they may complement each other well, helping to prevent some amino acid deficiencies.

The EAA/NEAA ratio of the proteins ranged from 0.58 to 0.79, with the lowest observed in potato flour and the highest in corn flour. These values indicate that the EAAs were in lower concentrations compared to NEAAs in all proteins. However, corn protein revealed the highest concentration in the amount of EAAs. Although lupin had the highest amino acid content, its EAA/NEAA ratio was not higher than corn protein due to its higher content of non-essential amino acids, mainly in glutamic acid, aspartic acid, and arginine, reported in literature as the most abundant in oilseed flours (Miedzianka et al., 2021).

**Table 2.** Amino acid profile of the flours

<b>Amino acid</b>	<b>Corn flour</b>	<b>Potato flour</b>	<b>Lupin flour</b>	<b>FAO*</b>
<i>Total</i> <sup>1</sup>	5.55	7.77	47.66	
<i>Non-essential</i> <sup>2</sup>				
Aspartic acid	6.73	25.21	10.03	-
Glutamic acid	18.98	14.04	22.44	-
Serine	4.69	3.44	5.59	-
Glycine	3.67	3.15	4.28	-
Arginine	3.88	9.74	9.83	-
Alanine	7.55	3.72	3.75	-
Proline	10.20	4.01	4.06	-
<i>Essential</i> <sup>2</sup>				
Histidine	3.27	2.15	2.78	1.60
Isoleucine	3.67	3.29	4.86	3.00
Leucine	12.65	5.30	7.46	6.10
Lysine	2.65	6.01	6.12	4.80
Methionine	1.43	1.47	0.64	-
Cysteine	1.02	0.86	1.33	-
Methionine + cysteine	-	-	-	2.30
Phenylalanine	4.90	4.15	3.95	-
Tyrosine	2.86	3.15	3.80	-
Phenylalanine + tyrosine	-	-	-	4.10
Threonine	3.47	3.29	3.82	2.50
Tryptophan	3.47	2.72	1.08	0.66
Valine	4.90	4.30	4.17	4.00
<b>LAA [EAA score (%)]</b>	Lys [55.27]	Leu [86.86]	Meth+cys [85.46]	
<b>EAA/NEAA</b>	0.79	0.58	0.67	

\* Reference protein (standard) for an adult human (FAO, 2013).

<sup>1</sup> Results are expressed in g /100 g flour.

<sup>2</sup> Results are expressed in g /16 g N, equivalent to g /100 g protein.

LAA: limiting amino acid, EAA: essential amino acid, NEAA: non-essential amino acid

### Mineral characterization

Significant differences ( $p < 0.05$ ) were observed for each mineral among the flours analyzed qualitatively using EDS-SEM, except for those identified in only one type of flour (Table 3). Macroelements such as potassium (*K*) predominated in corn and potato flours, while phosphorus (*P*) was more abundant in lupin and corn flours. Unlike the other flours, calcium (*Ca*) was identified only in lupin at a similar level than *P*, while sodium (*Na*) and sulfur (*S*) were found only in potato. Magnesium (*Mg*) was present in all flours to a lesser extent than *K* and *P*, standing out in corn flour. Regarding microelements, zinc (*Zn*) and iron (*Fe*) were identified in lupin and corn flours, and chlorine (*Cl*) exclusively in potato flour. Similarly, Shakpo & Osundahunsi (2016) identified *K* and *P* as the main macroelements in corn flour, while *Zn* and *Fe* were detected as microelements. In potato flour, Buzera et al. (2023) also found that *K* was the most abundant ion, followed by *Mg* and *P*. In lupin flour, *P*, *Ca*, *Fe*, and *Zn* were also highlighted by Roman et al. (2023), along with *K*, which was not emphasized in this study. Variations in the predominance of minerals could be related to pretreatments that can influence the partial leaching of certain minerals (Carvajal-Larenas et al., 2016).

The presence of *P*, mainly in corn and lupin flours, suggests the presence of phytic acid. The absorption of *Fe*, *Zn*, *Mg*, and *Ca* is inhibited by phytic acid, found mainly in cereals, pulses and oilseeds. However, potatoes have very low levels of phytates and a significant amount of ascorbic acid, which promotes the intestinal absorption of minerals, particularly *Fe* (Joshi et al., 2021). Although ascorbic acid could be partially reduced during thermal processing, a significant concentration remains after potato treatment (Kusur et al., 2020). Thus, the ascorbic acid from potato flour may help promote the intestinal absorption of *Fe* from corn and lupin flours when combined (since in potato was not detected), counteracting the inhibitory effects of compounds such as phytic acid. The presence of *S* and *Cl* in potato flour may partially reflect the fertilizers used during the tuber growth. As Roman et al. (2023) mentioned, *S* in adequate concentrations in soil is generally absorbed as sulfate, which is further reduced to sulfite and contributes to the amino acid synthesis of cysteine and methionine.



**Table 3.** Mineral characterization of the flours by EDS-MEV




<b>Element (%)</b>	<b>Corn flour</b>	<b>Potato flour</b>	<b>Lupin flour</b>
Potassium	37.25 ± 0.96 <sup>b</sup>	40.98 ± 1.08 <sup>a</sup>	0.51 ± 0.09 <sup>c</sup>
Phosphorus	19.46 ± 0.36 <sup>b</sup>	7.37 ± 0.32 <sup>c</sup>	25.05 ± 0.18 <sup>a</sup>
Magnesium	6.10 ± 0.34 <sup>a</sup>	4.38 ± 0.12 <sup>b</sup>	2.91 ± 0.12 <sup>c</sup>
Sodium	N.D	6.84 ± 0.65	N.D
Calcium	N.D	N.D	25.81 ± 0.50
Zinc	0.16 ± 0.28 <sup>NS</sup>	N.D	0.22 ± 0.38 <sup>NS</sup>
Iron	0.16 ± 0.27 <sup>NS</sup>	N.D	0.54 ± 0.21 <sup>NS</sup>
Chlorine	N.D	4.93 ± 0.69	N.D
Sulfur	N.D	4.93 ± 0.28	N.D
Oxygen*	(36.87 ± 0.39)	(30.55 ± 0.62)	(44.96 ± 0.09)

N.D: not detected (missing values or below the detection limit). Results are presented as mean ± SD (n = 3), and expressed as a percentage of the total elemental composition (100%). \*Element that forms complexes with the minerals and is part of the total elemental composition. Means not sharing the same letter within a row are significantly different by Tukey test ( $p < 0.05$ ,  $N = 3$ ) or by Student's t-test ( $p < 0.05$ ,  $N = 2$ ), where  $N$  represents the number of types of flours being compared. N.S: not significant.

### Color

Table 4 displays the color of the flours, both visually and through instrumental measurement. Different shades of yellow were visually observed in the flours, ranging from cream for lupin, pale yellow for corn to a more intense yellow for potato. The parameters of color using the CIELAB system were significantly different ( $p < 0.05$ ) among the flours. All flours exhibited a clear appearance, redness (+a\*) and yellowness (+b\*). The superior lightness of corn and lupin ( $L^* > 85$ ) makes them suitable for moderate addition to food formulations without significantly altering the final color. The yellowness (+b\*) of potato was the highest, possibly due to prolonged boiling or steaming as a pretreatment for flour preparation and/or the presence of xanthophylls, a carotenoid found in dark yellow, light yellow and white-fleshed potatoes (Buzera et al., 2022; Lachman et al., 2016). Since the color of flour is crucial for visual appeal, the color of these flours is suited to bakery and pasta products, ensuring consumer acceptance.

**Table 4.** Color representations of the flours

Color	Corn flour	Potato flour	Lupin flour
Visual			
CIELAB system			
L*	90.12 ± 0.03 <sup>a</sup>	80.62 ± 0.18 <sup>c</sup>	85.45 ± 0.09 <sup>b</sup>
a*	3.54 ± 0.03 <sup>a</sup>	0.43 ± 0.12 <sup>c</sup>	1.70 ± 0.05 <sup>b</sup>
b*	34.30 ± 0.18 <sup>b</sup>	41.26 ± 0.64 <sup>a</sup>	25.91 ± 0.12 <sup>c</sup>

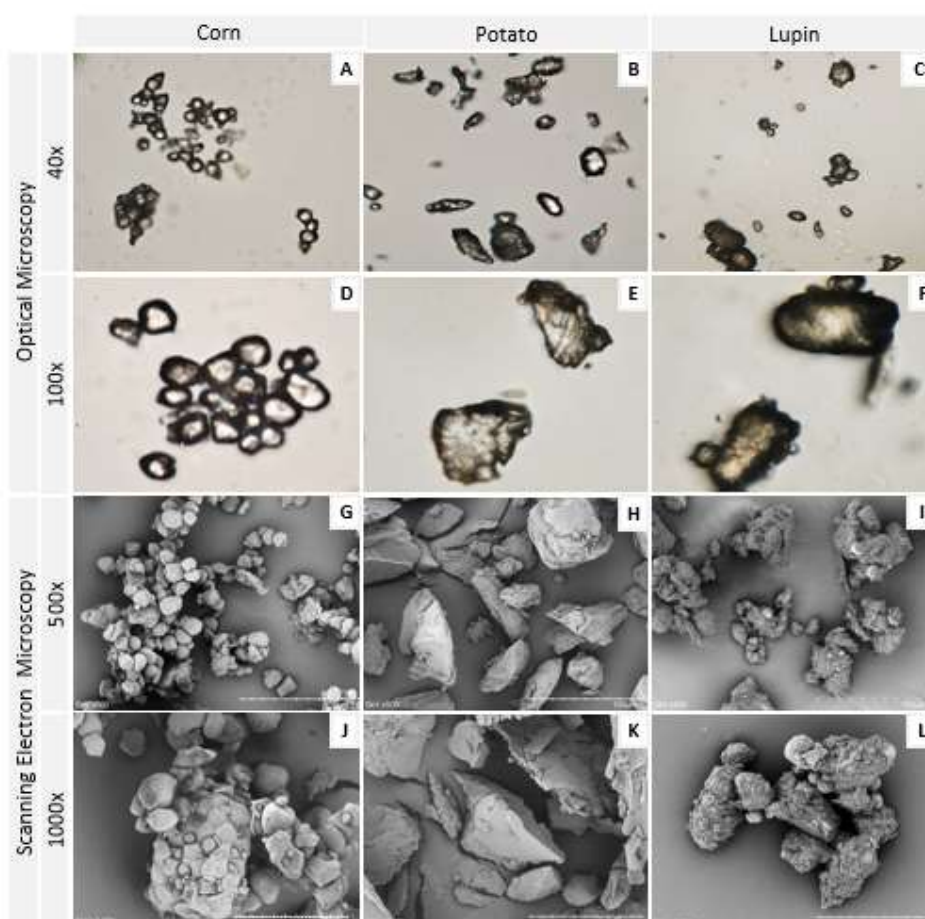
Results are presented as mean ± SD (n = 3). Means not sharing the same letter within a row are significantly different by Tukey test (p < 0.05).

### Morphology

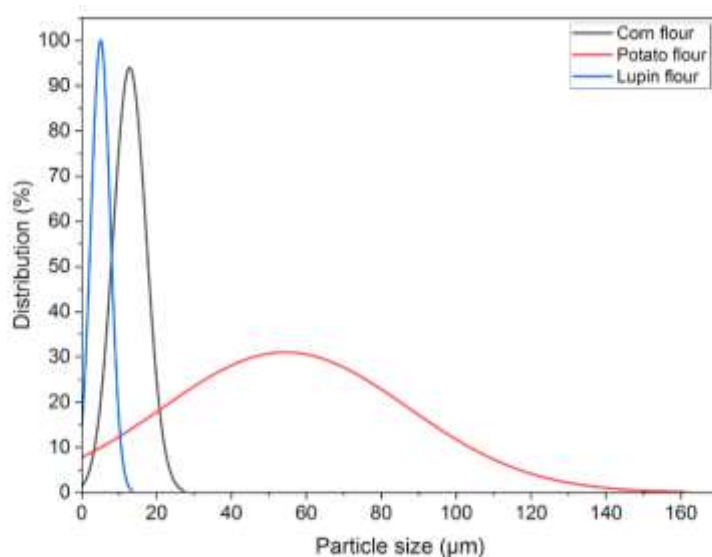
Fig. 1 displays images of the flours by optical microscopy (OM) and scanning electron microscopy (SEM). Corn flour exhibited medium-sized granules ( $12.77 \pm 4.46 \mu\text{m}$ ) with native starch, and both semi-crystalline and amorphous regions (Fig. 1A and 1G). Irregular-rounded and pear-shaped granules were observed by OM (Fig. 1D). SEM revealed closely clustered, branched granules, primarily polyhedral shapes with rounded corners (Fig 1J), indicating a semi-hard endosperm. These findings are consistent with micrographs from Cisse et al. (2013) for yellow ordinary corn. Corn granule morphology varies from the soft, spherical, and loosely packed granules of floury corn to the vitreous, polygonal, and tightly packed granules of hard corn (Xu et al., 2019).

Potato flour exhibited granules of irregular size ( $54.11 \pm 32.76 \mu\text{m}$ ) with pointed ends, flaky surfaces, and scarce native starch (Fig. 1B, 1E, 1H, 1K). This morphology differed from the oval-shaped granules reported for native potato flour by Wang et al. (2020), but was similar to those of pretreated potato flour reported by Buzera et al. (2022). This was likely due to the blanching pretreatment of the potato tuber used to inactivate enzymes and prevent microbial spoilage. Unlike low temperature blanching, high-temperature blanching ( $\sim 95^\circ\text{C}$ ) or boiling disrupts starch granules, which results in larger, asymmetrical granules (Buzera et al., 2022). In the present study, gelatinization of potato starch during heat exposure and its retrogradation during cooling and storage could have led to the rearrangement of soluble molecules into new structures resulting in heterogeneous particles after grinding.

Lupin flour showed small, globular granules ( $4.92 \pm 2.45 \mu\text{m}$ ) with a yellow-like content by MO (Fig. 1C, 1F). SEM revealed a highly dense, sponge-like matrix with amorphous protuberances (Fig. 1I, 1L). The globular bodies may be mostly protein aggregates held together by fatty acids and fiber fractions surrounding their membranes, since they are the main macromolecules in lupin composition. Similar morphologies were found in Australian lupins (Thambiraj et al., 2019). Lupin oil can form viscous and capillary connections promoting cohesion of individual powder particles (Pelgrom et al., 2014), which is explained by the agglomerates in lupin SEM images. Additionally, fibers particles have been reported as nearly indistinguishable with protein in granules of other legumes observed by SEM (Schlangen et al., 2022). Lupin flour had the narrowest particle size distribution ( $1.35\text{--}12.09 \mu\text{m}$ ), followed by corn ( $4.95\text{--}24.90 \mu\text{m}$ ) and potato ( $12.01\text{--}158.97 \mu\text{m}$ ), based on particle dimensions from SEM images (Fig. 2), indicating high homogeneity in lupin but wide dispersion in potato particles. Combining the flours in low-moisture products may lead to partial separation of potato granules.



**Fig. 1.** Micrographs of the flours. Corn (left: A, D, G, J), potato (middle: B, E, H, K) and lupin (right: C, F, I, L) using OM at 40x and 100x, and SEM at 500x and 1000x.



**Fig. 2.** Particle size distribution of flours analyzed by SEM

#### **4.3. Techno-functional properties**

##### **Emulsifying activity (EA) and emulsion stability (ES)**

EA and ES of the flours ranged from 4.42 to 18.34%, and from 5.93 to 57.72%, with lupin flour having the highest values, significantly different ( $p < 0.05$ ) from the others (Table 5). Mazumder et al. (2021) reported slightly higher EA (19.36–25.94%) but lower ES (20.76–43.41%) for Australian lupins, using canola oil in the analyses. These differences might be due to the type of oil and lupin variety used for the emulsifying analysis (Nawaz et al., 2021). Compared to the other flours, lupin formed a thicker emulsified layer in EA and resisted high temperatures (80°C) in ES. Its superior emulsifying properties are likely due to its higher crude protein and dietary fiber content. The amount and type of protein, due to their amphiphilic nature, are crucial for forming emulsions and decreasing surface tension between droplets, thereby maintaining emulsion stability (Raikos et al., 2014). Emulsion systems containing lupin are stabilized by the proteins and fiber components that are adsorbed on the oil-water interface, providing a layer with electrostatic and/or steric repulsive forces between emulsion droplets (Arzami et al., 2022).

Emulsifying properties of corn flour were different from those reported by Shakpo & Osundahunsi (2016) for cooked whole corn flour, who found higher EA (8.76%) and lower ES (7.43%). The absence of corn germ in the present study resulted in lower fat and protein, affecting EA. Hydroxyl groups in fat enhance emulsified layer formation by interacting with protein hydrophobic bonds (Li et al., 2024). Differences in ES may be associated with the nature of the flours, since raw corn was used in

the present study. The pretreated potato flour showed notably lower emulsifying properties compared to raw potatoes from the study of Ndungutse et al. (2019). Decreases in emulsifying properties of some flours as a result of heat treatments were also reported by Hasmadi et al. (2020).

#### **Water solubility (WSI), water absorption index (WAI), and oil absorption index (OAI)**

Potato flour exhibited significantly higher WAI and WSI compared to the other flours, absorbing about five times its weight in water and releasing 13.21% particles (Table 5). Lower WAI values were reported by Ndungutse et al. (2019) for raw potato flour (2.65–3.00 g/g), but similar results were found by Yadav et al. (2006) for thermally pretreated potato flour at 25°C. Pretreatment modified potato starch resulting in heterogeneous particle sizes, which could influence the water absorption rate. Modified starches have a higher water-holding capacity, more swollen granules and solubility than native ones (Gerçekaslan, 2021). Regarding corn and lupin flours, similar WAI values (2.35 g/g and 2.06 g/g) were found by Oladapo et al. (2017) and Maray (2023), respectively. WAI of lupin flour could be more related to its soluble dietary fibers and ratio of hydrophilic/hydrophobic amino acids, whereas WAI of corn flour is likely influenced by the characteristics of its native starch granules, mainly its amorphous and crystalline domains. Unlike other legumes where starch is mainly responsible for retaining water and thickening, in suspensions containing lupin it is attributed to pectin (Arzami et al., 2022). Lupin debittering can denature proteins, enhancing hydrophilicity and, therefore, water absorption (Sathe et al., 1982).

OAI ranged from 0.96 to 1.34 g/g, with only a small difference between potato and corn flours (Table 5). Similar OAI was reported by Ndungutse et al. (2019) for raw potato flour (0.99–1.93 g/g), Oladapo et al. (2017) for yellow corn flour (0.66 g/g), and by Maray (2023) for *L. albus* (1.64 g/g). OAI is related to the binding of fat by non-polar amino acid side chains, depending on its hydrophobic interactions (Hasmadi et al., 2020). All flours showed capacity to interact with lipids in formulations involving fat additions.

#### **Swelling capacity (SC)**

Potato flour exhibited significantly higher SC compared to the other flours, swelling to about eleven times its weight at 60°C, while corn and lupin flours swelled to about four times their weight (Table 5). Similar results were described by Cisse et al. (2013) for yellow corn (3–6 g/g), by Mazumder et al. (2021) for Australian lupin (4.0 g/g), and by Yadav et al. (2006) for pretreated potato flour (~12 g/g). There was no significant difference in SC between lupin and corn flours. The crystalline arrangement and degree of hydrogen bonding of the granules determine swelling (Nawaz et al., 2015). The low digestible carbohydrates content in lupin flour means that non-polysaccharides, rather than

starch, primarily contribute to granule swelling. However, the hydrophobic interactions of lipids in lupin can inhibit swelling (Devkota et al., 2024). In corn and potato granules, solubilized amylose could have leached during swelling, altering the amylose/amylopectin ratio and favoring water absorption. Starch swelling power is favored by the branched amylopectin, which facilitates water penetration, in contrast to the linear and tightly packed amylose, which tends to inhibit swelling (Lin et al., 2023).

### **Foaming capacity (FC) and foam stability (FS)**

Potato flour exhibited the highest FC among the flours, though its foam stability (FS) was lower compared to lupin and corn flours (Table 5). Potato flour foam had fewer, larger bubbles with thinner circumferences, resulting in higher foam height but with bubbles collapsing faster. Lupin and corn flours formed thicker foams with smaller bubbles, which were more stable over time. Larger bubbles are surrounded by thinner protein films that are more prone to collapse, thus losing foam stability (Chandra et al., 2015). FC results were in accordance with other authors for corn flour (Nawaz et al., 2015), pre-treated potato (Vaishali et al., 2020), and lupin flour (Monteiro et al., 2020). Similarly, FS values above 50% were consistent with findings for lupin (Kamran et al., 2021) and corn flours (Nawaz et al., 2015).

The higher FC in potato flour may be attributed to its larger bubbles, which provide a greater foam volume, however, heat-treatment could have induced denaturation of potato protein and reduced protein diffusion to the air-water interface, explaining the weak behavior of potato flour to keep foam over time. FC depends on the surface-active properties of its protein, while FS is linked to the amount of native protein and fiber (Hasmadi et al., 2020). Better FS obtained for corn and lupin flours could be related to the structure of its native protein and its quality in corn flour and to the surface hydrophilic interactions between high amounts of polypeptides and dietary fibers in lupin, when compared to potato flour. Higher FC was reported by defatted lupin (Devkota et al., 2024), but similar FS for other legumes (Badia-Olmos et al., 2023). The debittering process and fat content in lupin could have contributed to a lower FC compared to literature, probably due to partial unfolding of proteins upon alkaloids removal and the competitive effect of lipids in the interface (Carvajal-Larenas et al., 2016). In corn flour, its protein native structure and good quality of its essential amino acids may have contributed to FS.

**Table 5.** Techno-functional properties of the flours

Properties	Corn flour	Potato flour	Lupin flour
Emulsifying activity (%)	4.42 ± 0.54 <sup>c</sup>	9.00 ± 0.60 <sup>b</sup>	18.34 ± 0.87 <sup>a</sup>
Emulsion stability (%)	23.91 ± 4.77 <sup>b</sup>	5.93 ± 1.78 <sup>c</sup>	57.72 ± 5.55 <sup>a</sup>
Water absorption index	1.93 ± 0.05 <sup>c</sup>	4.76 ± 0.12 <sup>a</sup>	2.39 ± 0.03 <sup>b</sup>
Water solubility index (%)	3.52 ± 0.15 <sup>c</sup>	13.21 ± 0.26 <sup>a</sup>	7.56 ± 0.05 <sup>b</sup>
Oil absorption index	0.96 ± 0.04 <sup>b</sup>	1.34 ± 0.07 <sup>a</sup>	1.14 ± 0.18 <sup>ab</sup>
Swelling capacity	4.11 ± 0.16 <sup>b</sup>	11.12 ± 0.42 <sup>a</sup>	4.49 ± 0.01 <sup>b</sup>
Foaming capacity (%)	1.73 ± 0.06 <sup>c</sup>	5.14 ± 0.46 <sup>a</sup>	3.33 ± 0.26 <sup>b</sup>
Foam stability (%)	63.67 ± 7.46 <sup>a</sup>	33.47 ± 10.02 <sup>b</sup>	65.36 ± 6.70 <sup>a</sup>

Results are presented as mean ± SD (n = 3). Means not sharing the same letter within a row are significantly different by Tukey test (p < 0.05).

#### 4.4. Rheological properties

##### Pasting properties of the flours

The RVA results indicated that corn and potato flours showed a gradual increase in viscosity with rising temperature, while lupin flour did not form paste (Fig. 3). Potato flour had significantly lower (< 0.05) pasting temperature, breakdown, setback, and final viscosities, a similar peak viscosity, and a significantly higher (p < 0.05) trough viscosity compared to corn flour. The findings for each flour align with previous studies on pre-blanching potato flour (Buzera et al., 2023) and corn starches (Moses & Olanrewaju, 2018).

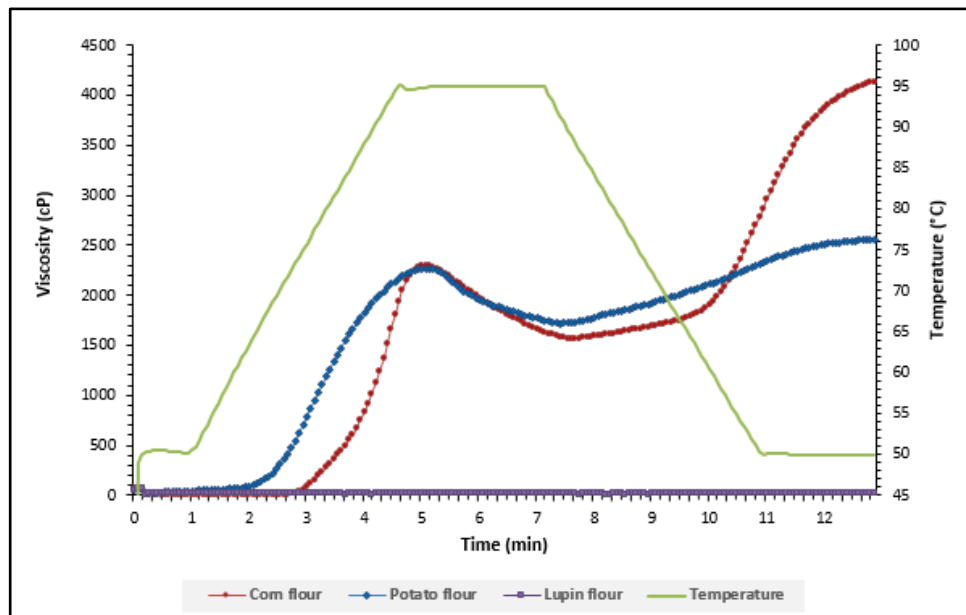
During gelatinization, starch granules swell and break, releasing amylose and forming a paste. The low viscosity in high-protein flours is explained by the competition between proteins and starch granules for water (Badia-Olmos et al., 2023). Also, the initial water absorption or hydration can be inhibited by fat and favored by fibers (Devkota et al., 2024). The low digestible carbohydrates (with minimal starch) and high lipid content in lupin flour made its granules difficult to swell and form a paste. In contrast, Mazumder et al. (2021) reported formation of weak pastes and gels in *L. angustifolius* and *L. albus*, which were characterized by having lower fat (4.4–11.9%), similar protein (41.5–48.2%), and higher fiber (32.6–42.96%) content compared to the values found in the present study for lupin flour. We noticed that the lupin flour concentration (12%, w/v) used in the RVA analysis was below the minimum required to form a gel, which is 14% (w/v) for *L. mutabilis* flour (Sathe et al., 1982). The gel-forming ability of lupin flour should be studied by measuring its 'least gelation concentration', a method

suitable for high-protein flours that determines the minimum quantity needed to form a gel in a given volume of water based on its protein concentration (Kamran et al., 2021). Compared to corn flour, potato flour has a less compact structure, allowing its starch to develop viscosity and begin to gelatinize at a lower pasting temperature and shorter time. Native potato starch has less crystallinity than native corn starch (Mishra & Rai, 2006). It is more notable in this study when working with retrograded potato starch, composed of realigned amylopectin and amylose chains.

Peak viscosity, or maximum viscosity in the heating phase, indicates the most swollen state of the hot granules, yet still intact (Mishra & Rai, 2006). Potato starch began to gelatinize earlier than corn flour but both flours reached similar peak viscosities simultaneously (5.2 min), because corn flour absorbed water and swelled faster once gelatinization started. According to Yadav et al. (2006), native flour hydrates rapidly above 60°C due to gelatinization, unlike the slower swelling in pretreated flour, possibly due to its retrograded starch. The similar peak viscosity of corn and potato flours indicates equivalent thickening capacities of their fully hydrated granules. In potato flour, this can result from the phosphate groups in the starch, which are closely associated with the flour's pasting properties. The covalent bonding between hydroxyl groups and phosphorus facilitates cross-linking, thereby impacting viscosity (Buzera et al., 2023).

The trough, minimum viscosity after reaching peak viscosity, and breakdown, difference between trough and peak viscosity, reflect the stability of the swollen granules under constant high temperature and shear, involving melting of starch crystalline regions, leaching of soluble polymers and water expulsion (Lin et al., 2023). Potato paste showed a smaller and slower drop in viscosity than corn flour, suggesting higher stability at 95°C, probably because its retrograded granules were in a recrystallized structure before thermal contact, disintegrating less at 95°C. Setback viscosity indicates the retrogradation tendency of the hot paste as it cools, until it reaches the final viscosity. In the cooling stage, viscosity rises again as the soluble amylose retrograde, forming gelatinized starch (Buzera et al., 2023). Potato flour had a reduced tendency to retrograde when cooled, possibly due to a lower amylose content available for retrogradation. This could be attributed to its previously retrograded starch, formed after the leaching and reassociation of amylose. Corn flour ended up with a more intense retrogradation, which was reflected in a more rigid gel structure, directly dependent on a possible better proportion of amylose content, forming more binding sites during the cooling stage.





**Fig. 3.** Pasting profile of the flours by RVA

### Texture properties of the gels

The texture of the gels after storage results from remnant granules, and re-association of leached starch and unfolded proteins (Badia-Olmos et al., 2023). Corn flour paste formed a gel with significantly higher ( $p < 0.05$ ) hardness and adhesiveness than the potato flour paste (Table 5). The higher hardness of the corn gel is attributed to greater starch retrogradation during RVA analysis, reflected in higher setback and final viscosity. Interactions of phosphorus with amylose can also impact rheological properties in starchy food, causing a rapid retrogradation, strengthening the molecular chain, and forming a more ordered and rigid gel structure (Lu et al., 2012). The prevalence of specific proteins in corn and potato flours could also impact on gel hardness, such as globulin (patatin) in potato and prolamin (zein) in corn. According to Lin et al. (2024), globulins cause more water retention, reducing viscosity and retrogradation, and resulting in a less compact gel. The lower adhesiveness of the potato gel is linked to its softer consistency and less sticky mouthfeel. Stickiness involves a combination of internal adhesive and cohesive forces influenced by surface tension and viscoelastic properties (Noren et al., 2019).

**Table 6.** Rheological properties of the flours

Parameters	Corn flour	Potato flour	Lupin flour
<b>Pasting parameters<sup>1</sup></b>			
Pasting temperature (°C)	74.3 ± 0.1 <sup>a</sup>	64.8 ± 0.5 <sup>b</sup>	N.A
Peak time (min)	5.2 ± 0.1 <sup>NS</sup>	5.2 ± 0.1 <sup>NS</sup>	N.A
Peak viscosity (cP)	2299 ± 26.7 <sup>NS</sup>	2264.3 ± 6.7 <sup>NS</sup>	N.A
Trough (cP)	1567.3 ± 11.9 <sup>b</sup>	1715.7 ± 26.6 <sup>a</sup>	N.A
Breakdown (cP)	731.7 ± 14.7 <sup>a</sup>	548.7 ± 25 <sup>b</sup>	N.A
Setback (cP)	2559.3 ± 26.4 <sup>a</sup>	839.3 ± 9.2 <sup>b</sup>	N.A
Final viscosity (cP)	4126.7 ± 28.1 <sup>a</sup>	2555 ± 17.4 <sup>b</sup>	N.A
<b>Texture parameters<sup>2</sup></b>			
Hardness (N)	1.23 ± 0.07 <sup>a</sup>	0.62 ± 0.03 <sup>b</sup>	N.A
Adhesiveness (N.s)	3.76 ± 0.69 <sup>a</sup>	2.28 ± 0.23 <sup>b</sup>	N.A

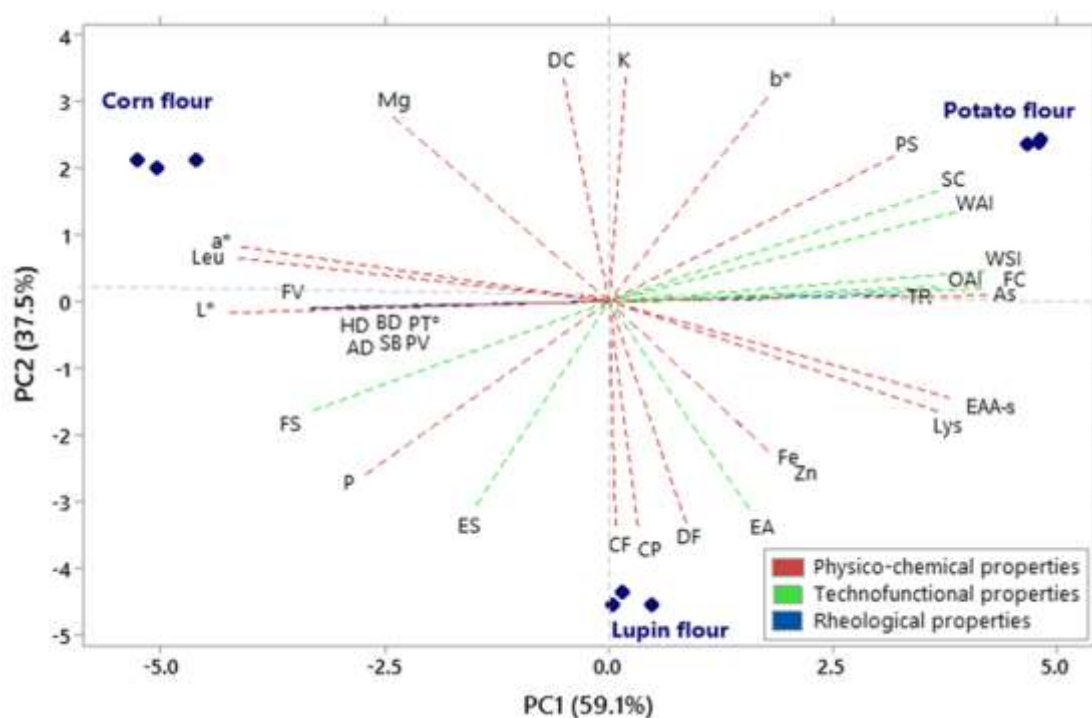
N.A: not available. Results are presented as mean ± SD (n = 3). Means not sharing the same letter within a row are significantly different by Tukey test (p < 0.05, N = 3) or by Student's t-test (p < 0.05, N = 2), where N represents the number of types of flours being compared. N.S: not significant.<sup>1</sup> Results correspond to the flour pastes. <sup>2</sup> Results correspond to the flour gels (after retrogradation).

### Principal Component Analysis (PCA)

PCA was used to reduce the dimensionality of the data (34 variables), and evaluate which properties significantly impacted the differences and similarities among the flours. Fig. 4 displays the plot of the first two principal components (PC1 = 59.1%, PC2 = 37.5%) that explains the majority of the total variance (96.6%). PC1 is mainly determined by L\*, ash, foaming capacity, water solubility index, leucine, a\*, water absorption index, swelling capacity, essential amino acid score, lysine, essential amino acid index and foaming stability, while PC2 is primarily associated with fat, protein, dietary fiber, emulsion stability, emulsifying activity, potassium, digestible carbohydrates, b\*, and magnesium. Corn and potato flours are more correlated with PC1, while lupin flour is more correlated with PC2. Only variables with data for at least two flours were included in the PCA (excluding Ca, Na, Cl, and S) to maintain precision in variable plotting. The farther a variable is from the origin, the better is its representation on the plot. Rheological properties, iron and zinc were obtained for only two flours,

resulting in shorter vectors closer to the origin, indicating that they are less well represented than the other variables.

As shown in the plot, the flours are located in different quadrants and are distant from each other, indicating that they have different relevant properties. Corn flour is mainly characterized by having more leucine,  $a^*$ , magnesium, and digestible carbohydrates but less lysine, essential amino acid score, emulsifying activity, dietary fiber, and protein. Potato flour is mostly distinguished by having more potassium,  $+b^*$ , particle size, swelling capacity, water absorption, water solubility, oil absorption, foaming capacity, and ash, but less emulsion stability, foaming stability, phosphorus, and  $L^*$ . Lupin flour is primarily defined by its fat, protein, dietary fiber, and emulsifying activity but has lower digestible carbohydrates, and magnesium. Different physico-chemical properties influenced a particular technological and rheological property in the flours due to the different nature and preprocessing of the flours, as their properties do not follow a specific pattern to all flours, but for each flour when compared with another similar one in the literature. The specific properties of each flour should be considered when selecting them for food applications.



**Fig. 4.** PCA plot of variables related to the physico-chemical, techno-functional and rheological properties of the flours. CP: protein, Ash: ash, CF: crud fat, DC: digestible carbohydrates, DF: dietary

fiber, EAA-s: essential amino acid score, Lys: lysine, Leu: leucine, L\*: lightness, +a\*: redness, +b\*: yellowness, P: phosphorus, K: potassium, Mg: magnesium, Fe: iron, Zn: zinc, PS: particle size, EA: emulsifying activity, ES: emulsifying stability, WAI: water absorption index, OAI: oil absorption index, WSI: water solubility index, SC: swelling capacity, FC: foaming capacity, FS: foaming stability, PT°: pasting temperature, PV: peak viscosity, BD: breakdown, TR: trough, SB: setback, FV: final viscosity, HD: hardness, AD: adhesiveness.

## 5. Conclusion

The research highlights the properties of corn, potato, and lupin flours, suggesting they have complementary attributes. The flours have light colors with yellowish tones and different amino acids and minerals. For potato flour, notable characteristics include non-uniform particles, retrograded starch, low levels of emulsion and foam stabilities, but high-water absorption, swelling, and release of solids into water. For corn flour, its native starch, high essential amino acids and good foam stability related to its protein nature are emphasized. For lupin flour, remarkable features such as good emulsifying properties and foam stability related to its high fiber and protein contents, as well as little capacity to form paste related to its low starch and high fat content. Potato and corn flours can act as good thickeners and stabilizers, as they form viscous pastes. Potato flour achieves this due to its ability to retain water, while corn flour, because of its greater retrogradation, forms firmer gels and can act as a better texture enhancer in bakery products. Lupin flour is suggested as a protein booster and potential emulsifier in products, including bakery, beverage and meat alternatives, and the high non-digestible carbohydrates content in lupin may make it a potential candidate for lowering the glycemic index. Hence, the physico-chemical, techno-functional, and rheological properties of potato and lupin flours from the Peruvian Andes are valuable for future research aimed at analyzing their potential blending with corn flour, focusing on the effects of flour proportions and their inclusion in special-purpose processed foods, including gluten-free products.

## 6. Acknowledgments

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## 7. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**CHAPTER 3 – Development of gluten-free corn pasta with lupin and potato flours from the Andes using a mixture design: Impact on physical, cooking, nutritional and sensory characteristics**

## ARTICLE II

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### **Development of gluten-free corn pasta with lupin and potato flours from the Andes using a mixture design: Impact on physical, cooking, nutritional and sensory characteristics**

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## Abstract

Pasta is a high-demand product, and alternative ingredients are being explored to create sustainable gluten-free pasta options. This study evaluated the impact of optimizing proportions of flint corn, *Yungay* potato, and Andean lupin on the physical, cooking, nutritional and sensory characteristics of gluten-free pasta. Twelve fusilli pasta formulations from corn (CF: 70-100%), potato (PF: 0-20%), and lupin (LF: 0-15%), monoglyceride (1%) and guar gum (1.5%) were developed using a D-optimal mixture design. Responses with well-fitted regression models included spiral distance, specks, deformation, optimal cooking time (OCT), and water absorption (WA) guided the multi-response optimization. Increasing LF increased spiral distance and deformation but reduced specks, OCT, and WA, while increasing PF and CF raised specks and OCT but reduced spiral distance and deformation. The effects were strongest for PF on specks and WA, LF on deformation, and CF on OCT. Optimum pastas, OP<sub>1</sub> (84.10% CF, 9.30% PF, 6.60% LF) and OP<sub>2</sub> (74.06% CF, 16.35% PF, 9.59% LF), demonstrated desired physical and cooking characteristics (intermediate spiral distance, low speck count, minimal deformation, minimal OCT, and intermediate WA). Additional analyses revealed OP<sub>1</sub> had lower cooking loss, both had similar hardness and displayed yellowish hues. Microstructures showed a compact starch-protein matrix in the dry pastas, which swelled and formed gelatinized starch and coagulated protein upon cooking. Acidity levels were not concerning, and moisture levels (<13%) met quality standards. OP<sub>2</sub> had higher protein, ash, fat, dietary fiber, and energy content, whereas OP<sub>1</sub> contained more carbohydrates. Both pastas were primarily limited by methionine+cysteine and contained prevalent minerals like potassium, phosphorus, calcium, magnesium and iron. Sensory evaluation showed both pastas with similar acceptability, with over 60% of participants expressed purchase intent. These results support the potential of both formulations as appealing gluten-free pasta alternatives.

**Keywords:** *Gluten-free pasta, corn-potato-lupin, celiac disease, mixture design, optimization.*



## 1. Introduction

Pasta is generally an affordable food choice with easy preparation, good appeal, and stable storage (Biernacka et al., 2018). Durum wheat (*Triticum durum*) is the preferred raw material for pasta production, especially for dry shapes of pasta. Durum wheat semolina provides a strong gluten and low extensibility to pasta (Edwards et al., 2001), resulting in a naturally viscoelastic product with unique characteristics. However, concerns about gluten-related disorders lead individuals with these specific dietary needs to consume gluten-free products (Aljada et al., 2021), which additionally are an alternative for countries facing unstable wheat imports and insufficient local production. Low dietary fiber, fat, and essential amino acid content in wheat (Albuja-Vaca et al., 2020) may also lead individuals to seek local ingredients that better meet their nutritional requirements.

The gluten-free pasta market primarily offers varieties made from corn, rice, and sorghum, which are widely accepted but have certain nutritional limitations (Giuberti et al., 2015). For example, corn flour is typically yellow in color, easily digested, good energy source and provides many B vitamins and essential minerals (Widelska et al., 2019; Ranum et al., 2014) but has lower protein content compared to wheat and rice, lacks some other nutrients (e.g., vitamin B12, vitamin C) and is poor source of lysine, calcium, folate, and iron (Li et al., 2022; Ranum et al., 2014). Meanwhile, in the last few years, strategies to enhance the acceptability and nutritional profile of gluten-free pasta have included the addition of pseudocereals (Huamán et al., 2024; Bouasla & Wójtowicz, 2019), legumes (Albuja-Vaca et al., 2020; Widelska et al., 2019; Bouasla et al., 2016), and tubers (Buzera et al., 2024; Ntukidem et al., 2023; Bao et al., 2021). Andean crops, in particular, have gained relevance for their potential in food technology applications (Salazar et al., 2021).

Andean lupin (*Lupinus mutabilis*) is a legume with pale beige seeds that visually blend well with various foods. It contains over 40% protein, high level of dietary fiber of which 75% is insoluble, fatty acids of which 80% are unsaturated, vitamins E, B1, B2 and B3, carotenoids, phenolic compounds, and a high level of lysine, but has a low starch content (Villacrés et al., 2020; Czubinski et al., 2021; Carvajal-Larenas et al., 2016), making it suitable to complement starch-rich formulations. Additionally, studies have reported potential hypolipidemic, hypoglycemic, hypotensive, anticarcinogenic, and anti-obesity activities in various lupin varieties (Carvajal-Larenas et al., 2016; Czubinski et al., 2021). Consumption of lupin is allowed once alkaloids naturally present in the raw seeds are removed by debittering technologies.

Potato (*Solanum tuberosum* L.) ranks as the fourth-largest staple worldwide (Bao et al., 2021). Andean varieties are considered starch-rich sources with low fat content and protein levels comparable

to wheat and relatively higher than rice or maize (Bártová et al., 2015). They contain chlorogenic acid, which helps regulate body fat, and offers high levels of vitamin C and low levels of phytates, which aid in iron and zinc absorption despite containing low levels of these minerals (Burgos & De Hann, 2019). The Peruvian *Yungay* potato—developed from crosses between native and exotic varieties— is highly adaptable, high-yielding, and commercially valuable, with a sale price similar to that of yellow corn in the Peruvian market (MIDAGRI-DGESEP 2024a,b). Potato flour has potential as a nutritional substitute for cereal flour, but commercial potato flour preparation usually involves boiling or steaming and heat-drying processes, leading to starch gelatinization and changes in the functional properties of potato starch (Bao et al., 2021).

Adding unconventional ingredients in gluten-free pasta can enhance nutritional quality and offer health benefits. However, maintaining technological and sensory quality is challenging due to the absence of gluten, making it essential to achieve a balanced proportion of ingredients in the pasta formulation. The mixture design methodology is a type of response surface design that helps predict responses for all possible formulations in a mixture experiment and identify optimal proportions of the components (factors), which must sum to a same total (Goos et al., 2016). This approach aids in analyzing the role of each ingredient in processed foods and their interactions. Among mixture designs, D-optimal approach is a flexible design structure that best estimates the effects of the factors (Stat-Ease Inc, 2024) and provides excellent guidance even with a small number of available runs (Goos et al., 2016), thus reducing the cost of experimentation.

This study was conducted to evaluate the followings: (a) the possibility of producing a gluten-free pasta using a combination of different concentrations of corn, potato and lupin flours, (b) the use of D-optimal mixture design to obtain two optimal formulations based on physical characteristics and cooking quality of the gluten-free pastas, and (c) to analyze the physical, cooking, nutritional and sensory characteristics of the optimum pastas.

## **2. Material and Methods**

### **2.1. Material**

Corn flour (CF) was provided by Douradinha da Fazenda S.A.C. (São Paulo, Brazil), while potato flour (PF) and lupin flour (LF) were provided by Agro Mi Perú Foods S.A.C. and Nutri Mix S.A.C., respectively, both located in Lima, Peru, and then transported to Brazil. Guar gum was provided by Adicel LTDA (MG, Brazil), and the monoglycerides of fatty acids (Mono 90) were supplied by Art

Alimentos (São Paulo, Brazil). As reported by the suppliers, the crops underwent preprocessing before being transformed into flours. This included degerming raw corn grains to prevent deterioration by germ lipids, blanching peeled potato tubers to inactivate polyphenol oxidase, and aqueous debittering of raw lupin seeds to remove alkaloids.

## 2.2. Experimental Design

A D-optimal Mixture experimental design (Design-Expert® v. 13.0.5.0, Stat-Ease Inc., USA) for a quadratic *Scheffé* model was used. The design included 3 lack-of-fit points and 3 additional center points to elaborate the mixtures. The concentration of CF, PF, and LF were the mixing factors, from 0% to 15% for LF, 0% to 20% for PF, and 70% to 100% for CF, for a total mixture equal to 100%. The design generated 12 trials (runs) of pasta formulations with both coded and actual values (Table 1), the latter being used experimentally to prepare the samples.

**Table 1.** Pasta formulation based on corn, potato and lupin flours

Sample	Coded values			Actual values		
	CF (%)	PF (%)	LF (%)	CF (%)	PF (%)	LF (%)
1	1	0	0	100	0	0
2	0.333	0.667	0	80	20	0
3	0.367	0.367	0.267	81	11	8
4	0.75	0	0.25	92.5	0	7.5
5	0.667	0.333	0	90	10	0
6	0.25	0.25	0.5	77.5	7.5	15
7	0	0.667	0.333	70	20	10
8	0.558	0.183	0.258	86.75	5.5	7.75
9	0	0.5	0.5	70	15	15
10	0.367	0.367	0.267	81	11	8
11	0.367	0.367	0.267	81	11	8
12	0.5	0	0.5	85	0	15

CF, corn flour; PF, potato flour; LF, lupin flour

## 2.3. Preparation of the dry pasta samples

Based on the experimental design, different proportions of CF, PF and LF were blended in a KitchenAid mixer (K45SS, Whirlpool, USA) for 15 min, resulting in a dry mix. Guar gum (1.5%), monoglycerides (1%), and water (45%) were weighed separately based on the total weight of the flours

and mixed for 1 min, resulting in a wet mix. The dry mix was added to the wet mix, kneaded for 5 min, and left to rest for 1 h for fully hydration. The dough was cold-extruded using a Pastaia 2 (Italvisa, Brazil) with a spiral-shaped die ( $\varnothing$ : 1 cm) for fusilli, passing the dough 4 times through the machine to achieve complete homogenization of its components. The pasta was cut manually to a length between 3.0 and 3.3 cm. The extruded pasta was dried in two circulating-air ovens (TE-394/2, Tecnal, Brazil) at  $50^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for 1 h, followed by drying at  $40^{\circ}\text{C} \pm 1^{\circ}\text{C}$  until the moisture content was below 13.5% (ranged from 11 to 13%). The dry pasta was cooled in perforated stainless-steel trays for 30 min at room temperature, packaged in Doy-pack metallic bags and stored in a dry and ventilated place.

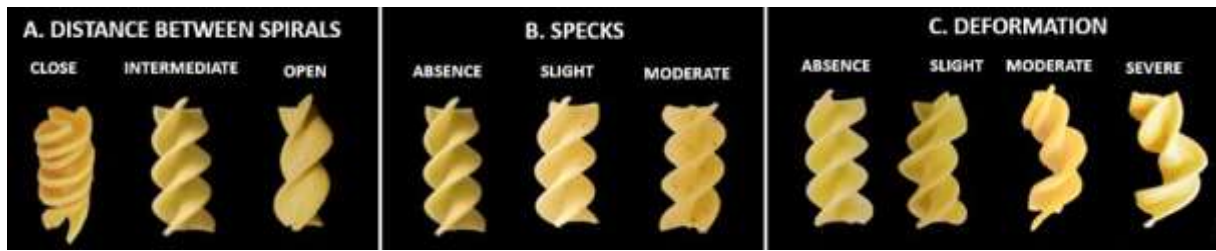
#### 2.4. Physical characteristics of the pasta

This assessment followed a modified method from Scarton et al. (2021). Physical characteristics that commonly determine the visual appeal of *fusilli* pasta, including spiral distance, specks, and deformation, were evaluated in twenty units of dry pasta from each formulation and classified according to Table 2. The spiral distance (the gap between two spirals) was measured using a vernier caliper, the specks (tiny superficial spots) were counted and summed, while deformation (crooked, bent and curved structures) was visually inspected and a specific score was assigned to enable numerical processing of the results (Fig. 1).

**Table 2.** Classification of physical characteristics in the dry pasta samples

Physical characteristics	Classification (measure)	Score <sup>1</sup>
Spiral distance (cm)	Close ( $< 0.65$ )	-
	Intermediate ( $0.65 - 0.74$ )	-
	Open ( $> 0.74$ )	-
Specks (N°)	Absence (0)	-
	Slight ( $\leq 7$ )	-
	Moderate ( $> 7$ )	-
Deformation	Absence	0
	Slight	1
	Moderate	2
	Severe	3

<sup>1</sup> Values assigned to evaluate physical deformation (visual).



**Fig. 1.** Physical characteristics in the dry pasta samples

### 2.5. Cooking quality of the pasta

The optimal cooking time (OCT) and cooking loss (CL) were assessed following the Method 66-50 from the AACC (2000). The dry pasta (5 g) was added to a 400-mL beaker containing boiling distilled water (60 mL) on a hotplate. OCT was determined by cooking ~5 g of dry pasta in boiling distilled water (60 mL) and testing pasta at 5-sec intervals by squeezing them between two acrylic plates until the white core was nearly translucent. After reaching the OCT, CL was measured by evaporating the cooking water in an oven at 105°C to dryness, and calculating the remaining solids as a percentage of the dry pasta weight. Water absorption (WA) was calculated by the weight difference between cooked and dry pasta, expressed as grams of water per gram of dry pasta. The hardness of the pasta was measured using a texture analyzer (TA-XT2, Stable Micro Systems, UK) with a flat-ended cylindrical probe (P/35) by a compression cycle (Larrosa et al., 2016), at a test speed of 1.0 mm/s and compression distance of 80% of the pasta size.

### 2.6. Analysis, optimization, and validation of the model

To determine the influence of CF, PF, and LF concentrations on the physical characteristics and cooking quality of the pasta, the experimental results from each response (spiral distance, specks, deformation, optimal cooking time, water absorption, cooking loss and hardness) were analyzed statistically (Design-Expert® v. 13.0.5.0, Stat-Ease Inc., USA). The regression models were expected to be linear or quadratic (Eqs. 1 and 2) and to meet the following: a statistically significant p-value ( $p < 0.05$ ), high coefficient of determination ( $R^2$ ), high adjusted coefficient of determination ( $R^2_{Adj}$ ), non-significant lack of fit (LOF) ( $p > 0.05$ ), low coefficient of variation (CV%), and good adequate precision ( $AP > 7$ ).

$$Y = b_1X_1 + b_2X_2 + b_3X_3 \text{ (linear model)} \quad (1)$$

$$Y = b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1X_2 + b_5X_1X_3 + b_6X_2X_3 \text{ (quadratic model)} \quad (2)$$

where  $X_1$  = A or CF,  $X_2$  = B or PF, and  $X_3$  = C or LF, while  $b_1$ ,  $b_2$ , and  $b_3$  are the regression coefficients for  $X_1$ ,  $X_2$ , and  $X_3$ , respectively, calculated by the analysis of variance.

The responses that were well-fitted to at least one regression model were chosen for individual optimization, giving an acceptable criterion (goals) to each chosen response (Table 3) with an equal level of importance for all responses. The acceptable criteria included an intermediate spiral distance, absence or slight number of specks, minimum deformation, minimum optimal cooking time, minimum cooking loss, maximum hardness, and an intermediate value (based on experimental results) for water absorption. The acceptable numerical range for ‘intermediate spiral distance’ and ‘slight specks’ was established based on preliminary evaluations of 50 commercial dry pasta units visually considered to have appropriate shape and appearance. The background information on the acceptable criteria for each response was included in the discussion of “Individual goal (desired conditions) for response variables”. From the multi-response optimization, two different formulations with high overall desirability were selected for experimental validation of the predicted responses.

**Table 3.** Goals for individual optimization of the physical and cooking characteristics

Physical characteristics			Cooking characteristics			
<u>Spiral distance</u>	<u>Speck</u>	<u>Deformation</u>	<u>OCT</u> <sup>1</sup>	<u>WA</u> <sup>2</sup>	<u>CL</u> <sup>3</sup>	<u>Hardness</u>
In range: 0.65 – 0.74	In range: 0–7	Minimize	Minimize	Target: 1.25*	Minimize	Maximize

<sup>1</sup>OCT: optimal cooking time, <sup>2</sup>WA: water absorption, <sup>3</sup>CL: cooking loss. \*: Intermediate experimental value chosen for WA.

## 2.7. Evaluation of Optimum Pasta

In addition to the physical characteristics and cooking quality, other analyses such as total titratable acidity, moisture, color, microscopy, nutritional value and sensory analysis were performed on the two selected optimum pastas. To analyze the dry pasta for acidity, color, microscopy and nutritional value, samples were ground in a blender (PRD-0450, Metvisa, Brazil) for 3 minutes and then sieved through a 250 µm mesh.

### Color

The ground dry pasta (20 g) was placed in a Petri dish and measured using a MiniScan XE 3500 45/0-L colorimeter (HunterLab, USA) in the CIELAB system ( $L^*$ ,  $a^*$ ,  $b^*$ ). The Yellowness Index (YI) was calculated using the formula (Eq. 3) provided by Francis & Clydesdales (1975) to better understand the variations in the yellow hue of the pasta.

$$YI = 142.86 \times \frac{b^*}{L^*} \quad (3)$$

### **Light Microscopy and Scanning Electron Microscopy**

Small fine fractions of dry and cooked optimum pasta were obtained using a scalpel. To examine the samples under a light microscope (BX51, Olympus, USA), they were placed on glass slides and observed at 100x magnification. To examine the samples under a scanning electron microscope (TM-4000 Plus, Hitachi High-Tech, Japan), they were fixed on a specimen stub with double-sided carbon tape, gold-coated with a sputter coater (K450, Emitech, UK), and observed at 1000x magnification, 10 kV acceleration voltage, 6.1 mm working distance and 92  $\mu$ A beam current.

### **Total titratable Acidity**

A solution composed of ground dry pasta and distilled water (1:20 w/v) was stirred on a magnetic stirrer at 1,000 rpm for 5 min, and centrifuged at 5,000 rpm for 10 min. The total titratable acidity (TTA) was determined by titrating the supernatant with 0.1 N NaOH, after adding 2-3 drops of phenolphthalein, measuring the pH of the solution with a pH meter (AK90, ASKO, Brazil) until it reached  $8.3 \pm 0.1$  (IAL, 2008). A blank test was prepared with only distilled water. The result was expressed in mL eq NaOH per 100 g of dry pasta.

### **Moisture content**

The air-oven drying method (925.10) of the AOAC International (AOAC, 2023) was used to determine the moisture content, which was calculated as the difference between the initial and final mass and expressed as a percentage of the initial mass.

### **Nutritional value**

#### **Proximate composition**

The proximate composition was carried out on the ground dry pastas according to AOAC International (AOAC, 2023). Methods such as micro-Kjeldahl for crude protein (960.52) with factor N = 6.25 (Jones, 1931), Soxhlet extraction for crude fat (920.85), direct method for ash (923.03), and enzymatic-gravimetric for dietary fiber (985.29) were used. The total carbohydrate content was determined by subtracting the percentages of moisture, crude protein, crude fat, and ash from 100%. The dietary fiber content was included in the total carbohydrate content. The energy content (EC) or caloric value of the pastas was estimated based on the contents of crude protein, fat and total carbohydrate using the Atwater conversion factors of 4.0, 9.0, and 4.0 kcal/g, respectively (FAO/WHO, 1973).

### **Amino acid profile**

To determine the amino acids of the optimum dry pasta, a set of steps including hydrolysis, PITC derivatization, buffer dilution, detection at 254 nm using a reversed-phase HPLC equipment

(Alliance 2695, Waters, USA), and quantification by external and internal standards were performed on the ground samples (Hagen et al., 1989; White et al., 1986). Tryptophan was an exception, as it was detected at 590 nm using a UV/VIS spectrophotometer (Genesys 50, Thermo Fisher, USA) and quantified by external standard, after treating the samples with enzymatic hydrolysis (Lucas & Sotelo, 1980). The amino acid composition was expressed as g of amino acid/16 g of nitrogen. The essential amino acid (EAA) score of the tested protein (TP) of the pasta relative to the EAAs of the reference protein (RP) for an adult human (FAO, 2013) was calculated according to Eq. (4) (Mitchell & Block, 1946), and those with lower values than the reference EAAs were used to identify the limiting amino acids (LAAs) in the pastas.

$$EAA\ score\ (\%) = \frac{mg\ EAA\ in\ 1\ g\ of\ TP}{mg\ EAA\ in\ 1\ g\ of\ RP} \times 100 \quad (4)$$

### Mineral characterization

The ashes from the optimum dry pastas were dispersed on a slide and attached to a specimen stub using double-sided carbon tape. The elements were identified with an Energy Dispersive X-ray detector (EDS-6070, Oxford Instruments, England) coupled to a SEM equipment (Leo 440i, Leo Electron Microscopy, England). The relative percentages of the elements, totaling 100%, were estimated by analyzing the relative peak heights in three selected areas of an image at 500x magnification.

### Sensory analysis

The sensory analysis was previously approved by the Ethics Committee of the University of Campinas (UNICAMP) through protocol No. CAAE-69240923.8.0000.5404, carried out in the Sensory Analysis Laboratory of the Department of Food Engineering and Technology – UNICAMP (São Paulo Brazil), with 129 untrained panelists aged 18 to 60 years, who were provided with a preliminary information sheet about the origin, nutritional value and health benefits of Andean lupin and potato to familiarize them with these Peruvian ingredients before receiving the samples. Two dry and cooked optimum pasta samples (7 units each) were presented on disposable plastic cups (2 oz) coded with three-digit random numbers, and served in individual testing booths, under white light with adequate ventilation, following a sequential monadic test within a completely randomized design.

The sensory test of the optimum dry and cooked samples was performed by using a 9-point hedonic scale (1 = dislike extremely, 9 = extremely like extremely) for acceptance and a 5-point scale (1 = certainly would not buy, 5 = certainly would buy) for purchase intent (Stone et al., 2004). The acceptance of the dry pasta was evaluated based on appearance and color, while that of the cooked pasta was evaluated based on appearance, color, aroma, taste, texture, and overall acceptability. To evaluate the cooked pasta, the dry pasta was cooked in boiling water (1:12 w/v) up to its OCT, with 1 mL of



soybean oil added beforehand to prevent sticking. It was then drained and served with a traditional homemade tomato sauce on top, along with a glass of water as a palate cleanser between samples.

### 3. Statistical analysis

Physical analyses, such as spiral distance, specks, and deformation of the pasta samples, were evaluated with twenty repetitions ( $n = 20$ ), while cooking analyses were performed in quadruplicate ( $n = 4$ ) and statistically assessed using analysis of variance (ANOVA) in the Design Expert software (v.13, Stat-Ease Inc., USA).

In the validation of the optimum models, differences between the experimental mean values and the predicted values were compared using a One-sample t-test. The optimum pasta samples were analyzed using Student's t-test. With the exception of the analyses mentioned above, all other analyses were conducted in triplicate ( $n = 3$ ), with results reported as the mean  $\pm$  standard deviation (SD), except for amino acids, which were analyzed only once ( $n = 1$ ). The analyses were performed using the statistical software Minitab (v. 13, Minitab Inc., USA).

### 3. Results

#### 3.1. Physical and cooking characteristics of the twelve pasta samples

Fig. 2 shows the development of the twelve pasta formulations generated by the Design Expert software, while Table 4 presents the physical and cooking characteristics of these samples.

##### *Spiral distance*

Intermediate and open spiral distances were observed in the pasta samples, ranging from 0.68 to 0.80 cm. The pastas with an intermediate spiral distance were primarily made with CF (sample 1) or a mixture of CF and PF (sample 2 and 5). These samples gave an impression of good quality and were more visually appealing. Conversely, the samples 6, 8, and 12, which primarily contained 7.7% to 15% LF showed an open spiral distance, especially pasta 12 (85% CF, 15% LF) which had a very stretched shape.

Starch can form a rigid structure in dried products especially due to amylopectin, which constitutes the major component in starch such as potato and corn (Singh et al., 2002). On the other hand, the plasticizing effect of water facilitates the handling of the mixture inside the extruder but excessive water significantly reduces the consistency of the dough and increases its fluidity, reducing extrusion pressure and causing shape issues (Peña et al., 2014). In the present study, the constant water

content (45%) used to hydrate the twelve doughs may have overhydrated the lupin-containing doughs, as the pasting behavior of LF differs from that of starchy flours, failing to form a paste at low concentrations (Navarro & Clerici, 2025, in pre-publication), which resulted in overly stretched pasta shapes. These findings align with those of Jayasena & Nasar-abbas (2011), who also found that lupin-semolina blends required lower hydration levels than semolina blends in spaghetti production. Therefore, using less amounts of water during kneading of LF-containing pasta would improve its spiral-like shape.

### *Specks*

The results showed that the pasta samples exhibited slight to moderate brown specks, ranging from 4 to 9 specks per pasta unit. Sample 1, 2, and 5 exhibited more specks than the other samples. Sample 2, which contained only CF and PF, with PF at the highest concentration (20%), exhibited the greatest speck count. In contrast, samples 4, 6, and 12, which contained 7.5% or less PF and 7.5% or more LF, had the fewest specks.

Speck count is an important quality control specification in milling, as brown or dark flecks in pasta are often caused by non-endospermic or non-fleshy fragments of raw ingredients, impacting the appearance of the pasta and potentially reducing consumer acceptability (Symons et al., 1996; Manthey & Twombly, 2006). According to the raw ingredients' suppliers, LF was made from whole lupin seeds, CF from degermed and partially dehulled corn, and PF from peeled and blanched potatoes. Unlike the semi-translucent, whitish coat of Andean lupin seeds; the semi-translucent gold bran in corn and brown skin in *Yungay* potato could lead to dark specks in pasta if accidentally included, with potato skin being especially likely to alter the pasta's appearance. Thus, the highest number of specks in pasta 1, 2 and 5 are likely due to the presence of corn bran and potato peel.

### *Deformation*

Deformation in pasta ranged from 0 to 2.63, indicating levels from no deformation to moderate deformation. Samples 2 and 5, made with CF and PF mixtures, displayed a straight structure. However, samples 9 and 12, which contained the highest concentration of LF (15%), exhibited the most pronounced deformations (crooked, bent and curved structures), particularly sample 12 (77.5% CF, 7.5% PF, 15% LF).

The use of unconventional ingredients can lead to structural irregularities in pasta (Manthey & Twombly, 2006). However, in the case of pastas containing only potato and corn flours, amylopectin chains may have contributed more significantly to a better structure due to their double-helical crystalline arrangement (Singh et al., 2002). In a previous study, potato, corn and lupin flours had the ability to absorb large amounts of water and absorb oil to a very small extent, however, lupin was not

able to form paste (Navarro & Clerici, 2025, in pre-publication). The high lipid content (up to 25 g/ 100 g dry weight) and good emulsifying properties in Andean lupin (Carvajal- Larenas et al., 2016), combined with constant amounts of hydrocolloid (guar gum) and emulsifier (monoglycerides) probably emulsified and lubricated too much the lupin-containing doughs during extrusion. In contrast to the study of Peña et al. (2014) who mentioned that the lubricant effect of flaxseed flour favorably reduced the friction in dough extrusion, in this study, the lupin flour, food additives and constant hydration level (45% of total flour weight) could have increased deformation in lupin-containing pastas. Thus, reducing or omitting the food additives could prevent deformation of LF-containing pasta, in addition to allowing this type of pasta to be classified as a clean label product.

#### *Optimal cooking time (OCT)*

The OCT ranged from 4.31 to 4.83 min, equivalent to 04:19–04:50 (mm:ss), with the longest time observed in pasta containing no LF (samples 1, 2 and 5), especially in sample 1, which only contained CF. Conversely, samples 9 (70% CF, 15% PF, 15% LF) and 7 (70% CF, 20% PF, 10% LF) showed the lowest OCT (4.30 and 4.33 min, respectively).

The addition of legume flours in cereal-based pasta can reduce overall starch levels, potentially lowering the hydration time required for cooking (Petitot et al., 2010). This may be due to the inability of lupin to form paste at concentrations below its gelation point. Additionally, lupin proteins may accelerate water diffusion since they contain hydrophilic side chains from albumin (Carvajal et al., 2016), unlike water-insoluble corn proteins (zein) (Zhang et al., 2022). During cooking, hydrated legume proteins denature, exposing polar amino-acid groups that enhance water affinity (Bouasla et al., 2016), thereby shortening the OCT. The shorter OCT with increased potato compared to corn in pasta formulations is probably due to the required time to reach pasting temperature (onset) in cereals, with corn needing a higher temperature to gelatinize than potato (Singh et al., 2002). Morreale et al. (2019) found similar results, where gluten-free pasta made with corn and potato starches and lupin flour had a shorter OCT (5 min) than pasta made only with corn starch (6–10 min).

#### *Water absorption (WA)*

The results for WA of the pastas ranged from 1.14 to 1.35 g water per g sample. The highest WA was observed for sample 2 (80% CF, 20% PF), while the lowest WA was found in sample 12 (85% CF, 15% LF). These results indicate that higher CF and PF concentrations enhanced WA, while higher concentrations of LF caused the opposite effect.

WA in pasta reflects how well it hydrates, with starch, protein, and fiber content playing crucial roles (Larrosa et al., 2016; Giuberti et al., 2015). Pastas with higher LF concentrations absorbed less

water due to reduced cooking times. The highest WA values may be due to potato starch with superior water-binding and swelling capacities that preprocessing (blanching) may have further intensified (Buzera et al., 2024; Navarro & Clerici, 2025, in pre-publication). Similarly, pasta fortified with 35 % legume flour had a lower water uptake than cereal-based pasta in the study of Petitot et al. (2010). In contrast, other authors have reported lower WA values for pasta made solely from cereals (Morreale et al., 2019; Bouasla & Wójtowicz, 2019). Variations in the nature and amount of the flours, other compounds, drying conditions, pasta dimension and shape, and moisture may explain the differences in our study compared to others.

#### *Cooking loss (CL)*

The results revealed that CL ranged from 3.62 to 7.00 %. Sample 2 (80% CF, 20% PF, 0% LF) had the highest CL (7%), while sample 12 (85% CF, 0% PF, 15% LF) and 4 (92.5% CF and 7.5% LF) showed the lowest values (3.62% and 3.85%, respectively).

CL reflects the release of soluble materials from pasta as its structure loosens during the cooking process (Petitot et al., 2010). In gluten-free pasta, this effect is more pronounced due to the lack of a gluten network that typically entraps starch molecules (Larrosa et al., 2016). since higher values can result in cloudy cooking water and sticky pasta. In this study, the maximum CL value was within the recommended acceptable range. The addition of LF did not increase CL, suggesting that its proteins, despite not forming a gluten-like network, helped to avoid the high loss of solids into the cooking water. Conversely, adding PF increased CL, probably due to the less stable granular structure and lower pasting temperature that increase the exposure of potato starch to hot water compared to corn starch. Similarly, Singh et al. (2002) reported that noodles made with native potato starch exhibited more amylose leaching compared to native corn starch, which was explained by their viscosity properties. In this work, the pre-processing of PF may have intensified the cooking loss in the PF-containing pastas through modification of the potato starch and increased particle size distribution, as observed in a previous study (Navarro & Clerici, 2025, in pre-publication).

#### *Hardness*

Hardness ranged from 3.31 to 7.58 N. Pasta 1 (100% CF) and pasta 5 (90% CF and 10% PF) exhibited the highest hardness, whereas pasta 9 (70% CF, 15% PF and 15% LF) and 6 (77.5% CF, 7.5% PF and 15% LF), which had the highest LF concentrations, showed the lowest values.

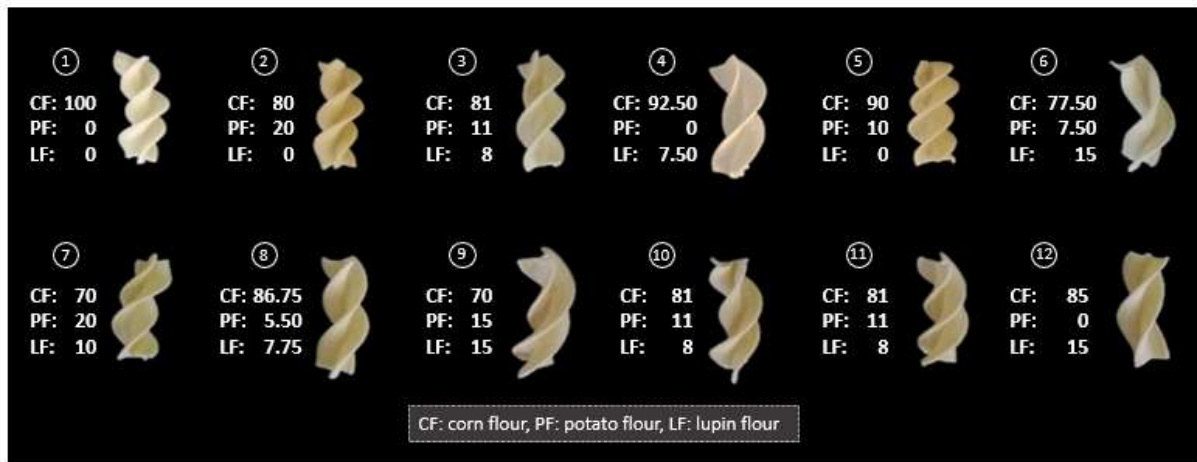
Gluten-free pasta faces challenges in maintaining a firm structure due to the absence of gluten, which is essential for forming structure in traditional pasta (Larrosa et al., 2016). The high dietary fiber content and in lupin may have contributed to a more fragile pasta texture. Fiber fractions in legume flour

may cause discontinuities inside the pasta, weakening its structure (Petitot et al., 2010). These results also suggest that the protein network in lupin is thick but relatively weak, as mentioned by Laleg et al. (2016). Conversely, retrograded starch from ingredients like corn and potato flour helps increase rigidity in cooked pasta, with starch structural organization playing a crucial role in this process (Lucisano et al., 2012). Corn is usually used to increase the hardness in pasta because of its high amylose content (Ntukidem et al., 2023). Therefore, the amount and type of starch in CF and retrograded PF can explain the higher hardness in the pastas containing these two flours.

**Table 4.** Experimental results for the response variables of the 12 pasta samples

Sample	Formula (CF/PF/LF)	Physical characteristics			Cooking characteristics			
		Spiral distance (cm)	Speck (N°)	Deformation (score)	Optimal cooking time (min)	Water absorption (g/g)	Cooking loss (%)	Hardness (N)
1	100/0/0	0.69	8.40	0.40	4.83	1.27	6.09	7.47
2	80/20/0	0.69	8.80	0.00	4.67	1.35	7.00	6.08
3	81/11/8	0.74	6.00	1.15	4.50	1.25	5.34	4.85
4	92.5/0/7.5	0.78	4.40	1.50	4.60	1.18	3.85	4.59
5	90/10/0	0.68	8.60	0.00	4.75	1.31	6.46	7.58
6	77.5/7.5/15	0.80	4.50	2.65	4.35	1.15	4.04	3.65
7	70/20/10	0.74	6.40	1.5	4.33	1.30	6.51	4.60
8	86.8/5.5/7.7	0.75	5.30	0.75	4.54	1.20	4.84	4.70
9	70/15/15	0.73	4.80	2.25	4.31	1.20	4.97	3.31
10	81/11/8	0.74	5.60	1.5	4.50	1.25	5.24	4.81
11	81/11/8	0.74	5.90	1.35	4.52	1.26	5.31	4.78
12	85/0/15	0.80	4.00	1.8	4.40	1.14	3.62	4.10

Results are presented as the mean (physical characteristics: n = 20, cooking characteristics: n = 4).



**Fig. 2.** Development of the twelve dry pasta samples

### 3.2. Individual goal (desired conditions) for response variables

The individual goal for each response was set earlier and discussed as follows:

An intermediate spiral distance in dry *fusilli* pasta is visually acceptable by consumers. While rarely addressed in academic literature, the industry aims to avoid overly shrunken or stretched pasta. A shrunken shape increases pasta's weight per length, which is less economical for the industry owners, while a stretched pasta is less likely to hold sauce in its grooves. A low speck count is expected in commercial pasta, as numerous specks may indicate lower flour purity and negatively impact the pasta's appearance. Physical deformations cause undesirable twisted pasta and often results from ingredient imbalances or process variations, though it's rarely discussed in the literature. Overly deformed pasta is often reprocessed in the industry by being grinded and reintegrated into the production process, which increases energy and time consumption.

Regarding cooking quality, a shorter optimal cooking time can help maintain the structure of cooked pasta, reducing its susceptibility to damage and energy consumption (Bolarinwa & Oyesiji, 2021). The rehydration characteristics of dry pasta are also a quality index (Jayasena & Nasar-abbas, 2011); however, excessive water absorption can result in overly swollen granules and a less desirable appearance. Nevertheless, businesses that offer ready-to-eat food prefer pasta that gains more weight per kilogram when cooked, as this can increase food prices and profitability. Additionally, minimal release of organic matter into the cooking water signals high-quality pasta that resists disintegration (Larrosa et al., 2016). Cooked gluten-free pasta with maximum hardness is also valued, as it maintains structural compactness and is less prone to disintegration (Larrosa et al., 2016).

### 3.3. Fitting the mathematical models

Table 5 shows the analysis of variance (ANOVA) for the response variables. Spiral distance and specks followed a quadratic model, while deformation, cooking time optimal, water absorption, cooking loss, and hardness followed a linear model. All the models presented  $p$ -values lower than 0.05, indicating their significance. Models for spiral distance, speck, deformation, OCT and WA had non-significant lack of fit (LOF) ( $p > 0.05$ ), indicating their accuracy to fit the experimental data. In contrast, LOF was significant ( $p < 0.05$ ) for cooking loss and hardness models, indicating poor fit and limiting their use as reliable predictors of their responses.  $R^2$  measures the variation explained by the fitted model, with values closer to 1 indicating smaller variation (Stat-Ease Inc., 2024). All the models showed coefficients of determination ( $R^2$ ) and adjusted  $R^2$  higher than 0.8, adequate precision (AP) values above 7, meeting the expected threshold of 4 (Stat-Ease Inc., 2024). The CV values were less than 25% for all responses showing a low data dispersion around the mean. Therefore, based on the ANOVA results, only the models for spiral distance, speck, deformation, OCT, and WA, which showed significant LOF, were selected for multi-response optimization and prediction.

**Table 5.** ANOVA results for the response variables of the twelve pasta samples

Source	Spiral Distance	Speck	Deformation	OCT	WA	CL	Hardness
	$p$ -value	$p$ -value	$p$ -value	$p$ -value	$p$ -value	$p$ -value	$p$ -value
<b>Model</b>	0.0019	0.0002	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Linear Mixture	0.0005	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
AB	0.4201	0.9589	-	-	-	-	-
AC	0.0479	0.0124	-	-	-	-	-
BC	0.9891	0.0658	-	-	-	-	-
LOF	0.0580	0.1582	0.2868	0.0726	0.1223	0.0146	0.0039
<b>Fit Statistics</b>							
CV (%)	1.95	6.91	23.70	0.41	1.15	7.09	9.79
$R^2$	0.9315	0.9665	0.8986	0.9899	0.9606	0.9044	0.8898
$R^2_{Adj.}$	0.8744	0.9386	0.8761	0.9876	0.9519	0.8832	0.8653
AP	13.16	18.05	14.96	59.89	33.50	20.99	15.44

Results were significant at  $p < 0.05$ . LOF: lack of fit, CV: coefficient of variation,  $R^2$ : coefficient of determination, AP: adequate precision, OCT: optimal cooking time, WA: water absorption, CL: cooking loss. A = corn flour (CF), B = potato flour (PF), and C = lupin flour (LF).

Table 6 presents the regression coefficients of the equations for the models that fit well their responses. For spiral distance and speck, the individual effect of the linear terms (A, B, C) and the interaction effect AC were significant ( $p < 0.05$ ), while the other responses showed only significant effects ( $p < 0.05$ ) for the linear terms. The largest coefficient estimates were as follows: +0.77 and +4.40 corresponding to the C term (LF) for spiral distance and deformation, respectively; +9.38 and +1.42 corresponding to the B term (PF) for speck and WA, respectively; and +4.83 corresponding to the A term (CF) for OCT. This indicated which flour had the most significant positive impact on the specified responses.

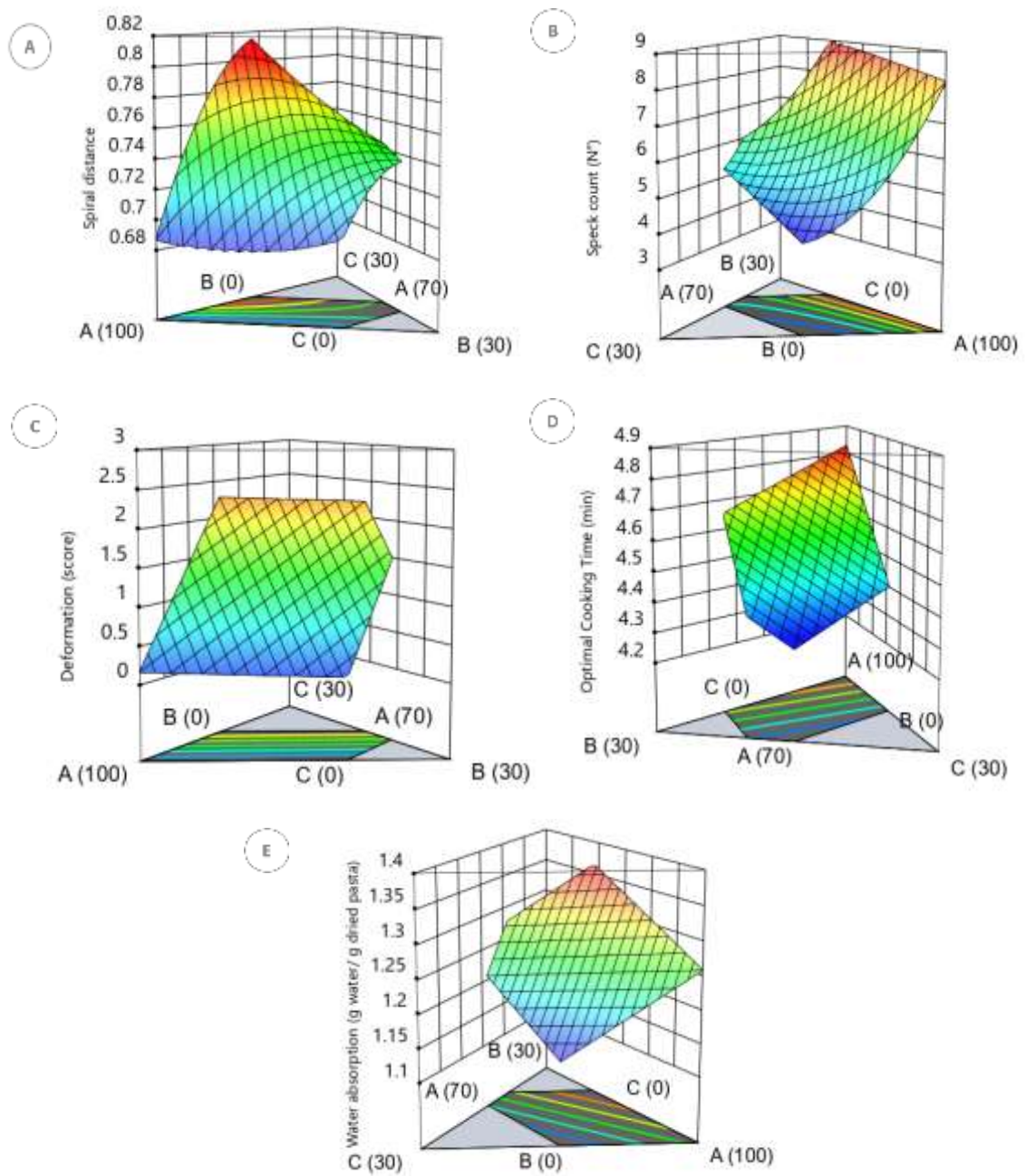
**Table 6.** Regression coefficient estimates for the selected responses of the 12 pasta samples

Term	Spiral distance	Speck	Deformation	Optimal cooking time	Water absorption
	Y <sub>1</sub>	Y <sub>2</sub>	Y <sub>4</sub>	Y <sub>5</sub>	Y <sub>6</sub>
<b>A</b>	+0.69	+8.15	+0.17	+4.83	+1.26
<b>B</b>	+0.72	+9.38	+0.05	+4.57	+1.42
<b>C</b>	+0.77	+6.38	+4.40	+3.98	+0.99
<b>AC</b>	+0.35	-14.41	-	-	-

A = corn flour (CF), B = potato flour (PF), and C = lupin flour (LF).

The 3D surface plots (Fig. 3) illustrate the effects of flour concentrations on selected response variables. Spiral distance and speck increased with an increase in LF, while an increase in CF and PF reduced these responses (Fig. 3.A, 3.B). Both with a significant quadratic effect in the CF–LF interaction. Spiral distance initially increased rapidly as CF decreased and LF increased, but this response slowed down as the interaction progressed, while the number of specks were initially slightly reduced as LF increased and CF decreased but the reduction became more noticeable as the interaction progressed. Deformation increased with an increase in LF, while increases in CF or PF led to a decrease in this response (Fig. 3.C). OCT and WA decreased with an increase in LF but an increase in CF led to an increase in OCT (Fig. 3.D), and an increase in PF led to an increase in WA (Fig. 3.E).





**Fig. 3.** 3D-surface plots for the selected response variables of the twelve pasta samples.

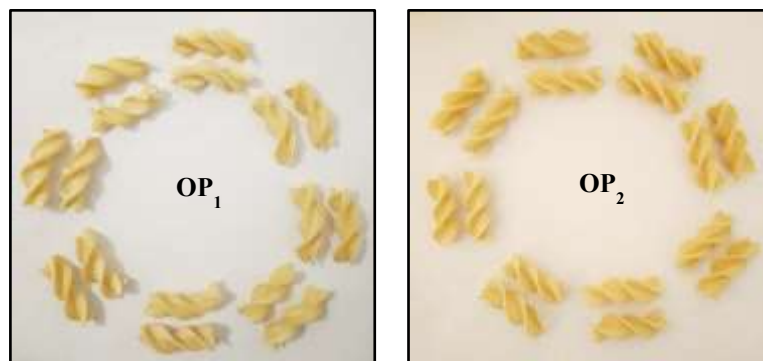
A = corn flour (CF), B = potato flour (PF), C = lupin flour (LF).

### Multi-response optimization and model validation

Based on the individual goals of the selected responses, multi-response optimization aimed to produce pasta with desired physical and cooking qualities. Fig. 4 shows the optimum pasta formulations (OP<sub>1</sub> and OP<sub>2</sub>), which coded and actual values are shown in Table 7, and their model validation are shown in Table 8. OP<sub>1</sub> and OP<sub>2</sub> achieved global desirability of 0.69 and 0.71, respectively, which are higher than those (0.58 and 0.53) for two optimum gluten-free pasta using lupin flour, rice flour, egg and guar gum (Albuja-Vaca et al., 2020), but are lower than those (0.84 to 0.90) for four optimum noodle formulations made with rice flour, soybean flour, corn starch and potato starch (Ntukidem et al., 2023). The goal of optimization is to find a balance that meets all response goals rather than aiming for a perfect desirability score of 1.0 (Stat-Ease Inc., 2024).

Actual (experimental) values closely matched predicted values ( $p > 0.05$ ), except for speck in OP<sub>1</sub> ( $p = 0.013$ ) and deformation in OP<sub>1</sub> ( $p = 0.031$ ) and OP<sub>2</sub> ( $p = 0.001$ ), where the actual values were lower than the confidence interval range but remained acceptable as they met optimization goals of low speck count and minimum deformation. Both OP<sub>1</sub> and OP<sub>2</sub> showed similar actual values ( $p > 0.05$ ) for spiral distance and specks; however, OP<sub>2</sub> had less deformation and a shorter OCT, while OP<sub>1</sub> showed a water absorption value that more closely aligned with the predicted value. These results indicate that both formulations effectively validated the selected models for multi-response optimization.

Moreover, cooking loss and hardness were independently tested for the optimum pasta formulations. Both OP<sub>1</sub> and OP<sub>2</sub> had similar hardness ( $p > 0.05$ ), suggesting that the increased PF and LF concentrations in OP<sub>2</sub> did not significantly increase hardness. However, OP<sub>1</sub> showed lower cooking loss due to the lower PF and LF contents. Since PF had higher concentration than LF in both pasta formulations, the results suggest that PF proportion played a larger role in influencing these responses. Yang et al. (2020) attributes reduced cooking loss to a stable protein-gelatinized starch matrix, whereas the addition of potato starch may lead to separation of starch from the matrix during cooking.



**Fig. 4.** Optimum pasta formulations

**Table 7.** Coded and actual values of the optimum pasta formulations

Optimum pasta formulations	Coded values			Actual values (%)		
	CF	PF	LF	CF	PF	LF
OP <sub>1</sub>	0.47	0.31	0.22	84.10	9.30	6.60
OP <sub>2</sub>	0.15	0.54	0.31	74.06	16.35	9.59

CF: corn flour, PF: potato flour, LF: lupin flour

**Table 8.** Validation of the models

Optimum pasta formulations	Responses	Predicted value	95% CI Low	95% CI High	Actual value <sup>1</sup>	<i>p</i> - value <sup>2</sup>
OP <sub>1</sub>	Spiral distance (cm)	0.74	0.72	0.76	0.74 ± 0.04 <sup>NS</sup>	0.934
	Specks (N°)	5.97	5.47	6.48	5.40 ± 0.82 <sup>NS</sup>	0.013
	Deformation (score)	1.06	0.87	1.26	0.90 ± 0.11 <sup>b</sup>	0.031
	Optimal cooking time (min)	4.56	4.55	4.58	4.56 ± 0.04 <sup>a</sup>	0.496
	Water absorption (g/g)	1.25	1.24	1.26	1.24 ± 0.02 <sup>b</sup>	0.239
	Cooking loss (%)	-	-	-	3.98 ± 0.27 <sup>b</sup>	-
	Hardness (N)	-	-	-	3.40 ± 0.32 <sup>NS</sup>	-
OP <sub>2</sub>	Spiral distance (cm)	0.74	0.72	0.76	0.72 ± 0.03 <sup>NS</sup>	0.071
	Specks (N°)	5.83	5.32	6.34	5.55 ± 0.69 <sup>NS</sup>	0.222
	Deformation (score)	1.43	1.14	1.71	0.70 ± 0.10 <sup>a</sup>	0.001
	Optimal cooking time (min)	4.42	4.40	4.44	4.40 ± 0.04 <sup>b</sup>	0.553
	Water absorption (g/g)	1.26	1.25	1.28	1.28 ± 0.01 <sup>a</sup>	0.103
	Cooking loss (%)	-	-	-	5.20 ± 0.40 <sup>a</sup>	-
	Hardness (N)	-	-	-	4.06 ± 0.56 <sup>NS</sup>	-

<sup>1</sup> Results are presented as the mean ± SD (spiral distance, specks, deformation: n = 20; optimal cooking time, water absorption, cooking loss, hardness: n = 4). Means not sharing the same letter within a row are significantly different by Tukey test ( $p < 0.05$ ,  $N = 3$ ) or by Student's t-test ( $p < 0.05$ ,  $N = 2$ ), where  $N$  represents the number of types of flours being compared. N.S: not significant

<sup>2</sup> Means of the actual values are not significantly different ( $p > 0.05$ ) from the predicted values within a row by One sample T-test.

### Color of the optimum samples

Color is one of the attributes that directly affects consumer preferences and selection. The results for the instrumental color of OP<sub>1</sub> and OP<sub>2</sub> are presented in Table 9. The color parameters (L\*, a\*, b\*) were significantly different ( $p < 0.05$ ) between the two samples. OP<sub>1</sub> showed L\* value slightly higher than OP<sub>2</sub>, while a\* and b\* values of both samples were positive, indicating reddish and yellowish colors, with the highest values for OP<sub>2</sub>. The yellowness index (YI) was more than 50% in both samples, demonstrating their yellowish hues, especially for OP<sub>2</sub>.

Pasta color is influenced by the flour properties, since those containing carotenoids provide a yellow color to pasta (Huamán et al., 2024). Heat pretreatments in flour preparation also affect the color of the final product (Buzera et al., 2022). The darker, more intense red and yellow hues in OP<sub>2</sub> likely result from its higher PF concentration and lower CF concentration. Due to the white-fleshed *Yungay* potato, its color is mainly attributed to the blanching process, as discussed in a previous study on these flours (Navarro & Clerici, 2025, in pre-publication). Similarly, Buzera et al. (2024) noted brightness reductions but increased red and yellow tones with potato additions in wheat-based pasta.

**Table 9.** Instrumental color measurements of the optimum dry pasta

Optimum sample	Formula (CF/PF/LF)	L*	a*	b*	YI
OP <sub>1</sub>	84.10/9.30/6.60	83.28 ± 0.49 <sup>a</sup>	2.52 ± 0.19 <sup>b</sup>	34.48 ± 0.31 <sup>b</sup>	59.15 ± 0.83 <sup>b</sup>
OP <sub>2</sub>	74.06/16.35/9.59	81.54 ± 0.43 <sup>b</sup>	3.46 ± 0.30 <sup>a</sup>	36.51 ± 0.49 <sup>a</sup>	63.97 ± 1.14 <sup>a</sup>

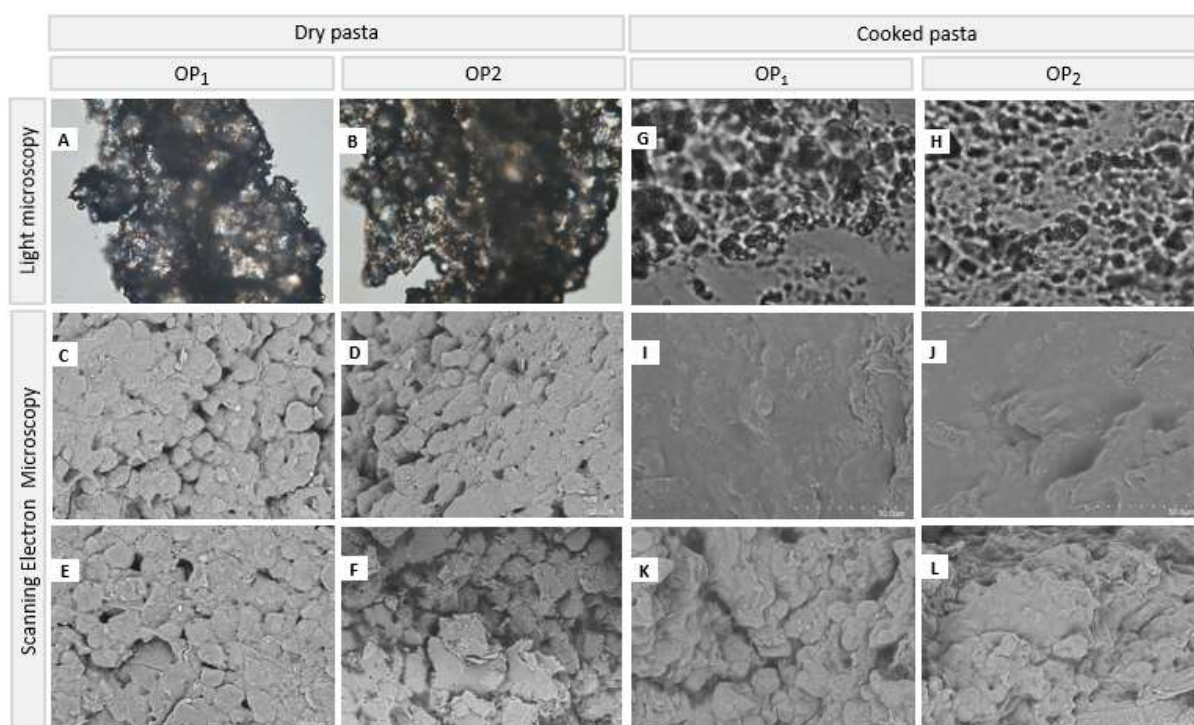
OP, Optimum pasta. YI, Yellowness Index. Results are presented as mean ± SD (n = 3). Means not sharing the same letter within a column are significantly different by Student's t-test ( $p < 0.05$ ).

### Microscopy of the optimum samples

The dry optimum samples (Fig. 5.A, 5.B) displayed agglomerates of round-like particles under light microscopy, while SEM images (Fig. 5.C–5.F) revealed bound starch granules semi-embedded in a dense matrix with tiny pores (dark holes) throughout the structure. These pores may have resulted from air bubbles formed during kneading (Jia et al., 2023), as it was not performed in a vacuum mixer, potentially influenced by the foaming properties of the flours, or may have resulted from water evaporation during low-temperature drying (Mercier et al., 2010). Proteins, especially from lupin, could have helped form the matrix and stabilize the granules, and guar gum might have enhanced particle agglomeration, as occurred similarly in a study of maize-field bean pasta (Widelska et al., 2019). While the outer layers of dry OP<sub>1</sub> and OP<sub>2</sub> were similar (Fig. 5.C, 5.D), the inner layer of OP<sub>1</sub> contained more

polyhedral to round native starch (Fig. 5.E), while the inner layer of OP<sub>2</sub> showed a combination of retrograded and native starch, with slightly larger gaps between granules (Fig. 5.F). As shown in a previous study on these flours, the retrograded starch corresponds to PF, while the native starch to CF, and the gaps between some granules in OP<sub>2</sub> may be explained by the high particle size distribution in PF, which may explain the greater cooking loss compared to OP<sub>1</sub>.

Light microscopy of the optimum cooked samples revealed swollen granules surrounded by ducts or fibrils (Fig. 5.G, 5.H), which probably indicate cooking losses as leached soluble compounds and trapped water. Cunningham et al. (2007) described the fibrillar features in cooked pasta as leached amylose chains. The outer zone of both cooked pastas (Fig 5.I, 5.J) showed a network with indistinguishable starch and protein due to the higher level of gelatinized starch and coagulated protein compared to the inner zone (Fig. 5.K, 5.L), which had more preserved granules as a result of less water absorption and cooking impact. These findings agree with that reported by Gopalakrishnan et al. (2011) on the micrographs of sweet potato-fortified pasta. During cooking, water penetrates the pasta, increasing the extent of starch gelatinization (which may have partially gelatinized during pasta preparation) and protein denaturation (Lucisano et al., 2012).



**Fig. 5.** Micrographs of dry and cooked pasta by light microscopy at 100x (dry pasta: A, B; cooked pasta: G, H) and SEM at 1000x (outer dry pasta: C, D; inner dry pasta: E, F; outer cooked pasta: I, J; inner cooked pasta: K, L)

### 3.4. Physicochemical and nutritional value of the optimum samples

The optimum dry pasta samples had total titratable acidity (TTA) values below 1.60 mL eq NaOH/100g and a moisture content of approximately 12%, with no significant difference ( $p > 0.05$ ) between samples (Table 10). Similar results for TTA (0.97 to 1.87 mL NaOH/ 100g) were obtained by Pagnussatt et al. (2014) in dry pasta made with oatmeal and microalga. Moisture content agrees with what was reported by Biernacka et al. (2018) for raw spaghetti (8.76–11.52 %). In line with industry standards, dry pasta typically targets a moisture content of 11–12%, although the Code of Federal Regulation (21 CFR Part 139) allows up to 13% (FDA, 2024). These specifications are essential for microbiological safety and maintaining sensory quality of the final product.

OP<sub>2</sub> had significantly higher ( $p < 0.05$ ) levels of protein, ash, fat and dietary fiber (Table 9). Upon cooking, the pasta samples increased in weight approximately 2.3 times due to water absorption, making their energy content similar per serving. The nutritional value of the pastas is directly linked to the nutritional properties of each flour component, as discussed in a previous study (Navarro & Clerici, 2025, in pre-publication) where LF revealed higher protein, fat and dietary fiber, while PF had higher ash content (indicator of mineral contribution). Thus, OP<sub>2</sub> displayed improved overall quality due to its higher LF and PF content. Similarly, other studies highlighted the impact of lupin on increasing protein, fats and fiber in rice pasta (Albuja-Vaca et al., 2019) and the effect of potato on enhancing mineral content in wheat cake (Akter & Alim, 2018). Additionally, similar energy content was observed by Bolarinwa & Oyesiji (2021) for rice-soy pasta (379.5–388.8 kcal/100g), which was higher than that of pasta made solely with rice.

**Table 10.** Physico-chemical and nutritional analyses of the optimum dry pasta

Sample	TTA <sup>1</sup> (%)	Moisture (%)	Protein (%)	Ash (%)	Fat (%)	TDF (%)	CH <sup>3</sup> (%)	EC <sup>4</sup>
OP <sub>1</sub>	1.36 ± 0.12 <sup>NS</sup>	12.02 ± 0.05 <sup>NS</sup>	10.42 ± 0.08	0.76 ± 0.01 <sup>a</sup>	2.73 ± 0.05 <sup>a</sup>	6.13 ± 0.01 <sup>b</sup>	74.1	362.5
OP <sub>2</sub>	1.50 ± 0.21 <sup>NS</sup>	12.09 ± 0.03 <sup>NS</sup>	11.50 ± 0.06 <sup>b</sup>	0.98 ± 0.01 <sup>b</sup>	3.37 ± 0.24 <sup>b</sup>	8.20 ± 0.01 <sup>a</sup>	72.1	364.6

<sup>1</sup>TTA: total titratable acidity (expressed in mL eq NaOH/100g dry matter), <sup>2</sup>TDF: total dietary fiber, <sup>3</sup>CH: carbohydrates, calculated by difference (includes TDF), <sup>4</sup>EC: energy content, expressed in kcal/100g of dry pasta (equivalent to 226 g cooked OP<sub>1</sub> and 228 g cooked OP<sub>2</sub>).

### **Amino acid profile of the optimum samples**

The amino acid profile of the optimum pasta samples is shown in Table 11. The total amino acid (TAA) composition was 10.11% for OP<sub>1</sub> and 11.38% for OP<sub>2</sub>, closely aligning with crude protein values obtained by the Kjeldahl method (10.42% for OP<sub>1</sub> and 11.50% for OP<sub>2</sub>). In a previous study, the amino acid content of LF was the highest (47.66 %), followed by PF (7.77 %) and LF (5.55 %) (Navarro & Clerici, 2025, in pre-publication). Thus, the higher TAA in OP<sub>2</sub> is linked to a higher content of LF and PF in the pasta. The essential amino acids (EAAs) were slightly higher in OP<sub>1</sub> (43.19%) than in OP<sub>2</sub> (42.20%), with leucine as the most abundant EAA. The limiting amino acids (LAAs), based on the FAO reference EAAs for adults (FAO, 2013), were meth+cys in OP<sub>1</sub> and OP<sub>2</sub>, with EAA scores of 53.80% and 47.79%, respectively, and lysine only in OP<sub>1</sub>, with EAA score of 91.39%.

The most abundant EAA in corn is leucine while potato and lupin are good sources of lysine (Li et al., 2022; Bártová et al., 2015; Carvajal-Larenas et al., 2016). Leucine promotes muscle protein synthesis and boosts human immunity, while lysine aids in calcium absorption and collagen formation (Li et al., 2022). The higher CF concentration in OP<sub>1</sub> explains its higher levels of leucine and essential amino acids, while the higher PF and LF concentrations in OP<sub>2</sub> explain its higher level of lysine. On the other hand, corn, lupin and potato are all considered poor sources of sulfur-containing amino acids (Li et al., 2022; Bártová et al., 2015; Carvajal-Larenas et al., 2016), which explains the meth+cys deficit in OP<sub>1</sub> and OP<sub>2</sub>.

**Table 11.** Amino acid profile and Essential amino acid score of the optimum pastas

Amino acid	FAO*	OP <sub>1</sub>		OP <sub>2</sub>	
<i>NEAA</i> <sup>1</sup>					
Asp	-	9.00	-	11.49	-
Glu	-	19.46	-	22.18	-
Ser	-	4.77	-	5.46	-
Gly	-	3.86	-	4.44	-
Arg	-	6.82	-	8.64	-
Ala	-	5.68	-	5.91	-
Pro	-	7.50	-	7.62	-
<i>EAA</i> <sup>1</sup>					
		<i>EAA score (%)</i>		<i>EAA score (%)</i>	
Hist	1.60	3.49	217.94	3.40	212.50
Ile	3.00	3.82	127.48	3.90	130.00
Leu	6.10	11.36	186.25	10.60	173.77
Lys	4.80	4.39	91.39	4.80	100
Meth	-	1.24	-	1.10	-
Cys	-	< 0.01	-	< 0.01	-
(Meth + cys)	2.30	(~1.24)	53.80	(~1.10)	47.83
Phe	-	4.50	-	4.40	-
Tyr	-	4.17	-	4.10	-
(Phe + tyr)	4.10	(8.66)	211.25	(8.49)	207.32
Thr	2.50	4.39	175.48	4.78	168.00
Trp	0.66	1.35	204.52	1.48	196.97
Val	4.00	4.50	112.49	5.00	110.00
<i>TAA</i> <sup>2</sup>					
	-	10.11	-	11.38	-
<i>EAA</i> s (%)	-	43.19	-	42.20	-
<i>1st LAA</i>	-	-	Meth+cys	-	Meth+cys
<i>2nd LAA</i>	-	-	Lys	-	-

\* Reference protein for an adult human (FAO, 2013).

<sup>1</sup> Results are expressed in g /16 g N, equivalent to g /100 g of protein.

<sup>2</sup> Results are expressed in g /100 g of dry pasta.

TAA: total amino acid, NEAA: non-essential amino acid, EAA: essential amino acid, LAA: limiting amino acid.



### Mineral qualitative characterization

In both OP<sub>1</sub> and OP<sub>2</sub>, potassium, phosphorus, calcium, and magnesium were the main macroelements, while lesser percentages of chlorine and sulfur were observed, and iron was the only microelement detected (Table 12). Both pastas showed similar mineral percentages ( $p > 0.05$ ), except for phosphorus, which was higher in OP<sub>1</sub>, and chlorine and sulfur, which were higher in OP<sub>2</sub>.

Prior research on these flours detailed their mineral content: calcium was mainly found in LF, iron in both LF and CF, and chlorine and sulfur in PF, while phosphorus, potassium, and magnesium were present in the three flours (Navarro & Clerici, 2025, in pre-publication). The mineral content in the optimized pasta is thus attributed to the mentioned flours. In a study, it was mentioned that lupin increased the levels of certain minerals, including calcium, iron, magnesium, and zinc in noodles (Bilgiçli & İbanoglu, 2014), whereas chlorine and sulfur content in potatoes may reflect fertilization practices (Oliveira et al., 2022). Dietary deficiencies of microelements bring some raising concerns for human nutrition. A quantitative analysis of the minerals in the optimum pastas would help determine their proximity to the acceptable values in the current legislation.

**Table 12.** Mineral characterization of the optimum pastas by EDS-MEV

Element (%)	OP <sub>1</sub>	OP <sub>2</sub>
Potassium	35.04 ± 1.63 <sup>NS</sup>	35.19 ± 0.92 <sup>NS</sup>
Phosphorus	16.09 ± 0.19 <sup>a</sup>	14.64 ± 0.07 <sup>b</sup>
Calcium	4.73 ± 0.38 <sup>NS</sup>	4.70 ± 0.55 <sup>NS</sup>
Magnesium	4.62 ± 0.20 <sup>NS</sup>	4.32 ± 0.71 <sup>NS</sup>
Iron	0.53 ± 0.17 <sup>NS</sup>	0.31 ± 0.29 <sup>NS</sup>
Chlorine	0.33 ± 0.06 <sup>b</sup>	1.39 ± 0.14 <sup>a</sup>
Sulfur	2.23 ± 0.47 <sup>b</sup>	3.40 ± 0.34 <sup>a</sup>
Oxygen*	(36.43 ± 0.60)	(36.04 ± 0.52)

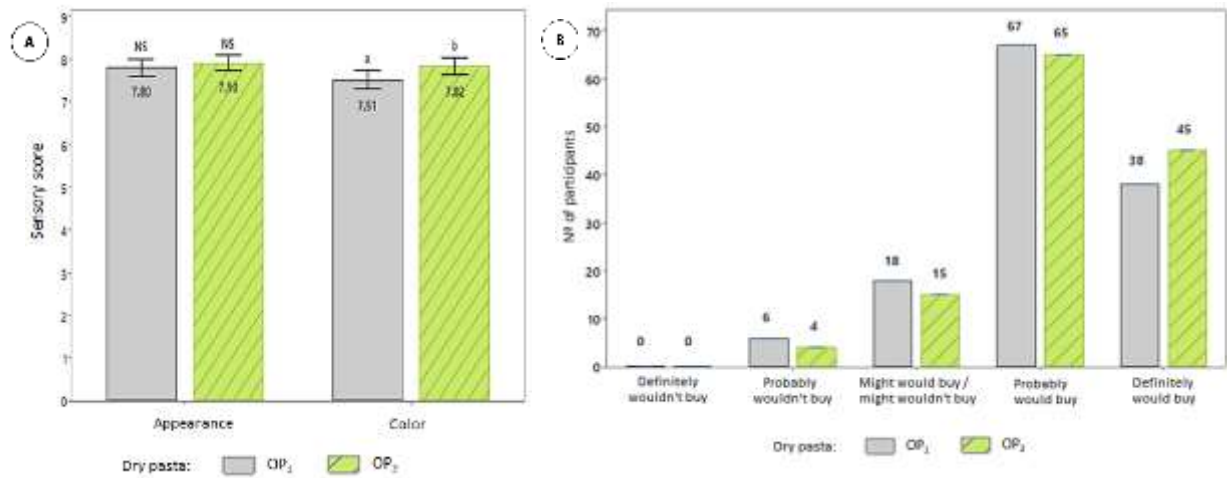
Results are presented as mean ± SD ( $n = 3$ ), and expressed as a percentage of the total elemental composition (100%). \*Element that forms complexes with the minerals and is part of the total elemental composition. Means not sharing the same letter within a row are significantly different by Tukey test ( $p < 0.05$ ,  $N = 3$ ) or by Student's t-test ( $p < 0.05$ ,  $N = 2$ ), where  $N$  represents the number of types of flours being compared. N.S: not significant.

### 3.5. Consumer test

Fig. 6.A shows the average sensory scores for the attributes in the dry optimum pastas, evaluated using a 9-point hedonic scale. Both dry samples were rated over 7 (like moderately) for appearance and color, being statistically similar ( $p > 0.05$ ) in appearance but different ( $p < 0.05$ ) in color, with the highest scores for OP<sub>2</sub>. The yellowness of the studied flours followed the order PF > CF > LF in a previous

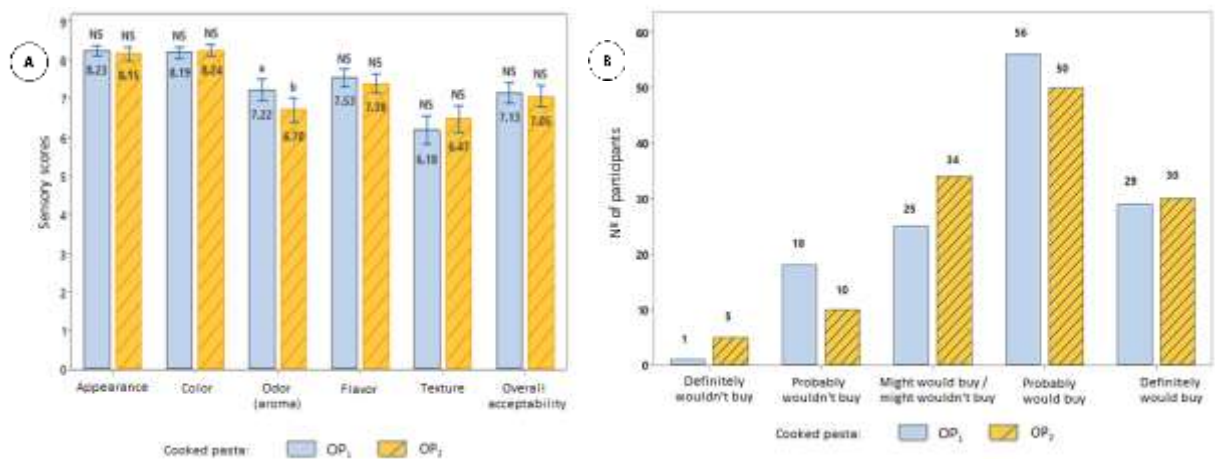
study (Navarro & Clerici, 2025, in pre-publication), thus PF enhance the yellow color of the pastas. A bright yellow color in pasta, often preferred by consumers, is typically achieved with durum wheat semolina (Biernacka et al., 2018). This may explain the participants' higher scores for the attributes of dry OP<sub>2</sub>, as it had a more pronounced yellow hue due to its higher content of PF, as discussed previously. Fig. 6.B shows participant's purchase intent after the sensory evaluation. A total of 105 participants indicated they would buy OP<sub>1</sub> (67 participants stated they would probably buy it and 38 stated they would definitely buy it), while 110 participants indicated they would buy OP<sub>2</sub> (65 participants stated they would probably buy it and 45 stated they would definitely buy it). The higher purchase intent for OP<sub>2</sub> dry pasta may be linked to its better color acceptance.

Fig 7.A. shows the average sensory scores for the attributes in the cooked optimum pastas. Both cooked samples, served with tomato sauce, scored above 6 for all the sensory attributes on the 9-point hedonic scale, being statistically similar ( $p > 0.05$ ) in appearance, color, flavor, texture, and overall acceptability but OP<sub>1</sub> had significantly higher score ( $p < 0.05$ ) for odor (aroma). The most liked attributes of the cooked pastas were appearance and color (scores above 8), while the least liked attribute was texture (scored above 6). The lowest sensory score for texture in the cooked samples suggests they differed from the ideal firm and non-sticky mouthfeel expected in pasta (Biernacka et al., 2018), as the absence of gluten can cause the pasta to disintegrate more easily in the mouth. The higher sensory score for odor in OP<sub>1</sub> may be attributed to its lower PF and LF concentrations, as the typically naturally subtle earthy smell of *Yungay* potato or the beany odor of Andean lupin are unfamiliar for Brazilians. Enzymatic reactions during the boiling of potatoes (Petersen et al., 1998), or oxidation of fatty acids in legumes (Jayasena & Nasar-abbas, 2011) could be responsible for the formation of particular volatile compounds in these foods. These results agree with other authors who reported that potato (Buzera et al., 2024) and lupin (Jayasena & Nasar-abbas, 2011) incorporations affected the odor and texture (mouthfeel) of noodles. The higher scores for color and appearance in both cooked samples compared to the dry ones may suggest that the addition of tomato sauce on the top enhanced their visual appeal. Regarding the purchase intent after the evaluation of the cooked pastas, Fig 7.B shows that a total of 85 participants would buy OP<sub>1</sub>, while 80 participants would buy OP<sub>2</sub>. The number of participants who indicated they would buy the optimum pasta samples decreased after tasting the cooked samples, possibly influenced by the texture attribute, which received the lowest sensory scores. The higher purchase intent for OP<sub>1</sub> cooked pasta could be due to its better odor acceptance.



**Fig 6.** Acceptance tests for the optimum dry pasta samples (participants = 129)

A) Sensory test (9-point hedonic scale), B) Purchase intent test (5-point scale).



**Fig 7.** Acceptance tests for the optimum cooked pasta samples (participants = 129)

A) Sensory test (9-point hedonic scale), B) Purchase intent test (5-point scale).

Future research could benefit for examining sensory responses and purchase intent for cooked pastas with and without tomato sauce, as well as with and without the information about their origin, nutritional value and health benefits, which was provided to participants at the beginning of this consumer test.

#### 4. Conclusion

This study successfully optimized gluten-free pastas using flint corn, *Yungay* potato, and Andean lupin flours resulting in two optimal formulations (OP<sub>1</sub> and OP<sub>2</sub>) with desired physical (intermediate spiral distance, low speck count, minimal deformation) and cooking (minimal optimal cooking time, intermediate water absorption) characteristics. Microstructure analysis showed that the compact starch-protein matrix seen in dry pastas became less distinct in cooked pastas due to granule swelling and compound leaching. OP<sub>2</sub> demonstrated lower cooking loss and higher protein, fat, ash, and dietary fiber, without lysine as a limiting amino acid, unlike OP<sub>1</sub>. However, OP<sub>1</sub> provided higher essential amino acid content and more phosphorus. Both pastas were well accepted sensorially and were intended to be purchased by the majority of participants, who showed openness to pasta made with diverse ingredients. These optimized formulations demonstrate the feasibility of using Peruvian-sourced Andean flours as sustainable ingredients in gluten-free corn pasta, offering acceptable physical, cooking and sensory qualities to support inclusive nutrition.

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#### 6. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## CHAPTER 4 – General discussion

The general aim of this study was to evaluate the potential of Andean potato, Andean lupin and hard yellow corn flours on the quality characteristics of gluten-free pasta formulations. After analyzing corn, potato and lupin flours individually, twelve pasta formulations were elaborated and evaluated for physical and cooking characteristics, including spiral distance, specks, deformation, optimal cooking time, water absorption, cooking loss, and hardness, for being relevant for the optimization. However, the models for cooking loss and hardness were not statistically accurate ( $LOF < 0.05$ ) and were excluded from the optimization process. Two optimum pastas were obtained ( $OP_1 = 84.10\%$  CF,  $9.30\%$  PF,  $6.60\%$  LF, and  $OP_2 = 74.06\%$  CF,  $16.35\%$  PF,  $9.59\%$  LF) by multi-response optimization. Physical and cooking analyses, including the excluded responses for optimization, as well as nutritional and sensory evaluations were performed on the optimum pasta formulations.

Regarding the impact of the flour properties on the physical characteristics of the twelve fusilli pasta samples, the desired intermediate spiral distances and minimum deformations were achieved with higher proportions of corn and potato flours, attributed to the structure of their starches, particularly the retrograded starch in potato flour due to pretreatment. Conversely, constant hydration during kneading negatively affected lupin-containing doughs, leading to open spirals in pastas, which might be related to the negligible pasting viscosity of lupin flour at low concentrations. The high lipid content and good emulsifying properties in lupin, combined with guar gum and monoglycerides, intensified the emulsifying and lubricant effect in lupin-containing doughs, causing deformations. Lupin flour may act similarly to these additives, suggesting that reducing hydration or removing additives could address these defects and support a clean-label product. The increased specks with higher potato flour levels may result from its high ash content, probably included those from non-endospermic parts, affecting the pasta's appearance.

Concerning the influence of the flour properties on the cooking characteristics of the twelve pasta samples, higher lupin concentrations diluted the total starch content, reducing pasta hydration and optimal cooking time. Pastas with increased potato flour content had shorter optimal cooking times compared to those with more corn flour, which might be attributed to potato flour's lower pasting temperature, allowing earlier gelatinization. Additionally, the wide particle size distribution of non-native starch in potato flour contributed to greater solubility of this flour, which in pasta increased the release of soluble particles into the water, leading to increased cooking loss. Additionally, the higher water absorption and swelling indexes when increasing potato flour resulted in pastas with greater water

uptake and swollen granules. Shorter cooking times led to lower water absorption and lower cooking loss. Hydrated dietary fiber softened lupin-containing cooked pastas, whereas the native starch in corn flour and the retrograded starch in potato flour similarly increased hardness.

The optimum pasta formulations (OP<sub>1</sub> and OP<sub>2</sub>) had similar spiral distance, speck and hardness. However, OP<sub>2</sub> exhibited lower deformation and optimal cooking time, while OP<sub>1</sub> had better water absorption and lower cooking loss. The moisture content and low acidity in the pastas ensured proper preservation. Lupin flour was the main contributor to increased dietary fiber, protein, and fat, while potato flour increased ash content. Potato and lupin flours increased lysine and total amino acid content, while corn flour enhanced the essential amino acid content and leucine. Both pastas were limited by methionine and cysteine, as these amino acids were present in low levels in the flours, particularly in lupin flour, where they were deficient. OP<sub>1</sub> was additionally limited in lysine due to its lower potato and lupin contents. The calcium in lupin flour and iron in corn and lupin flours were also in the pastas, along with minerals like potassium, phosphorus and magnesium. However, the zinc detected in corn and lupin flours was not detected in the pastas. Predominance of phosphorus in lupin and corn flours could suggest high phytic acid levels, which may inhibit mineral absorption.

The cream-to-yellow hues of the flours contributed to a general yellow tone of the optimum pastas, being more intense to OP<sub>2</sub>, which contained more potato flour. Since *Yungay* potato is classified as a 'white' variety, its flour color was primarily influenced by compounds formed during the high-temperature blanching pretreatment. Morphologically, native corn starch was predominant due to its higher proportion in both pastas, while lupin aggregates and retrograded potato starch were present in minor extent. Gaps between granules in the dry pastas were linked to the irregular particle size distribution of the retrograded potato granules, while tiny pores inside the granules may be result of air incorporation during kneading or water removal during drying, partially influenced by the foaming properties of the flours. Potato flour likely produced larger bubbles due to its higher foam capacity, whereas corn and lupin flours' greater foam stability created longer-lasting bubbles. The predominantly starch-protein structure in the dry pastas was partially disintegrated during cooking due to starch coagulation, amylose leaching and protein denaturation.

Regarding sensory characteristics, the optimum pastas showed similar scores for the evaluated attributes, except for color in the dry pastas and odor in the cooked ones. The more intense yellow hue of dry OP<sub>2</sub> was more appealing due to its higher potato flour content, while the more pleasing odor of cooked OP<sub>1</sub> was linked to Brazilians' familiarity with corn-based products, as OP<sub>1</sub> contained a higher proportion of corn flour, suggesting that the acceptance of the pastas may vary depending on the

geographical origin of the participants. Both dry and cooked pastas were rated as acceptable, scoring above 6. More participants were confident about purchasing the pasta after evaluating the dry samples than the cooked ones. Texture negatively impacted the overall acceptability of the cooked pastas, partially reflecting the participants' limited consumption with gluten-free pasta, as the recruitment guidelines excluded individuals with celiac disease or gluten-related disorders due to the pastas were prepared in a machine commonly used for wheat-based pasta.

The study proposes using Andean crop flours in corn pasta to create a gluten-free alternative, promoting food inclusion for those with gluten-related disorders. Future research could address the following aspects, considered as limitations in the present study: 1. Investigate the potential effect of vitamin C from potato flour to inhibit phytic acid levels from lupin and corn flours. 2. Quantify the mineral contributions from the flours and pastas. 3. Analyze starch, amylose, and amylopectin content to better understand their roles in pasta properties. 4. Examine the gelling properties of lupin flour at concentrations equal or higher than 14% (w/v). 5. Assess the water absorption rate of the flours in pasta development. 6. Test similar pasta formulations with and without food additives. 7. Develop new techniques to standardize the evaluation of short pasta characteristics. 8. Conduct sensory tests of pasta with Peruvian and Brazilian consumers with gluten-related disorders, evaluating the sample both with and without tomato sauce, and with and without a brochure about characteristics of the raw materials provided beforehand.

## **CHAPTER 5 – General conclusion**

The evaluation of physico-chemical, techno-functional, and rheological properties of corn, potato and lupin flours revealed unique properties for each flour that had significant influence on the physical, cooking, nutritional and sensory quality of the pasta, with each flour contributing distinct benefits and challenges. Higher proportions of corn and potato flours favored typical spiral distance and prevented deformation in pasta, while, higher proportions of lupin flour reduced specks, optimal cooking time, and water absorption. The optimum pasta formulations offered desirable physical and cooking characteristics, along with a balanced nutritional composition that make them a good source of energy, minerals and amino acids source, except for methionine and cysteine. Moreover, they demonstrated acceptable sensory characteristics, with texture being the most challenging attribute to improve. They differed in color for the dry forms and in odor when cooked, and were appealing to the majority of participants, who expressed interest in purchasing them. Thus, Andean potato and Andean lupin flours are promising ingredients for gluten-free pasta from hard yellow corn flour, primarily enhancing its nutritional value and color. In addition, this approach fosters sustainable synergy between the two Andean crops from Peru and the widely used corn from Brazil, promoting bilateral support and strengthening ties between the two countries.

## CHAPTER 6 – Bibliographical references

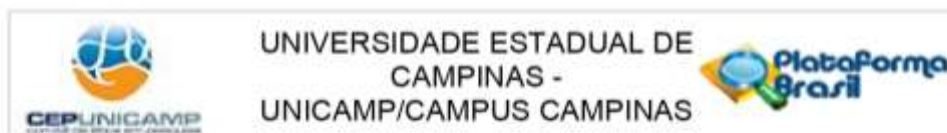
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## ANNEX

## A. Document approval for sensory analysis of pasta (in Article II).



## PARECER CONSUBSTANCIADO DO CEP

## DADOS DO PROJETO DE PESQUISA

**Título da Pesquisa:** Qualidade tecnológica, nutricional e sensorial de massas alimentícias de milho (*Zea mays*) contendo misturas de farinhas de tremço (*Lupinus mutabilis* Sweet) e batata (*Solanum tuberosum*) andinos.

**Pesquisador:** Katherine Kelly Navarro Valdez

**Área Temática:**

**Versão:** 2

**CAAE:** 69240923.8.0000.5404

**Instituição Proponente:** Faculdade de Engenharia de Alimentos

**Patrocinador Principal:** FUND COORD DE APERFEICOAMENTO DE PESSOAL DE NIVEL SUP  
Programa Nacional de Crédito Educativo

## DADOS DO PARECER

**Número do Parecer:** 6.251.814

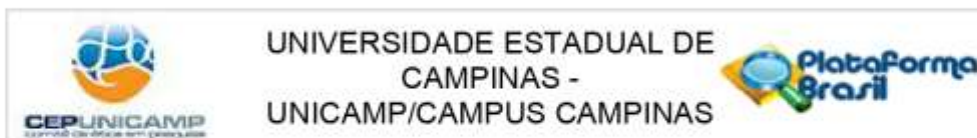
## Apresentação do Projeto:

As informações contidas nos campos "Apresentação do Projeto", "Objetivo da Pesquisa", "Avaliação dos Riscos e Benefícios" e "Comentários e Considerações sobre a Pesquisa" foram obtidas dos documentos apresentados para apreciação ética pelo CEP e das informações inseridas pelo/a PESQUISADOR/A RESPONSÁVEL pelo estudo, na Plataforma Brasil.

## Introdução:

A massa alimentícia é um dos alimentos mais populares e acessíveis do mundo, e é tradicionalmente feita de semolina e/ou farinha de trigo hard (*Triticum durum*) ou soft (*Triticum aestivum* L.). Grandes quantidades de trigo e seus derivados são importados principalmente da Rússia, União Europeia, Austrália e Canadá por países que não conseguem satisfazer a demanda interna (TREGO, 2023), e seu uso é atribuído ao glúten, responsável pelo comportamento viscoelástico da massa que desenvolve produtos com boas características de qualidade. Porém, a ingestão do glúten, seja de trigo, centeio, cevada ou triticale, traz consigo reações de intolerância e sensibilidade para pessoas com a síndrome celíaca e sensibilidade ao glúten não-celíaca; fazendo com que as mucosas intestinais fiquem inflamadas e não absorvam os nutrientes, causando vários desconfortos físicos a curto prazo (distensão abdominal, náuseas, vômitos e diarreia), longo prazo (osteoporose, anemia, crescimento atrofiado e doenças associadas) (ALJADA;

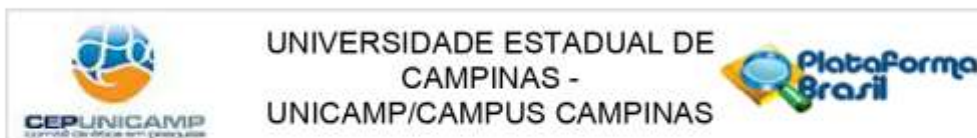
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ZOHNI; EL-MATARY, 2021; CATASSI et al., 2017) e outros sintomas relacionados (dor de cabeça, déficit de atenção e manifestações cutâneas) (MAKHARIA; CATASSI; MAKHARIA, 2015). Na alergia ao glúten o risco é imediato logo após ao consumo, podendo levar a choque anafilático e morte (CIANFERONI, 2016). Somado a necessidade de saúde, um número cada vez maior de indivíduos que não têm distúrbio relacionado ao glúten está se interessando em adotar uma dieta sem glúten, mas são motivados por suas percepções de que uma dieta sem glúten é uma escolha alimentar saudável. Estes fatores fizeram o mercado global de produtos sem glúten ser avaliado em US\$ 5,9 bilhões em 2021 e com projeção para crescer a uma taxa composta anual de 9,2%, com previsão de atingir US\$ 13,7 bilhões em 2030 (GRAND VIEW RESEARCH INC., 2022). Portanto, é importante ter no mercado uma variedade de produtos sem glúten, sobretudo daqueles mais consumidos na dieta, como as massas alimentícias. A utilização de cereais tem sido uma alternativa alimentar para o preparo de massas sem glúten. Na América Latina, destaca-se a farinha de milho como base substituta para tais fins, especialmente aquela que provém de grãos da variedade indurata por serem amarelos, duros e brilhantes. Para potencializar a ação da farinha de milho nas massas alimentícias, a utilização combinada deste cereal com tubérculos e/ou leguminosas têm sido estudada por vários autores devido à complementaridade dos seus nutrientes (PADALINO et al., 2015; FERREIRA et al., 2016; GIMÉNEZ et al., 2016; VELASCO-RODRÍGUEZ et al., 2018; SUO et al., 2022). A batata (*Solanum tuberosum*), na forma de farinha, é uma alternativa que complementa a farinha do milho por ser boa fonte de energia e relativamente baixa em calorias (MUÑOZ, 2014), considerada valiosa aliada na luta contra a desnutrição e fome no mundo (CIP, 2018). O tremço andino (*Lupinus mutabilis* Sweet) é outra alternativa que pode ser incluída na elaboração de massas alimentícias depois destes serem desamargados e transformados em farinhas, tem importância estratégica na alimentação por seu alto teor de proteína e óleo, pois correspondem a mais da metade do seu peso, tornando-se uma fonte alimentar moderna e sustentável para uma população que cresce rapidamente, bem como no combate da desnutrição infantil (CHIRINOSARIAS, 2015; MENEZES et al., 2014; PROINPA, 2019). Ao utilizar uma mistura de farinhas que não sejam de trigo, a qualidade nutricional do produto tende a melhorar, mas a qualidade sensorial e tecnológica podem ser comprometidas. Portanto, o objetivo deste trabalho será avaliar a qualidade tecnológica, nutricional e sensorial de massas alimentícias à base de milho e misturas de farinha de tremço e farinha de batata andinos, visando gerar novo conhecimento e divulgação

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de informação sobre as farinhas de tremço e batata, utilizando a farinha de milho como base sinérgica de mistura que ajude a impulsionar a industrialização destas matérias-primas peruanas.

Hipótese:

Análise sensorial: As farinhas de batata, tremço andino e milho permitem a elaboração de massas alimentícias sem glúten de qualidade sensorial aceitável.

#### **Objetivo da Pesquisa:**

Objetivo Primário:

Análise sensorial: Avaliar o efeito da concentração das farinhas de batata e tremço de origem peruana e farinha de milho de origem brasileira sobre a qualidade sensorial de massas alimentícias sem glúten.

#### **Avaliação dos Riscos e Benefícios:**

Riscos:

Considera-se que não vai haver riscos ou desconfortos previsíveis aos participantes desta pesquisa, salvo eventuais casos de alergia ou intolerância a algum dos ingredientes mencionados no "Critério de Exclusão".

Benefícios:

Não haverá benefícios diretos aos participantes deste estudo. Porém, o participante poderá saber de todos os benefícios que esta análise sensorial trará. Os resultados desta análise permitirão conhecer o nível de aceitação de massas alimentícias otimizadas, ajudando a saber quanto elas são desejáveis para o consumidor, bem como a intenção de compra e de consumo. Através dos resultados, será deduzido se as massas têm potencial para ser comercializadas no mercado visando fornecer uma opção inovadora e nutritiva de massa alimentícia sem glúten que pode ser adequada não apenas para celíacos e pessoas com alergia ao glúten ou trigo, mas também para consumidores que veem neste tipo de produto uma opção saudável para ser degustada, bem como divulgar a inclusão de ingredientes andinos como a batata e o tremço em produtos similares.

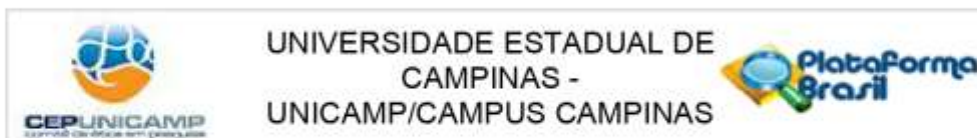
#### **Comentários e Considerações sobre a Pesquisa:**

Trata-se de um projeto de pesquisa que irá constituir a Dissertação (Mestrado), área de Tecnologia de Alimentos, de uma aluna regularmente matriculada no programa de pós graduação-Faculdade de Engenharia de Alimentos, orientada por uma docente do Departamento de Ciência de Alimentos e Nutrição (DECAN) dessa Unidade.

Metodologia Proposta:

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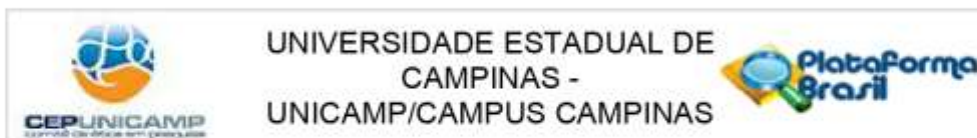
Antes da análise sensorial, tendo em vista que as matérias-primas de batata e tremoço andino são de origem peruana e pouco conhecidas pelo consumidor brasileiro, uma ficha informativa de cada matéria prima sobre as características e benefícios para a saúde será fornecida ao participante para sua leitura. A análise sensorial será realizada conforme a metodologia proposta por SCARTON et al. (2021), com pequenas modificações. As amostras (15 g) serão apresentadas em pratos brancos descartáveis codificados com números aleatórios de três dígitos e servidos em cabines de testes individuais para permitir a privacidade de cada provador, bem como evitar a interação e distração dos provadores. Cada provador receberá inicialmente até três amostras de massa seca e depois, até três amostras de massa cozida. Dentre o grupo das três amostras, até duas serão as obtidas por otimização e uma será o controle. As amostras serão entregues em sequência monádica em uma única sessão, seguindo um delineamento experimental de bloco completo balanceado. Para avaliação das massas secas, junto com as amostras será entregue uma ficha (ANEXO 01) contendo escalas de 9 pontos para avaliar os atributos de aparência e cor, de 1 = "desgostei extremamente" a 9 = "gostei extremamente"; e uma escala de 1 = "certamente não compraria" a 5 = "certamente compraria" para a intenção de compra. Será permitido atribuir o mesmo valor às escalas para mais de uma amostra, se assim for percebido pelo provador. Para avaliação das massas cozidas, elas serão colocadas em água fervente até atingir o tempo ótimo de cozimento (TOC), escorridas em peneira e servidas juntamente com um copo de água para lavar o paladar entre as massas. A ficha de avaliação (ANEXO 02) conterá escalas de 9 pontos para avaliar os atributos de aparência, cor, aroma, sabor, textura e impressão global das massas cozidas, de 1 = "desgostei extremamente" a 5 = "gostei extremamente". Será utilizada escalas de 5 pontos para avaliar a intenção de compra, de 1 = "certamente não consumiria" a 5 = "certamente consumiria". Será permitido atribuir o mesmo valor às escalas para mais de uma amostra, se assim for percebido pelo provador.

#### Metodologia de Análise de Dados:

Os dados que serão obtidos a partir do teste de aceitação sensorial serão avaliados utilizando ANOVA e reportados como a média  $\pm$  desvio padrão (Microsoft Excel 2016, Microsoft®). A diferença significativa entre as médias de cada experimento será calculada de acordo com o teste de Tukey (Minitab Inc., State College, EUA) com um nível de significância  $< 0,05$ . Esta pesquisa armazenará os dados coletados em repositório de dados, e a identidade dos participantes não será revelada nesses dados, pois os dados só serão armazenados de forma anônima e não haverá possibilidade de reversão da anonimização.

#### Critério de Inclusão:

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Estudantes e funcionários da UNICAMP entre 18 e 60 anos de idade.

**Critério de Exclusão:**

O participante não deve participar deste estudo se possuir pelo menos uma das seguintes questões:

- Alergia, intolerância ao glúten (doença celíaca) e/ou sensibilidade ao glúten não-celíaca que pode se agravar com a ingestão de glúten causando desconfortos físicos, pois este produto foi produzido em equipamento que produz massas a partir de farinha de trigo, tendo o risco de contaminação cruzada por traços de glúten.
- Intolerância ou efeitos adversos ao consumo de milho e/ou a sua proteína zeína, pois os produtos contêm milho como ingrediente.
- Intolerância ou efeitos adversos ao consumo de batata, pois os produtos contêm batata como ingrediente.
- Intolerância ou efeitos adversos ao consumo de tremoço, pois os produtos contêm tremoço como ingrediente.
- Intolerância ou efeitos adversos ao consumo do aditivo INS-471 (mono e diglicerídeos de ácidos graxos), pois os produtos contêm esse aditivo. O participante não pode participar deste estudo se tiver menos de 18 anos.

**Desfecho Primário:**

- Pontuação média de aceitação das massas alimentícias secas.
- Pontuação média de aceitação das massas alimentícias cozidas.
- Pontuação média de intenção de compra das massas alimentícias secas.
- Pontuação média de intenção de consumo das massas alimentícias cozidas.

**Cronograma de execução**

Características sensoriais das massas alimentícias otimizadas: 16/08/2023 a 31/08/2023

Qualificação de projeto: 26/05/2023 a 26/05/2023

**Orçamento**

Copos de plástico 180 mL/Custeio: R\$ 18,00

Pratos descartáveis 15 cm/Custeio: R\$ 29,00

Guardanapo/Custeio: R\$ 15,00

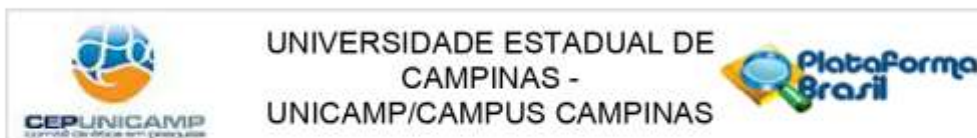
Caneta/Custeio: R\$ 55,00

Pote de plástico redondo PP 145mL com tampa/Custeio: R\$ 25,00

Xerox/Custeio: R\$ 57,00

Total: R\$ R\$ 199,00

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A proponente informa que o projeto será financiado pela Capes.

Equipe da pesquisa:

Orientadora: Profa. Dra. Maria Teresa Pedrosa Silva Clerici-Departamento de Ciência de Alimentos e Nutrição-Faculdade de Engenharia de Alimentos-Unicamp.

Mestranda: Katherine Kelly Navarro Valdez-Departamento de Ciência de Alimentos e Nutrição-Faculdade de Engenharia de Alimentos-Unicamp.

**Considerações sobre os Termos de apresentação obrigatória:**

Foram apresentadas a Folha de Rosto, assinada pela Diretora Associada-Faculdade de Engenharia de Alimentos-Unicamp; o documento com Informações Básicas do projeto; o projeto detalhado; o projeto detalhado relativo à parte sensorial; o Termo de Consentimento Livre e Esclarecido; o comprovante de vínculo acadêmico da proponente do estudo com a Unicamp; Ficha de avaliação sensorial de amostras secas (Anexo 2); Ficha de avaliação sensorial de amostras cozidas (Anexo 3).

**Recomendações:**

Nenhuma

**Conclusões ou Pendências e Lista de Inadequações:**

Orçamento

Pendência 1: ATENDIDA

O orçamento, postado como documento separado, na Plataforma Brasil difere do que consta no arquivo "PB\_INFORMAÇÕES\_BÁSICAS\_DO\_PROJETO\_2129482.pdf". Solicita-se adequação.

Resposta:

O orçamento, postado como documento separado, foi modificado. O arquivo "Orçamento\_(versão\_final\_atualizada)" foi postado como novo documento na Plataforma Brasil. O orçamento (marcado em amarelo) neste documento é igual do que consta no arquivo "PB\_INFORMAÇÕES\_BÁSICAS\_DO\_PROJETO\_2129482.pdf".

Projeto completo detalhado

Pendência 2: ATENDIDA

Apresentar o mesmo cronograma que consta no arquivo

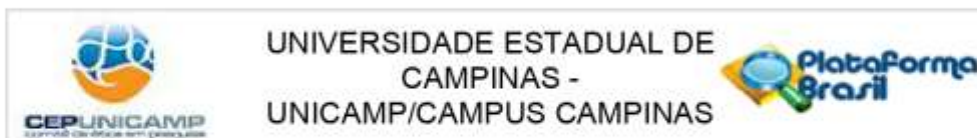
"PB\_INFORMAÇÕES\_BÁSICAS\_DO\_PROJETO\_2129482.pdf" ou o que consta no arquivo

"Projeto\_detalhado\_parte\_sensorial".

Resposta:

**Endereço:** Rua Tessália Vieira de Camargo, 126, 1º andar do Prédio I da Faculdade de Ciências Médicas  
**Bairro:** Barão Geraldo **CEP:** 13.083-887  
**UF:** SP **Município:** CAMPINAS  
**Telefone:** (19)3521-8936 **Fax:** (19)3521-7187 **E-mail:** cep@unicamp.br





Continuação do Parecer: 8.251.814

O cronograma no arquivo "Projeto\_completo\_detalhado" foi modificado. O arquivo "Projeto\_completo\_detalhado\_(versão\_final\_atualizada)" foi postado como novo documento na Plataforma Brasil. O cronograma neste documento é igual ao que consta no arquivo Projeto\_detalhado\_parte\_sensorial.

Recrutamento dos/as participantes do estudo

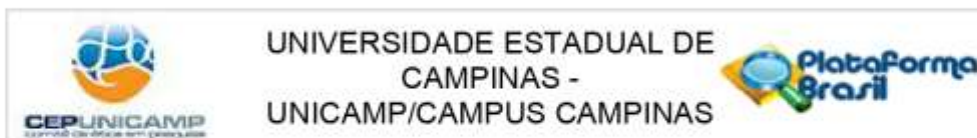
Pendência 3: ATENDIDA

Informar de que maneira serão recrutados os/as participantes da pesquisa, se via cartazes, redes sociais, emissoras de rádio, envio de e-mails, etc. No caso de cartaz, enviar o texto impresso (modelo de cartaz) a este CEP, lembrando que deve ser colocado no mesmo o CAAE, a informação que o projeto foi aprovado pelo CEP-Unicamp, o nome da pesquisadora responsável e o da orientadora e a data de aprovação do projeto pelo CEP. Informar também o(s) local(ais) em que os cartazes

serão afixados. No caso do recrutamento ser realizado via redes sociais, envio de e-mails, etc., encaminhar a este CEP o texto que será usado para essa finalidade. Em relação aos procedimentos que envolvem contato através de meio virtual ou telefônico com possíveis participantes de pesquisa, solicita-se anexar o convite que será disponibilizado ao/as potenciais participantes da pesquisa, sendo que:

- a) O convite para participação na pesquisa não deve ser feito com utilização de listas que permitam identificação do/as convidado/as nem visualização de seus dados de contato (e-mail, WhatsApp, telefone, redes sociais, etc.) por terceiros.
- b) Qualquer convite individual enviado online (email, WhatsApp, redes sociais) só poderá ter um/a remetente e um/a destinatário/a ou ser enviado na forma de lista oculta.
- c) Qualquer convite individual deve esclarecer o/a candidato/a a participante de pesquisa que, antes de responder as perguntas do/a pesquisador/a, disponibilizadas em ambiente não presencial ou virtual (questionário/formulário ou entrevista), será apresentado o Termo de Consentimento Livre e Esclarecido (ou Termo de Assentimento, quando for o caso) para sua anuência.
- d) No convite, deve ficar claro ao/à participante da pesquisa que o consentimento será previamente apresentado e, caso concorde em participar, será considerada anuência quando este/a responder o questionário/formulário ou entrevista da pesquisa.
- e) O convite para participação na pesquisa deverá conter, obrigatoriamente, link para endereço eletrônico ou texto com as devidas instruções de envio, que informem ser possível, a qualquer

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**UF:** SP **Município:** CAMPINAS  
**Telefone:** (19)3521-8936 **Fax:** (19)3521-7187 **E-mail:** cep@unicamp.br



Continuação do Parecer: 8.251.814

momento e sem nenhum prejuízo, a retirada do consentimento de utilização dos dados do/a participante da pesquisa. Nessas

situações, o/a pesquisador/a responsável fica obrigado a enviar ao/a participante de pesquisa, a resposta de ciência do interesse do/a participante de pesquisa retirar seu consentimento.

Resposta:

A seguinte informação sobre o recrutamento foi adicionada em azul e marcada em amarelo nos arquivos "Projeto\_completo\_detalhado\_(versão\_final\_atualizada)" e "Projeto\_detalhado\_parte\_sensorial\_(versão\_final\_atualizada)": "O painel será composto por 60 participantes (18 - 60 anos de idade) entre estudantes e funcionários da UNICAMP, quem serão convidados por meio de cartazes que serão afixados nos murais da Faculdade de Engenharia de Alimentos e dos refeitórios da universidade."

O modelo de cartaz foi adicionado em azul e marcado em amarelo nos arquivos "Projeto\_completo\_detalhado\_(versão\_final\_atualizada)" e "Projeto\_detalhado\_parte\_sensorial\_(versão\_final\_atualizada)". Os arquivos "Modelo\_de\_cartaz", e "Projeto\_detalhado\_parte\_sensorial\_(versão\_final\_atualizada)" foram postados como novos documentos na Plataforma Brasil.

2) No Termo de Consentimento Livre e Esclarecido (documento com pendência):

Nada que informar

3) Nas Informações Básicas do Projeto (etapas da Plataforma Brasil) (documento com pendência):

Nada que informar

#### Considerações Finais a critério do CEP:

-Este estudo só pode ser iniciado após aprovação pelo CEP, conforme compromisso assumido pelo/a PESQUISADOR/A RESPONSÁVEL, cumprindo a Resolução 466/2012, item XI.2 letra a e a Norma Operacional nº 001/2013 do CNS.

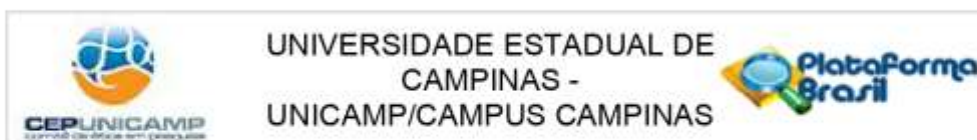
-Relatórios parciais e final devem ser apresentados, respectivamente seis meses após a data deste parecer de aprovação e ao término do estudo, por meio da Plataforma Brasil, via notificação do tipo "relatório", para que sejam devidamente apreciados pelo CEP, conforme Resolução CNS nº 466/12, item XI.2.d e Resolução CNS nº 510/16, art. 28. item V.

-O/A pesquisador/a deve manter os dados da pesquisa em arquivo, físico ou digital, sob sua guarda e responsabilidade, por um período de 5 anos após o término da pesquisa.

-O/A pesquisador/a deve desenvolver a pesquisa conforme delineada no protocolo aprovado. Se o/a pesquisador/a considerar a descontinuação do estudo, esta deve ser justificada e somente ser

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Continuação do Parecer: 8.251.814

realizada após análise das razões da descontinuidade pelo CEP que o aprovou.

-O/A pesquisador/a deve aguardar o parecer do CEP quanto à descontinuação, exceto quando perceber risco ou dano não previsto ao/à participante ou quando constatar a superioridade de uma estratégia diagnóstica ou terapêutica oferecida a um dos grupos da pesquisa, isto é, somente em caso de necessidade de ação imediata com intuito de proteger o/as participantes.

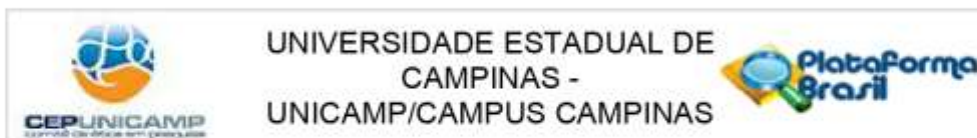
-O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo. É responsabilidade do/a pesquisador/a assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido e enviar notificação ao CEP e, se for o caso, à Agência Nacional de Vigilância Sanitária (ANVISA) junto com seu posicionamento.

-Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas e aguardando aprovação do CEP para continuidade da pesquisa.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_2129482.pdf	17/08/2023 21:28:10		Aceito
Outros	Carta_resposta.docx	17/08/2023 21:22:40	Katherine Kelly Navarro Valdez	Aceito
Outros	Modelo_de_cartaz.pdf	17/08/2023 21:21:51	Katherine Kelly Navarro Valdez	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_detalhado_parte_sensorial_versao_final_atualizada.docx	17/08/2023 21:20:35	Katherine Kelly Navarro Valdez	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_detalhado_parte_sensorial.docx	17/08/2023 21:18:57	Katherine Kelly Navarro Valdez	Aceito
Outros	Projeto_completo_detalhado_versao_final_atualizada.docx	17/08/2023 21:18:27	Katherine Kelly Navarro Valdez	Aceito
Outros	Projeto_completo_detalhado.docx	17/08/2023 21:17:05	Katherine Kelly Navarro Valdez	Aceito
Orçamento	Orcamento.xlsx	17/08/2023 21:12:05	Katherine Kelly Navarro Valdez	Aceito
Orçamento	Orcamento_versao_final_atualizada.xlsx	17/08/2023 21:10:43	Katherine Kelly Navarro Valdez	Aceito
Outros	AtestadoMatricula.pdf	28/04/2023	Katherine Kelly	Aceito

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Continuação do Parecer: 8.251.814

Outros	AtestadoMatricula.pdf	12:56:40	Navarro Valdez	Aceito
Outros	Carta_de_encaminhamento.pdf	27/04/2023 20:09:55	Katherine Kelly Navarro Valdez	Aceito
Outros	Fichas_sensoriais_de_massas.pdf	27/04/2023 20:07:31	Katherine Kelly Navarro Valdez	Aceito
Declaração de Pesquisadores	Declaracao_dos_envolvidos.pdf	27/04/2023 20:04:27	Katherine Kelly Navarro Valdez	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	27/04/2023 20:00:14	Katherine Kelly Navarro Valdez	Aceito
Folha de Rosto	Folha_de_Rostoassinada.pdf	27/04/2023 19:59:53	Katherine Kelly Navarro Valdez	Aceito

**Situação do Parecer:**

Aprovado

**Necessita Apreciação da CONEP:**

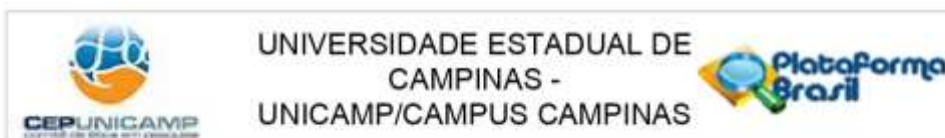
Não

CAMPINAS, 21 de Agosto de 2023

Assinado por:  
Renata Maria dos Santos Celeghini  
(Coordenador(a))

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## B. Amendment approval for sensory analysis of pasta (in Article II).



### PARECER CONSUBSTANCIADO DO CEP

#### DADOS DA EMENDA

**Título da Pesquisa:** Qualidade tecnológica, nutricional e sensorial de massas alimentícias de milho (*Zea mays*) contendo misturas de farinhas de tremço (*Lupinus mutabilis* Sweet) e batata (*Solanum tuberosum*) andinos.

**Pesquisador:** Katherine Kelly Navarro Valdez

**Área Temática:**

**Versão:** 3

**CAAE:** 69240923.8.0000.5404

**Instituição Proponente:** Faculdade de Engenharia de Alimentos

**Patrocinador Principal:** FUND COORD DE APERFEICOAMENTO DE PESSOAL DE NIVEL SUP  
Programa Nacional de Crédito Educativo

#### DADOS DO PARECER

**Número do Parecer:** 6.311.900

#### Apresentação do Projeto:

Trata-se de uma emenda que visa assemelhar a avaliação sensorial de todas as massas alimentícias cozidas em estudo à forma mais comum e tradicional de consumir massa, que é com a adição de molho de tomate.

#### Objetivo da Pesquisa:

Mantidos em relação ao projeto original.

#### Avaliação dos Riscos e Benefícios:

Mantidos em relação ao projeto original.

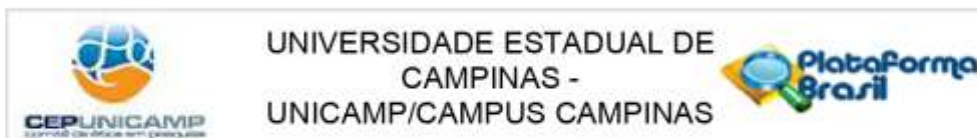
#### Comentários e Considerações sobre a Pesquisa:

O pesquisador responsável informou no Formulário de Informações Básicas da Plataforma Brasil:

"Justificativa da Emenda: Assemelhar a avaliação sensorial de todas as massas alimentícias cozidas em estudo à forma mais comum e tradicional de consumir massa, que é com a adição de molho de tomate.". O CEP-Unicamp aprovou este projeto (CAAE 69240923.8.0000.5404) conforme Parecer: 6.251.814, datado de 21Agosto2023. Esta emenda propõe as seguintes alterações na parte experimental do estudo:

1) Adição de molho de tomate comercial "Pomarola" nas porções de massas alimentícias cozidas que serão servidas aos participantes na análise sensorial.

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Continuação do Parecer: 6.311.900

- 2) Modificação do TCLE devido a adição de molho de tomate comercial nas porções de massas alimentícias cozidas que serão servidas aos participantes na análise sensorial.
  - 3) Modificação do Projeto\_detalhado\_parte\_sensorial devido a adição de molho de tomate comercial nas porções de massas alimentícias cozidas que serão servidas aos participantes na análise sensorial.
  - 4) Modificação do Projeto\_completo\_detalhado devido a adição de molho de tomate comercial nas porções de massas alimentícias cozidas que serão servidas aos participantes na análise sensorial.
  - 5) Modificação da imagem direita no Modelo\_de\_cartaz (foi colocada uma figura de massa alimentícia cozida em molho de tomate) devido a adição de molho de tomate comercial nas porções de massas alimentícias cozidas que serão servidas aos participantes na análise sensorial.
- Não há outras modificações no projeto, além das mencionadas, não sendo introduzidas outras alterações no conjunto do protocolo, razão pela qual não foram acrescentados outros arquivos, além daqueles mencionados na Carta enviada ao CEP.

**Considerações sobre os Termos de apresentação obrigatória:**

Na avaliação desta emenda foi analisado o documento:

"PB\_INFORMAÇÕES\_BÁSICAS\_2207095\_E1.pdf 01/09/2023 13:43:27"

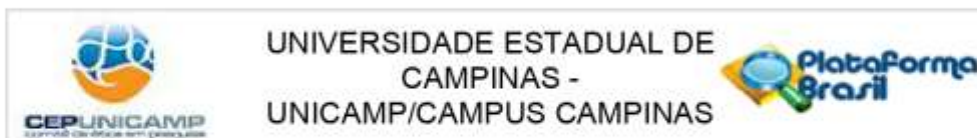
e a carta encaminhada ao CEP-Unicamp, postada na Plataforma Brasil, com a justificativa da Emenda e contendo as alterações introduzidas na parte experimental do projeto detalhado e, em consequência, no TCLE.

**Recomendações:**

- a) Solicita-se correção, no TCLE, do endereço eletrônico (mclerici@fea.unicamp.br) da orientadora do estudo. Há muito tempo a Unicamp alterou o formato dos endereços eletrônicos de docentes, pesquisadore(a)s e funcionário(a)s para o tipo: "nome@unicamp.br".
- b) Caso seja usado molho Pomarola com ingredientes que alteram o sabor do molho tradicional (sem os ingredientes) pergunta-se se os ingredientes não irão interferir nas análises sensoriais mascarando os efeitos, visto que afetam substancialmente os sabores dos mesmos.
- c) Caso a proponente não vá usar o molho "Pomarola" tradicional e considerando que os diversos tipos de molho "Pomarola" apresentam ingredientes que eventualmente podem causar alergias a(à)s participantes, solicita-se que ela apresente Notificação ao CEP inserindo esta informação como critério de exclusão no TCLE.
- d) Com relação ao cartaz para recrutamento de participantes do estudo, ao contrário do que afirma a proponente, a imagem direita apresentada no "novo" cartaz não mostra a massa alimentícia cozida em molho de tomate, pois esta imagem é idêntica à que está no cartaz.

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Continuação do Parecer: 6.311.900

anteriormente enviado e que consta no projeto originalmente aprovado pelo CEP. Ao que se sabe sobre preparação de massas alimentícias via cozimento ("macarronada"), a massa é cozinhada em água fervente (com um pouco de óleo e com ou sem adição de sal) e somente APÓS o cozimento se acrescenta molho, não sendo a massa cozinhada diretamente no molho.

**Conclusões ou Pendências e Lista de Inadequações:**

Emenda aprovada.

**Considerações Finais a critério do CEP:**

- O participante da pesquisa deve receber uma via do Termo de Consentimento Livre e Esclarecido, na íntegra, por ele assinado (quando aplicável).

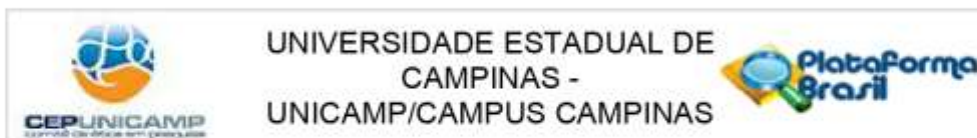
- O participante da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado (quando aplicável).

- O pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado. Se o pesquisador considerar a descontinuação do estudo, esta deve ser justificada e somente ser realizada após análise das razões da descontinuidade pelo CEP que o aprovou. O pesquisador deve aguardar o parecer do CEP quanto à descontinuação, exceto quando perceber risco ou dano não previsto ao participante ou quando constatar a superioridade de uma estratégia diagnóstica ou terapêutica oferecida a um dos grupos da pesquisa, isto é, somente em caso de necessidade de ação imediata com intuito de proteger os participantes.

- O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo. É papel do pesquisador assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.

- Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas e aguardando a aprovação do CEP para continuidade da pesquisa. Em caso de projetos do Grupo I ou II apresentados anteriormente à ANVISA, o pesquisador ou patrocinador deve enviá-las também à mesma, junto com o parecer aprovatório do CEP, para serem juntadas ao protocolo inicial.

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Continuação do Parecer: 6.311.900

- Relatórios parciais e final devem ser apresentados ao CEP, inicialmente seis meses após a data deste parecer de aprovação e ao término do estudo.

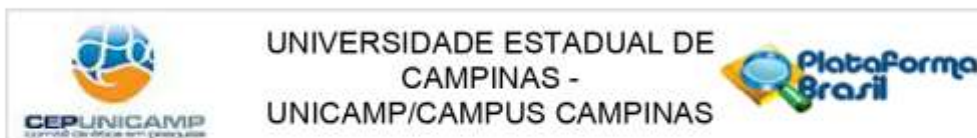
-Lembramos que segundo a Resolução 466/2012, item XI.2 letra e, "cabe ao pesquisador apresentar dados solicitados pelo CEP ou pela CONEP a qualquer momento".

-O pesquisador deve manter os dados da pesquisa em arquivo, físico ou digital, sob sua guarda e responsabilidade, por um período de 5 anos após o término da pesquisa.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_2207095_E1.pdf	01/09/2023 13:43:27		Aceito
Parecer Anterior	PB_PARECER_CONSUBSTANCIADO_CEP_6251814.pdf	01/09/2023 13:41:04	Katherine Kelly Navarro Valdez	Aceito
Outros	CEP_Carta_emenda_CEP.docx	01/09/2023 13:40:02	Katherine Kelly Navarro Valdez	Aceito
Outros	E_Modelo_de_cartaz_versao_3.pdf	01/09/2023 13:38:07	Katherine Kelly Navarro Valdez	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	E_TCLE_versao_3.docx	01/09/2023 13:33:43	Katherine Kelly Navarro Valdez	Aceito
Projeto Detalhado / Brochura Investigador	E_Projeto_detalhado_parte_sensorial_versao_3.docx	01/09/2023 13:31:56	Katherine Kelly Navarro Valdez	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_detalhado_parte_sensorial_versao_2.docx	01/09/2023 13:28:29	Katherine Kelly Navarro Valdez	Aceito
Brochura Pesquisa	E_Projeto_completo_detalhado_versao_3.docx	01/09/2023 13:28:07	Katherine Kelly Navarro Valdez	Aceito
Brochura Pesquisa	Projeto_completo_detalhado_versao_2.docx	01/09/2023 13:26:22	Katherine Kelly Navarro Valdez	Aceito
Outros	Modelo_de_cartaz.pdf	17/08/2023 21:21:51	Katherine Kelly Navarro Valdez	Aceito
Orçamento	Orcamento.xlsx	17/08/2023 21:12:05	Katherine Kelly Navarro Valdez	Aceito
Orçamento	Orcamento_versao_final_atualizada.	17/08/2023	Katherine Kelly	Aceito

**Endereço:** Rua Tessália Vieira de Camargo, 126, 1º andar do Prédio I da Faculdade de Ciências Médicas  
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Continuação do Parecer: 6.311.900

Orçamento	xlsx	21:10:43	Navarro Valdez	Aceito
Outros	AtestadoMatricula.pdf	28/04/2023 12:56:40	Katherine Kelly Navarro Valdez	Aceito
Outros	Carta_de_encaminhamento.pdf	27/04/2023 20:09:55	Katherine Kelly Navarro Valdez	Aceito
Outros	Fichas_sensoriais_de_massas.pdf	27/04/2023 20:07:31	Katherine Kelly Navarro Valdez	Aceito
Declaração de Pesquisadores	Declaracao_dos_envolvidos.pdf	27/04/2023 20:04:27	Katherine Kelly Navarro Valdez	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	27/04/2023 20:00:14	Katherine Kelly Navarro Valdez	Aceito
Folha de Rosto	Folha_de_Rosto_assinada.pdf	27/04/2023 19:59:53	Katherine Kelly Navarro Valdez	Aceito

**Situação do Parecer:**

Aprovado

**Necessita Apreciação da CONEP:**

Não

CAMPINAS, 20 de Setembro de 2023

Assinado por:  
Renata Maria dos Santos Celeghini  
(Coordenador(a))

**Endereço:** Rua Tessália Vieira de Camargo, 126, 1º andar do Prédio I da Faculdade de Ciências Médicas  
**Bairro:** Barão Geraldo **CEP:** 13.083-887  
**UF:** SP **Município:** CAMPINAS  
**Telefone:** (19)3521-8936 **Fax:** (19)3521-7187 **E-mail:** cep@unicamp.br

### C. Consumer consent form for sensory analysis (in Article II)

#### TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

##### Título da pesquisa:

Qualidade tecnológica, nutricional e sensorial de massas alimentícias de milho (*Zea mays*) contendo misturas de farinhas de tremçoço (*Lupinus mutabilis*) e batata (*Solanum tuberosum*) andinos

##### Nome do(s) responsável(is):

Mestranda Katherine Navarro Valdez  
Orientadora: Prof. Dra. Maria Teresa Pedrosa Silva Clerici

Número do CAAE: 69240923.8.0000.5404

Você está sendo convidado a participar de uma pesquisa. Este documento, chamado Termo de Consentimento Livre e Esclarecido, visa assegurar seus direitos como participante da pesquisa e é elaborado em duas vias, assinadas e rubricadas pelo pesquisador e pelo participante/responsável legal, sendo que uma via deverá ficar com você e outra com o pesquisador.

Por favor, leia com atenção e calma, aproveitando para esclarecer suas dúvidas. Se houver perguntas antes ou mesmo depois de assiná-lo, você poderá esclarecê-las com o pesquisador. Se preferir, pode levar este Termo para casa e consultar seus familiares ou outras pessoas antes de decidir participar. Não haverá nenhum tipo de penalização ou prejuízo se você não aceitar participar ou retirar sua autorização em qualquer momento.

##### Justificativa e objetivos:

Esta pesquisa tem como um dos seus objetivos, a avaliação sensorial de massas alimentícias sem glúten feitas principalmente de farinha de milho (fubá mimoso), com ou sem adição de farinhas de batata e de tremçoço andino, as quais foram obtidas trás o processamento de moenda do tubérculo de batata e semente de tremçoço limpos no Peru. A batata (*Solanum tuberosum* var. *Yungay*) é de cor amarela, muito conhecida como acompanhante nos guisados peruanos, e é considerada boa fonte de carboidratos, enquanto o tremçoço andino (*Lupinus mutabilis* Sweet) é reconhecido por seu alto teor de proteína e gorduras insaturadas que, juntamente com a batata, são fontes promissórias para o combate contra a desnutrição. Um aditivo regulamentado pela ANVISA que foi utilizado para o preparo destas massas alimentícias é um estabilizante chamado ésteres de mono e diglicerídeos de ácidos graxos (INS-471).

##### Procedimentos:

Participando do estudo você será convidado a ler uma ficha informativa sobre dois alimentos peruanos (batata "Yungay" e tremçoço andino). Depois, será convidado a avaliar visualmente os atributos de aparência e cor de duas amostras de massa alimentícia sem glúten em estado seco e utilizar uma escala de 9 pontos para marcar com [X] o quanto você gostou ou desgostou de cada atributo, bem como utilizar uma escala de 5 pontos para marcar com [X] a certeza ou incerteza de sua intenção de compra. Quando essa avaliação acabar, você será convidado a avaliar os atributos de aparência, cor, aroma, sabor, textura e impressão global de duas amostras de massa alimentícia sem glúten em estado cozido, todas com um mesmo molho de tomate, e utilizar uma escala de 9 pontos para marcar com [X] o quanto você gostou ou desgostou de cada atributo, bem como utilizar uma escala de 5 pontos para marcar com [X] a certeza ou incerteza de sua intenção de consumo. O tempo total previsto da avaliação é de 15 a 20 minutos.

Rubrica do pesquisador: \_\_\_\_\_ Rubrica do participante: \_\_\_\_\_



**Desconfortos e riscos:**

Você **não** deve participar deste estudo se possuir pelo menos uma das seguintes questões:

- Alergia, intolerância ao glúten (doença celíaca) e/ou sensibilidade ao glúten não-celíaca que pode se agravar com a ingestão de glúten causando desconfortos físicos, pois este produto foi produzido em equipamento que produz massas a partir de farinha de trigo, tendo o risco de contaminação cruzada por traços de glúten.
- Intolerância ou efeitos adversos ao consumo de milho e/ou a sua proteína zeína, pois os produtos contêm milho como ingrediente.
- Intolerância ou efeitos adversos ao consumo de batata, pois os produtos contêm batata como ingrediente.
- Intolerância ou efeitos adversos ao consumo de tremoço, pois os produtos contêm tremoço como ingrediente.
- Intolerância ou efeitos adversos ao consumo de goma guar, pois os produtos contêm esse coadjuvante.
- Intolerância ou efeitos adversos ao consumo do aditivo INS-471 (mono e diglicerídeos de ácidos graxos), pois os produtos contêm esses lipídios.
- Intolerância ou efeitos adversos ao consumo de molho de tomate ou qualquer um dos seus ingredientes, pois o produto contém tomate, cebola, amido modificado, sal, salsa e alho.

**Benefícios:**

Não haverá benefícios diretos aos participantes deste estudo. Porém, o participante poderá saber de todos os benefícios que esta análise sensorial trará. Os resultados desta análise permitirão conhecer o nível de aceitação de massas alimentícias otimizadas, ajudando a saber quanto elas são desejáveis para o consumidor, bem como a intenção de compra e de consumo. Através dos resultados, será deduzido se as massas têm potencial para ser comercializadas no mercado visando fornecer uma opção inovadora e nutritiva de massa alimentícia sem glúten que pode ser adequada não apenas para celíacos e pessoas com alergia ao glúten ou trigo, mas também para consumidores que veem neste tipo de produto uma opção saudável para ser degustada, bem como divulgar a inclusão de ingredientes andinos como a batata e o tremoço em produtos similares.

**Acompanhamento e assistência:**

O participante tem o direito à assistência integral e gratuita devido a danos diretos e indiretos, imediatos e tardios, pelo tempo que for necessário. O pesquisador estará à disposição para esclarecer as dúvidas do participante relacionadas ao produto e à avaliação sensorial durante todo o estudo. O participante pode ser descontinuado do estudo caso apresente algum sintoma que lhe cause desconforto, principalmente de tipo alérgico ou de intolerância, logo após consumir as massas alimentícias, cabendo ao pesquisador o acompanhamento do participante para que seja providenciado atendimento médico imediato.

**Sigilo e privacidade:**

Você tem a garantia de que sua identidade será mantida em sigilo e nenhuma informação será dada a outras pessoas que não façam parte da equipe de pesquisadores. Na divulgação dos resultados desse estudo, seu nome não será citado.

**Ressarcimento e indenização:**

Nenhum custo econômico será gerado aos participantes da pesquisa e, portanto, não haverá forma de reembolso, pois os participantes serão alunos e funcionários da UNICAMP que,

Rubrica do pesquisador: \_\_\_\_\_ Rubrica do participante: \_\_\_\_\_

dentro de sua rotina de estudos ou trabalho, comparecerão ao estudo. O participante terá a garantia ao direito à indenização diante de eventuais danos decorrentes da pesquisa.

#### **Tratamento dos dados:**

Esta pesquisa prevê o armazenamento dos dados coletados em repositório de dados, em local virtual de acesso público, com o objetivo de possível reutilização, verificação e compartilhamento em trabalhos de colaboração científica com outros grupos de pesquisa. Sua identidade não será revelada nesses dados, pois os dados só serão armazenados de forma anônima (isto é, os dados não terão identificação), utilizando mecanismos que impeçam a possibilidade de associação, direta ou indireta com você. Cabe ressaltar que quem compartilhar os dados também não terá possibilidade de identificação dos participantes de quem os dados se originaram. Sendo assim, não haverá possibilidade de reversão da anonimização.

#### **Contato:**

Em caso de dúvidas sobre a pesquisa, você poderá entrar em contato com os pesquisadores Katherine Navarro Valdez, e-mail: [k203682@dac.unicamp.br](mailto:k203682@dac.unicamp.br), telefone 51-923470193 e Dra. Maria Teresa Pedrosa Silva Clerici, e-mail: [mcclerici@fea.unicamp.br](mailto:mcclerici@fea.unicamp.br), telefone 55-19-3521-3434, pertencentes à Universidade Estadual de Campinas (UNICAMP) - Faculdade de Engenharia de Alimentos - Departamento de Ciência de Alimentos e Nutrição, localizado em Rua Monteiro Lobato, 80 – Cidade Universitária, Campinas – SP, 13083-862.

Em caso de denúncias ou reclamações sobre sua participação e sobre questões éticas do estudo, você poderá entrar em contato com a secretaria do Comitê de Ética em Pesquisa (CEP) da UNICAMP, de segunda a sexta-feira, das 08:00hs às 11:30hs e das 13:00hs às 17:30hs à Rua: Tessália Vieira de Camargo, 126; CEP 13083-887 Campinas – SP; telefone (19) 3521-8936 ou (19) 3521-7187; e-mail: [cep@unicamp.br](mailto:cep@unicamp.br)

#### **O Comitê de Ética em Pesquisa (CEP).**

O papel do CEP é avaliar e acompanhar os aspectos éticos de todas as pesquisas envolvendo seres humanos. A Comissão Nacional de Ética em Pesquisa (CONEP), tem por objetivo desenvolver a regulamentação sobre proteção dos seres humanos envolvidos nas pesquisas. Desempenha um papel coordenador da rede de Comitês de Ética em Pesquisa (CEPs) das instituições, além de assumir a função de órgão consultor na área de ética em pesquisas.

#### **Consentimento livre e esclarecido:**

Após ter recebido esclarecimentos sobre a natureza da pesquisa, seus objetivos, métodos, benefícios previstos, potenciais riscos e o incômodo que esta possa acarretar, aceito participar:

Nome do (a) participante: \_\_\_\_\_

Data: \_\_\_\_/\_\_\_\_/\_\_\_\_.

(Assinatura do participante ou nome e assinatura do seu RESPONSÁVEL LEGAL)

#### **Responsabilidade do Pesquisador:**

Asseguro ter cumprido as exigências da resolução 466/2012 CNS/MS e complementares na elaboração do protocolo e na obtenção deste Termo de Consentimento Livre e Esclarecido. Asseguro, também, ter explicado e fornecido uma via deste documento ao participante. Informo que o estudo foi aprovado pelo CEP perante o qual o projeto foi apresentado. Comprometo-me a utilizar o material e os dados obtidos nesta pesquisa exclusivamente para as finalidades previstas neste documento ou conforme o consentimento dado pelo participante.

Data: \_\_\_\_/\_\_\_\_/\_\_\_\_.

(Assinatura do pesquisador)

Rubrica do pesquisador: \_\_\_\_\_ Rubrica do participante: \_\_\_\_\_

# D. Informative sheets about the Andean raw materials used in the sensory analysis



### E. Evaluation sheets used in the sensory analysis to assess consumer acceptance

#### TESTE DE ACEITAÇÃO SENSORIAL DE MASSAS ALIMENTÍCIAS SEM GLÚTEN

Nome: \_\_\_\_\_

Data: \_\_\_\_/\_\_\_\_/\_\_\_\_

Nacionalidade: \_\_\_\_\_

#### Instruções:

- 1) Você recebeu uma amostra codificada de massa alimentícia seca. Por favor, utilize a escala abaixo para avaliar os atributos da amostra. Marque com [ X ] o quanto você gostou ou desgostou de cada atributo. Indique apenas uma opção para cada atributo.

	Número de 3 dígitos da AMOSTRA _____	Atributos	
		Aparência	Cor
▲	Gostei extremamente		
↑	Gostei muito		
—	Gostei moderadamente		
—	Gostei ligeiramente		
—	Indiferente		
—	Desgostei ligeiramente		
—	Desgostei moderadamente		
—	Desgostei muito		
▼	Desgostei extremamente		

- 2) Baseado na avaliação acima, se esta massa fosse comercializada, marque com [ X ] a resposta mais adequada com sua intenção de compra:

	Intenção de compra
▲	Certamente compraria
↑	Provavelmente compraria
—	Talvez compraria / Talvez não compraria
—	Provavelmente não compraria
▼	Certamente não compraria

### TESTE DE ACEITAÇÃO SENSORIAL DE MASSAS ALIMENTÍCIAS SEM GLÚTEN

Nome: \_\_\_\_\_

Data: \_\_\_\_/\_\_\_\_/\_\_\_\_

Nacionalidade: \_\_\_\_\_

#### Instruções:

- 1) Você recebeu uma amostra codificada de massa alimentícia cozida. Por favor, prove a amostra e utilize a escala abaixo para avaliar os atributos da amostra. Marque com [ X ] o quanto você gostou ou desgostou de cada atributo. Indique apenas uma opção para cada atributo. Para passar de uma amostra para outra, beba um pouco de água.

	Número de 3 dígitos da AMOSTRA _____	Atributos					Impressão global
		Aparência	Cor	Aroma	Sabor	Textura	
↑	Gostei extremamente						
↑	Gostei muito						
↑	Gostei moderadamente						
↑	Gostei ligeiramente						
↑	Indiferente						
↑	Desgostei ligeiramente						
↑	Desgostei moderadamente						
↑	Desgostei muito						
↓	Desgostei extremamente						

- 2) Baseado na sua impressão global da amostra, marque com [ X ] a resposta mais adequada com sua intenção de compra:

	Intenção de compra
↑	Certamente compraria
↑	Provavelmente compraria
↑	Talvez compraria / Talvez não compraria
↑	Provavelmente não compraria
↓	Certamente não compraria