# Breakfast snacks made from extruded leguminous flour

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## List of abbreviations

**AGE**: Rice: Chickpea (50:50, *w/w*)

**AOAC**: Association of Official Analytical Chemists

**ARE**: Rice Flour (100%)

**ATCC**: American Type Culture Collection

**ANOVA**: Analysis of Variance

**BB :** Black Bean

**CAGR**: Compound Annual Growth Rate

**CB :** Carioca Bean

**CFU**: Colony Forming Units

**CIE**: International Commission on Illumination

**CPC :** Chickpea Protein Concentrate

**CSW**: Chromatographic Software for Windows

**CW :** Cowpea

**DMSO**: Dimethyl Sulfoxid

**dw**: Dry Weight

**ER :** Expansion Ratio

**EUFIC**: European Food Information Council

**FA :** Fatty Acids

**FAME**: Fatty Acid Methyl Esters

**FAO**: Food and Agriculture Organization

**fw**: Fresh Weight

**GAE**: Gallic Acid Equivalent

**GAE**: Rice: Chickpea (60:40, *w/w*)

**GC-FID**: Gas Chromatography - Flame Ionization Detector

**GE**: Chickpea Flour (100%)

**HME :** High-Moisture Extrusion

**HMMA :** High-Moisture Meat Analogues

**HPC :** Hempseed Protein Concentrate

**HPLC-RI**: High-Performance Liquid Chromatography - Refractive Index

**INT**: Iodonitrotetrazolium Choloride

**Kcal**: Kilocalories

**kg**: Kilogram

**KJ**: Kilojoules

**LDL** : Low-Density Lipoprotein Cholesterol

**MA**: Malt Agar

**MBC**: Minimum Bactericidal Concentration

**MIC**: Minimum Inhibitory Concentration

**mL**: Milliliter

**MUFA:** Monounsaturated Fatty Acid

**n.a.**: Non Applicable

**NCTC**: National Collection of Type Cultures

**NPGA**: Northern Pulse Growers Association

**pH**: Potential of Hydrogen

**PUFA:** Polyunsaturated Fatty Acids

**RB :** Red Bean

**rpm**: Revolutions Per Minute

**SBE**: Semolina: Chickpea (60:40, w/w)

**SD**: Standard Deviation

**SE**: Semolina Flour (100%)

**SGE**: Semolina: Chickpea (50:50, w/w)

**SFA:** Saturated Fatty Acids

**S.L.**: Limited Liability Company

**SME :** Specific Mechanical Energy

**TFA :** Trans Fatty Acids

**TSB**: Tryptic Soy Broth

**USA**: United States of America

**USDA**: United States Department of Agriculture

**USD**: United States Dollar

**WG :** Wheat Gluten

**w/w**: Weight by Weight

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## Abstract

Although legumes are recognized as nutritionally richer than cereal grains, contributing a higher content of protein, dietary fiber, complex carbohydrates, and bioactive compounds, their global consumption often remains below recommended levels. Many countries, particularly in Europe, do not meet the suggested intake targets. In this regard, it becomes crucial to address alternatives that allow for offering nutritionally more attractive, convenient, and healthy foods that can be included in the daily diet and contribute to the maintenance of consumer health.

From this perspective, the present study aims to address the need for highly nutritious snacks, leveraging extrusion technology to enrich corn semolina flour and rice flour with chickpea flour in order to obtain protein-enriched ready-to-eat snacks. The specific objectives encompassed defining the optimized extrusion parameters as well as the proportions of each flour in the mixture to ensure not only the improvement of the protein content but also the organoleptic and texture characteristics expected by the consumer of this type of product. Additionally, the study intended to characterize the resulting snacks physically and nutritionally (fat, ash, protein, carbohydrates, and energy value) as well as to determine the chemical composition, and evaluate the bioactive properties, specifically antibacterial and antifungal activities.

The extrusion process was achieved using a twin-screw extruder to combine chickpea flour with rice flour or corn semolina. The process parameters, including screw speed, water flow, and a multi-zone temperature profile, were meticulously recorded to correlate with the resulting product characteristics, such as desirable color, crunchy texture, homogeneous flow, particle diameter, and protein content. For the Rice:Chickpea (AGE) flour mixture, the optimized characteristics (white color, crunchy texture, homogeneous flow, and good particle diameter) were achieved using a high screw speed of 600 rpm and a die temperature of 180°C (AGE4). In turn, the Corn Semolina:Chickpea (SGE1) flour mixture achieved optimized physical attributes (golden color, crunchy texture, homogeneous flow, and medium particle diameter) at a lower screw speed of 400 rpm and a relatively lower die temperature of 120°C.

Protein content was evaluated using the Kjeldahl method. Among all tested conditions, measured protein levels ranged from 10.23 g/100 g PS (DW) to a maximum of 13.39 g/100 g

PS (AGE 4). Specifically, the Corn Semolina:Chickpea (50:50) mixtures reached up to 12.58 g/100 g PS (SGE 1). The two formulations with the best performance in terms of physical characteristics and protein content were selected for complete nutritional and chemical characterization: SGE1 (Corn Semolina:Chickpea, 50:50) and AGE4 (Rice:Chickpea, 50:50).

Both SGE and AGE are classified as ready-to-eat snacks with low fat content, high carbohydrate content, and high protein content. They exhibited very low moisture (SGE: 3.97%; AGE: 3.63%) and very low fat content (SGE: 0.048 g/100 g fresh weight; AGE: 0.043 g/100 g fresh weight), which are desirable attributes for shelf stability and health. The AGE formulation demonstrated a statistically higher protein content (13.39 ± 0.01 g/100 g dry weight) compared to SGE (12.58 ± 0.10 g/100 g dry weight), suggesting that the Rice:Chickpea mixture offers a more protein-rich snack option. Carbohydrates were the main macronutrient (SGE: 83.28 g/100 g fresh weight; AGE: 82.81 g/100 g fresh weight), resulting in similar overall energy values (AGE: 385 Kcal/100 g; SGE: 384 Kcal/100 g). Chemical analysis of free sugars revealed sucrose as the only free sugar present in both snacks. The low free sugar content is favorably aligned with public health recommendations. The fatty acid profiles were distinct: SGE exhibited a lipid profile rich in saturated fatty acids (AGS: 55.3 ± 0.1%), with palmitic acid (C16:0) being the most abundant (45.287 ± 0.42%). Meanwhile, AGE demonstrated a profile rich in polyunsaturated fatty acids (AGPI: 48.4 ± 0.1%), predominantly linoleic acid (C18:2n6c: 48.438 ± 0.07%). Regarding bioactive properties, the evaluation of the extracts showed limited antibacterial activity against most tested foodborne pathogens (including Gram-negative E. coli and Gram-positive S. aureus). Minimum Inhibitory Concentrations (MICs) generally exceeded the maximum concentration tested (> 10 mg/mL). However, both SGE and AGE extracts showed fungistatic activity against Aspergillus species. The MIC values for fungal inhibition against A. brasiliensis and A. fumigatus ranged between 5 mg/mL and 10 mg/mL, although neither reached a fungicidal concentration (MFC > 10 mg/mL).

This study successfully demonstrated that extrusion technology can be effectively applied in the development of ready-to-eat snacks, rich in protein, low in fat, and with energy balance, by combining chickpea flour with rice and semolina flours. The rice and chickpea formulation (AGE) showed the highest protein content and a favorable fatty acid profile, confirming the potential of legumes to increase the nutritional value of extruded products. These findings highlight the relevance of legume mixtures in formulating healthy, protein-rich snacks

that meet modern consumer demands for nutritious and practical plant-based foods, while also supporting strategies to improve global protein intake and diet quality.

**Keywords:** Chickpea Flour, Extrusion Cooking, Free Sugars, Legumes, Nutritional Characterization, Protein-Enriched, Ready-to-Eat Snacks

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## Resumo

Apesar de as leguminosas serem reconhecidas como nutricionalmente mais ricas que os grãos de cereais, contribuindo com maior teor de proteína, fibra dietética, hidratos de carbono complexos e compostos bioativos, o seu consumo global permanece frequentemente abaixo dos níveis recomendados. Muitos países, particularmente na Europa, não atingem as metas de ingestão sugeridas. Neste sentido torna-se crucial abordar alternativas que permitam oferecer alimentos nutricionalmente mais atrativos, convenientes e saudáveis que possam ser incluidos na dieta diária e contribuir para a manutenção da saúde dos consumidores.

Nesta prespetiva, o presente estudo visa abordar a necessidade de snacks altamente nutritivos, alavancando a tecnologia de extrusão para enriquecer farinha de semolina de milho e de arroz com farinha de grão-de-bico de forma a obter snacks prontos a consumir enriquecidos em proteínas. Os objetivos específicos englobaram a definição dos parâmetros otimizados de extrusão bem como, das proporções de cada farinha na mistura para garantir não só a melhoria do teor de proteínas como também as características organoléticas e de textura espectáveis pelo consumidor deste tipo de produtos. Adicionalmente, o estudo pretendeu caracterizar física e nutricional (gordura, cinzas, proteína, hidratos de carbono e valor energético) os snacks resultantes bem como, determinar a composição química, e avaliar as propriedades bioativas, especificamente as atividades antibacteriana e antifúngica.

O processo de extrusão foi alcançado utilizando um extrusor de rosca dupla para combinar farinha de grão-de-bico com farinha de arroz ou semolina de milho. Os parâmetros do processo, incluindo a velocidade da rosca, o fluxo de água e um perfil de temperatura multi-zona, foram meticulosamente registados para correlacionar com as características do produto resultante, tais como cor desejável, textura crocante, fluxo homogéneo, diâmetro de partícula e teor em proteinas.

Para a mistura de farinhas Arroz:Grão-de-bico (AGE), as características otimizadas (cor branca, textura crocante, fluxo homogéneo e bom diâmetro de partícula) foram alcançadas utilizando uma velocidade de rosca elevada de 600 rpm e uma temperatura da matriz de 180°C (AGE4). Por sua vez, a mistura de farinhas Semolina de milho:Grão-de-bico (SGE1) alcançou atributos físicos otimizados (cor dourada, textura crocante, fluxo homogéneo e diâmetro de

partícula médio) a uma velocidade de rosca mais baixa de 400 rpm e uma temperatura da matriz relativamente mais baixa de 120°C.

O teor de proteína foi avaliado utilizando o método Kjeldahl. Entre todas as condições testadas, os níveis de proteína medidos variaram de 10,23 g/100 g PS (DW) a um máximo de 13,39g/ 100g PS (AGE 4). Especificamente, as misturas Semolina de milho:Grão-de-bico (50:50) atingiram até 12,58 g/100 g PS (SGE 1). As duas formulações com melhor desempenho a nível de características físicas e teor em proteínas fora selecionadas para a caracterização nutricional e química completa: SGE1 (Semolina de milho:Grão-de-bico, 50:50) e AGE4 (Arroz:Grão-de-bico, 50:50).

Tanto SGE como AGE são classificados como snacks prontos a consumir com baixo teor de gordura, alto teor de hidratos de carbono e alto teor de proteína. Apresentaram humidade muito baixa (SGE: 3,97%; AGE: 3,63%) e teor de gordura muito baixo (SGE: 0,048 g/100 g peso fresco; AGE: 0,043 g/100 g peso fresco), atributos desejáveis para a estabilidade em prateleira e para a saúde. A formulação AGE demonstrou um teor de proteína estatisticamente mais elevado (13,39 ± 0,01 g/100 g peso seco) em comparação com SGE (12,58 ± 0,10 g/100 g peso seco), sugerindo que a mistura Arroz:Grão-de-bico oferece uma opção de snack mais rica em proteínas. Os hidratos de carbono foram o principal macronutriente (SGE: 83,28 g/100 g peso fresco; AGE: 82,81 g/100 g peso fresco), resultando em valores energéticos globais semelhantes (AGE: 385 Kcal/100 g; SGE: 384 Kcal/100 g). A análise química dos açúcares livres revelou a sacarose como o único açúcar livre presente em ambos os snacks. O baixo teor de açúcar livre está favoravelmente alinhado com as recomendações de saúde pública. Os perfis de ácidos gordos foram distintos: SGE exibiu um perfil lipídico rico em ácidos gordos saturados (AGS: 55,3 ± 0,1%), com o ácido palmítico (C16:0) como o mais abundante (45,287 ± 0,42%). Enquanto isso, AGE demonstrou um perfil rico em ácidos gordos polinsaturados (AGPI: 48,4 ± 0,1%), com predominância do ácido linoleico (C18:2n6c: 48,438 ± 0,07%). Relativamente às propriedades bioativas, a avaliação dos extratos demonstrou uma atividade antibacteriana limitada contra a maioria dos agentes patogénicos de origem alimentar testados (incluindo E. coli Gram-negativa e S. aureus Gram-positiva). As Concentrações Inibitórias Mínimas (CIM) ultrapassaram, de um modo geral, a concentração máxima testada (> 10 mg/mL). No entanto, tanto os extratos de SGE como de AGE apresentaram atividade fungistática contra espécies de Aspergillus. Os valores de CIM para a inibição fúngica contra A. brasiliensis e A. fumigatus

variaram entre 5 mg/mL e 10 mg/mL, embora nenhum deles tenha atingido uma concentração fungicida (CFM > 10 mg/mL).

Este estudo demonstrou com sucesso que a tecnologia de extrusão pode ser aplicada eficazmente no desenvolvimento de snacks prontos a consumir, ricos em proteína, com baixo teor de gordura e com equilíbrio energético, combinando farinha de grão-de-bico com farinhas de arroz e sêmola. A formulação de arroz e grão-de-bico (AGE) apresentou o maior teor proteico e um perfil favorável de ácidos gordos, confirmando o potencial das leguminosas para aumentar o valor nutricional dos produtos extrudidos. Estas descobertas destacam a relevância das misturas de leguminosas na formulação de snacks saudáveis e ricos em proteínas que satisfaçam as exigências modernas dos consumidores por alimentos vegetais nutritivos e práticos, ao mesmo tempo que apoiam estratégias para melhorar a ingestão global de proteínas e a qualidade da dieta.

**Palavras-chave:** Caracterização Nutricional, Cozedura por Extrusão, Enriquecido com Proteína, Farinha de Grão-de-Bico, Leguminosas, Snacks Prontos a Consumir

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# 1. Introduction

## 1.1 Legumes: relevance and potential

### 1.1.1 Uses and consumption of legumes

Of the thousands of known legume species, less than 20 are planted extensively today (Basheer-Salimia et al., 2021). Those in common use include peanuts (groundnuts), soybeans, peas, lentils, pigeon peas, chickpeas, mungbeans, kidney beans (also known as common or dry beans), cowpeas, alfalfa (lucerne), clovers, and vetches (Nhu et al., 2024). They represent subfamilies of the *Leguminosae*. The *Papilionoideae*, with a worldwide distribution, are the largest subfamily. They are mostly herbs and include the most important species for human food. The *Mimosoideae* and *Caesalpinoideae* are mostly woody trees and shrubs. Many are valuable for lumber, fuelwood, tannins, and animal fodder. **Table 1** summarizes the uses of some of the important legumes.

**Table 1.** The uses of some of the important legumes (Fan et al., 2015).

| Common name | Species | Main Uses | Distribution |
|---|---|---|---|
| Carob or locust | *Ceratonia siliqua* | Food, gum | Europe, Mediterranean region |
| Chickpea | *Cicer arietinum* | Food | Mediterranean, Europe |
| Soybean | *Glycine max* | Asian dishes (tofu) roasted snacks, milk, yoghurt, sprouted beans, yuba, soy sauce | Worldwide |
| Lentil | *Lens culinaris* | Food | Europe, Middle East, India, warm temperate regions |
| Trefoils | *Lotus spp.* | Forage | Europe, Middle East, Central Asia, Australia, South America |
| Lupines | *Lupinus spp* | Forage, green manure, soil improvement | Europe, USA, Mediterranean |
| Sweet clover | *Melilotus spp* | Forage | Worldwide |
| Scarlet runner bean | *Phaseolus coccineus* | Food | Europe, Central America |
| Bean, common bean | *Phaseolus vulgaris* | Food | Worldwide |
| Peas | *Pisum sativum* | Food | Worldwide |
| Black gram | *Vigna mungo* | Food | Europe, North Africa, Egypt |

Legumes play a pivotal role in global food systems, contributing significantly to human and animal nutrition, as well as serving diverse industrial and agricultural purposes (European Journal of Public Health, 2023). As seeds that are nutritionally superior to cereal grains in protein content, legumes are fundamental to achieving balanced diets, particularly in regions where access to diverse food sources may be limited. Their inclusion in dietary practices around the world highlights their adaptability and value. They also serve as the basis for widely consumed products such as tofu, peanut butter, and soymilk, emphasizing their relevance in both conventional and modern food processing (European Journal of Public Health, 2023).

In addition to human consumption, legumes are indispensable in meeting the rising demand for high-quality animal feed, which is closely linked to improving global standards of human nutrition (Ibáñez et al., 2020). Soybeans, among the most extensively utilized grain legumes in animal feed, exemplify the critical role of legumes in livestock production systems. Moreover, forage legumes such as clovers, trefoils, and tropical pasture species enrich grass-legume mixtures, supporting sustainable livestock management across temperate and tropical regions. These uses underscore the adaptability of legumes in agricultural systems, promoting productivity and environmental stewardship (Ibáñez et al., 2020).

Beyond their direct nutritional applications, legumes are prized for their versatility in providing raw materials for several industries. Species within the *Mimosoideae* and *Caesalpinoideae* subfamilies yield essential commodities, including timber, dyes, tannins, resins, and fibers, while also contributing to environmental sustainability through practices such as agroforestry, soil restoration, and erosion control (Bruneau et al., 2024). The ability of certain legumes to serve as green manure further exemplifies their contribution to regenerative agricultural practices and their utility in enhancing soil health and fertility (Bruneau et al., 2024).

Despite their acknowledged benefits, legume consumption globally remains below recommended levels, as evidenced by data from the Global Dietary Database. While legumes are a staple in regions such as South Asia and parts of Africa, many countries, particularly in Europe, Asia, and North America, fall short of the suggested intake targets of 50 g/day (Marinangeli et al., 2017). Variability in consumption patterns is largely influenced by cultural dietary practices, accessibility, and awareness. Addressing this gap is critical, as even moderate daily portions, such as 100 g of cooked legumes, can significantly contribute to meeting nutritional requirements, particularly in populations with nutrient deficiencies (Marinangeli et al., 2017).

### 1.1.2 Health Roles Associated with Legumes

Many diseases (cardiovascular diseases, type 2 Diabetes, obesity, hypertension) of lifestyle are a result of a poor diet, high in animal products and low in plant matter (Kim et al., 2018). Legumes are high in dietary fiber, high in complex, low glycemic carbohydrates, high in bioactive compounds, low in saturated fat and no cholesterol. These dietary components promote health and longevity by decreasing insulin production and preventing chronic diseases such as diabetes, cancer, cardiovascular disease and obesity (Kim et al., 2018). As such, a legume-based diet can result in a longer, healthier life.

Although, legumes are the second most important crops after cereals, the inadequacy of the knowledge of their nutritional and functional benefits has resulted in them not being given enough attention (Kumar et al., 2018) **(Figure 1).** Therefore, future studies should investigate harnessing the many desirable properties of legumes in the development of inexpensive legume products that are available to all income groups (Jukanti et al., 2012). Most legumes are cultivated by low-income groups at household level. The increased use of legumes would increase their demand and in turn would encourage local farmers to increase legume production, hence resulting in increased financial stability and food security.

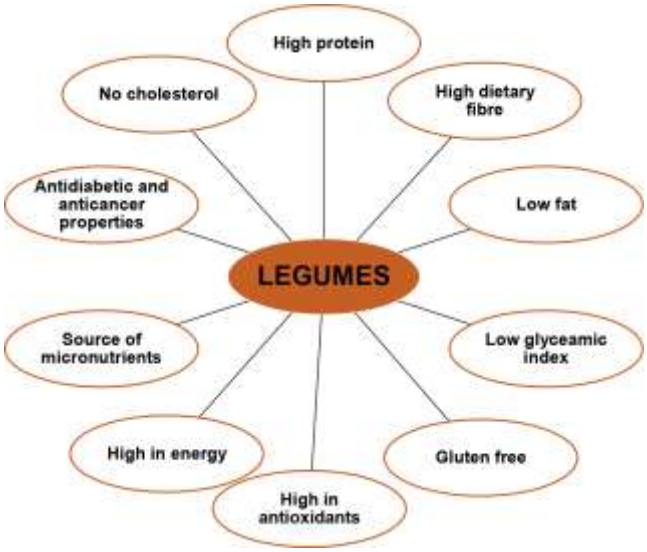


**Figure 1.** Desirable attributes of legumes (Mullins et al., 2021).

The functional properties of legumes such as water binding, oil binding, emulsion stabilization and gelling could be harnessed in the development of several food products (Kumar et al., 2024). There is urgent need to educate communities worldwide about the

nutritional value of legumes, methods of detoxifying legumes of anti-nutrients and various methods of making legumes more attractive to consumers. In addition, genetic modification could be explored in developing transgenic leguminous species that cook faster and have low levels of anti-nutrients. (Modupe et al., 2022).

Taking their nutritional superiority into consideration, it is expected that dieticians and nutritionists encourage the public through mass media such as television, press and radio, to increase their consumption of legumes (Modupe et al., 2022).

### 1.1.3 The specific case of different legumes

Legumes are consumed in many different forms, some of which have cultural relevance. However, pulses are recognized as nutritious and, thus, are consumed widely in countries with limited meat consumption (Shah et al., 2004).

The protein content of legumes generally falls between 15 and 30% **(Table 2).** Great northern bean had protein content of 23–27%, depending on growing location (Wang et al., 2010a). Kidney beans had approximately the same (27%) mean protein content (Wang, et al., 2010a). Among the kidney beans, mean protein contents of red, white, and dark red kidney beans were 27.2, 24.0, and 23.0%, respectively (Shah et al., 2004). El-Hady and Habiba (2003) reported 25.7% protein content in Egyptian kidney beans. Small white and navy beans had an approximate protein content of 22.7–26.0%. Haricot pinto and red bean protein contents were 21.1–25.3%, whereas pink bean protein content was 18.9–22.9%, depending on location  Black bean protein content was 22.9–27.7% (Shah et al., 2004). Cowpea protein variability was attributed to cultivar. Gupta et al. (2010) reported that protein content was 22–28% in 21 cowpea varieties. Others have reported similar protein contents (20–27%) in different cultivars (Horax et al., 2004; Wang and Daun, 2004).

Pea, lentil, and chickpea have protein contents like those of cowpeas and beans. Protein contents of 19-30% have been reported in numerous research articles. Data between 2008 and 2015 show that the mean protein values for pea, lentil, and chickpea were 24, 25, and 21%, respectively (NPGA, 2016). The protein content was 20–25 and 20–24% for yellow and green pea cultivar, respectively (NPGA, 2016).

**Table 2.** Macronutrient profile of legumes (Ganesan et al., 2017).

| | Chickpeas (100g dry) | Lentils (100g dry) | Peas (100g dry) | Lupin (100g dry) |
|---|---|---|---|---|
| Calories | 383 | 360 | 81 | 130 |
| Carbohydrates | 60 | 62 | 55-72 | 47 |
| Dietary fiber | 17 | 31 | 10 | 4 – 6 |
| Fats | 6 | 2 | 1 – 4 | 5 – 15 |
| Protein | 21 | 23 | 14 – 31 | 32 – 34 |

The mineral content of legumes is generally high in potassium, magnesium, iron, and manganese. Other minerals of interest for the pulse family include zinc, copper, selenium, and calcium **(Table 3).** Guzma´n-Maldonado et al. (2000) reported mineral data on 70 accessions of wild and weedy common beans from two different growing locations (states of Jalisco and Durango in Mexico). They reported that variability in mineral composition existed among accessions and location (Guzma´n-Maldonado et al., 2000). However, many of the beans tested by Paredes et al. (2009) were cultivated varieties and, thus, the variability would be expected to be lower than for the wild type.

**Table 3.** Micronutrient profile of legumes (Wallace et al., 2016).

| | Chickpeas (100g dry) | Lentils (100g dry) | Peas (100g dry) | Lupin (100g dry) |
|---|---|---|---|---|
| Iron | 34 | 41 | 60 | 24 |
| Vitamin B6 | 25 | 25 | 18 | 25 |
| Magnesium | 28 | 30 | 27 | 91 |
| Calcium | 10 | 5 | 5 | 14 |
| Manganese | 92,6 | 61 | 67 | 91 |
| Folate (B9) | 13,9 | 12 | 69 | 89 |

However, mineral content was not consistent across legume type or species. Field peas averaged 1,040, 117, 5.4, 3.1, 1.3, and 0.047 mg of potassium, magnesium, iron, zinc, manganese, and selenium, respectively, per 100 g of pea, with only trace amounts of copper

and nickel (Dahl et al., 2012; Ray et al., 2014). Wang and Daun (2004) reported ranges of various minerals that included calcium (60-107 mg/100 g), potassium (876-1,463 mg/100 g), and iron (4-8 mg/100 g) in peas. The soil mineral composition has been well documented as the leading reason for differences in mineral in plants. However, variability in the mineral content was observed among pea cultivars (Zia et al., 2013). Furthermore, Ray et al. (2014) reported that, when different pulse species were grown at the same locations, some pulses accumulated more minerals. For example, lentils accumulated more iron, manganese, selenium, and zinc compared with beans, which accumulated more copper, magnesium, and nickel when lentil and beans were grown together (Ray et al., 2014).

The mean mineral composition for a several chickpea cultivars included 170 mg/100 g of magnesium, 47 mg/100 g of calcium, 5.2 mg/100 g of iron, 2.5 mg/100 g of zinc, 2.4 mg/100 g of manganese, and trace amounts of copper (Ray et al., 2014). El-Adawy (2002) observed magnesium, calcium, and potassium contents of 176, 176, and 870 mg/100 g for chickpea obtained from Egyptian markets. Zia-Ul-Haq et al. (2007a, 2007b, 2011) observed greater variability in the mineral content of chickpea cultivars compared with variability across lentil cultivars. Lentil mineral composition of eight different lentil cultivars, including calcium (59-76 mg/100 g), potassium (844-943 mg/100 g), and iron (6-9 mg/100 g), was comparable with other pulses (Wang et al., 2009).

## 1.2 Extrusion cooking in the production of snacks and ready-to-eat products

The word extrusion has its origin from the Latin word “extrude” which means “thrust out” or “force out”. It is a technique in which a material (either metal or plastic or a group of mixed food ingredients), is squeezed under high temperature and pressure through a die or an orifice of the desired cross-section. In the food processing sector, this technology is referred to as food extrusion (Riaz, 2019).

Food extrusion **(Figure 2)** is a process in which a material is forced to flow under different conditions as a combination of thermal and mechanical treatment through a die (shaped hole) at a predetermined rate to produce a variety of products. Extrusion cooking is defined as a combination of thermal and mechanical treatment by which protein and/or starch-rich ingredients, are plasticized and are cooked in a cylinder to obtain a predefined shape. The final product is thus obtained by passing it through the die opening at the extruder outlet (Harper, 2019; Riaz, 2019).

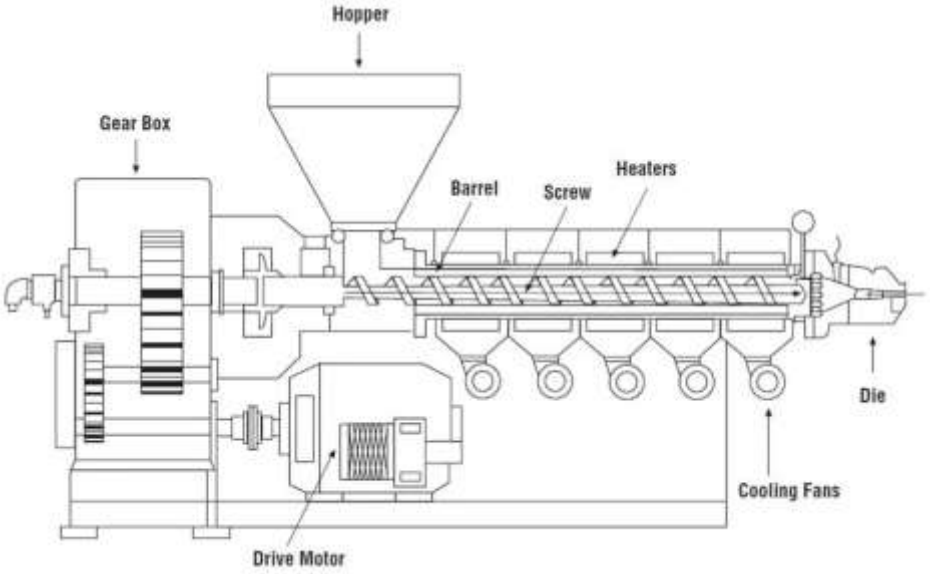


**Figure 2.** The basic components of a screw extruder (Abeykoon et al., 2011).

Food extrusion is a high-temperature short-time method which brings changes in texture, fluid flow and size reduction. It brings changes by caramelizing, mixing, cooking, heat and mass transfer, plasticizing, shearing, kneading, shaping and forming (Yu, 2011; Aktas et al., 2020) of the final product. Today in the food processing sector, the application of this technology is extended to a wide range such as shearing, separation, encapsulation, shaping, mixing, flavour generation, cooling or heating, sterilization, venting moisture and volatiles, conveying and co-extrusion (Menis-Henrique et al., 2020).

Many equipment modifications and upgradations have taken place in the field of extrusion for developing food products efficiently and sustainably with functional properties. Some of these include, extrusion using supercritical carbon dioxide, for nutrient retention (Paraman et al., 2015; Lohani et al., 2017) and hot-melt extrusion for improving solid dispersion stability and bioavailability of food and drugs (Maniruzzaman et al., 2012; Ottoboni et al., 2017). In hot-melt extrusion, heat is applied to the material for viscosity control and to allow smooth flow of the food through the die (Chokshi et al., 2004; Maniruzzaman et al., 2012; Ottoboni et al., 2017). The advent of robotics has paved way for the introduction of an innovative technology called 3-D printing, to develop personalized and customized food products with complex geometries and structures through layer-by-layer deposition, via extrusion by rapid prototyping (Nachal et al., 2019).

### 1.2.1 Mechanisms of extrusion technology

The extrusion process commences with the milling of the selected raw materials to achieve the appropriate particle size, typically resembling coarse flour. Subsequently, the dry mixture undergoes mixing and preconditioning, during which additional ingredients like liquid sugar, fats, dyes, meats, or water are incorporated, depending on the intended product. Steam is introduced to initiate the cooking process, and the preconditioned mix is then propelled through an extruder. During the extrusion process, the extruded product often undergoes puffing and textural changes due to the reduction of forces and the release of moisture and heat, termed the expansion ratio (Karwe, 2008). The product generates its friction and heat under pressures ranging from 10 to 20 bars resulting in protein denaturation and starch gelatinization, contingent upon various inputs and parameters. At the output of the extruder, blades rotating around the die openings cut the extrudate to the desired length. The cut products are then dried and cooled to achieve rigidity while retaining porosity **(Figure 3).** The dried extruded product may further be subjected to surface treatments such as seasonings, coatings and polishing. They may also undergo additional processing such as cooking, baking or frying to enhance flavour and improve nutritional characteristics. (Karwe, 2008).

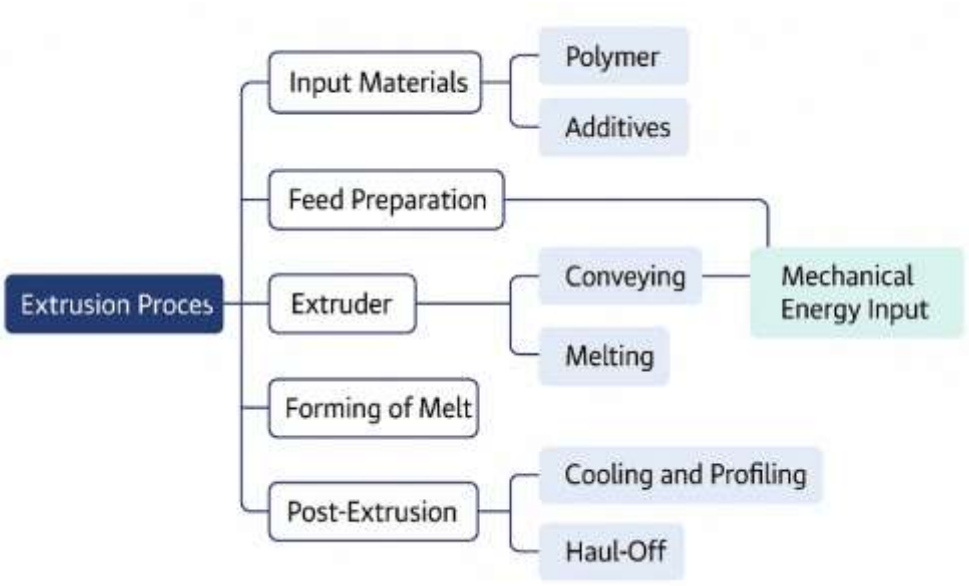


**Figure 3.** Flow chart for an extrusion process (Choton et al., 2020).

Numerous food extrusion processes involve high temperatures over a short duration. (Karwe, 2008). Critical factors influencing the extrusion process include the composition of

the extrudate, the length and rotational speed of the screw, barrel temperature and moisture content, die shape, and the speed of the blade's rotation. These parameters are meticulously controlled based on the desired product to ensure the uniformity of the output. (Rao, 2015).

### 1.2.2 Advantages and disadvantages of extrusion

Extrusion processing has continued to gain popularity as one of the most energy efficient and environmentally friendly processes for a wide range of food products **(**Deenanath et al., 2017). Extrusion cooking is thus one of the preferred food-processing techniques due to its continuous process with high productivity, high temperature and short time cooking period while destroying both harmful microbial organisms and anti-nutrient enzymes, resulting in significant nutrient-retention products with longer shelf life. Furthermore, nutritious foods can be designed through extrusion to meet societal needs in addressing malnutrition and food and nutrition insecurity (Backus et al., 2014).

Disadvantages of extrusion food processing include a costly initial financial investment, and careful selection of process parameters such as moisture content, feed particle size, feed rate, screw speed, temperature, screw configuration and die shape to avoid reactive and harmful substance formation (Backus et al., 2014).

### 1.2.3 Types of extruded foods

Extrusion cooking is an important food processing technology and has been used since the mid-1930s in the production of breakfast cereals, also known as ready-to-eat snacks, and other textured foods. These products not only improve digestibility (Pismag et al., 2021) but also enhance the bioavailability of nutrients present in raw materials **(**Zhou et al., 2020). Consequently, during the last decades, it has been highly investigated to produce a wide range of special foods with various characteristics **(Table 4).**

Cold extrusion involves processing materials with product temperatures generally around 50°C. This method is utilized for shaping various products such as food pasta, processed meat items, and gums. However, it's worth noting that some researchers categorize cold extrusion as involving intermediate temperatures, reaching approximately 80°C in certain applications like bread dough or semi-moist animal feed. According to Gamel et al. (2015), the temperatures in cold extrusion are observed to increase.

**Table 4.** Typical food products produced by extrusion technology (Brennan et al., 2011).

| Food Product | Extrusion Technology |
|---|---|
| ***Baby food*** | Puffing, expansion for cereals and snacks |
| ***Confectionery products*** | Toffees, caramels, etc. |
| ***Dehydrated soups*** | Agglomeration and texturization of powdered ingredients |
| ***Dry beverage mixes*** | Agglomerated powders for instant drinks |
| ***Food for companion animals (pet foods)*** | Dry, semi-humide |
| ***Pasta products*** | Extrusion of shaped pastas (macaroni, spaghetti) |
| ***Ready-to-eat breakfast cereals*** | Expanded flakes, puffs, multi-grain cereals |
| ***Snack products*** | Expanded snacks, corn curls, crispbreads, co-extruded products, etc. |
| ***Textured proteins*** | Meat analogues based on vegetable proteins, textured soy proteins, surimi |

Regarding extruders specifically, several recent studies classify them into three types: those that operate cold, performing mixing and melding; those that operate under low pressure, involving cooking and production at temperatures below 100°C; and those that operate under high pressure, involving cooking and production at temperatures above 100°C (Liu et al., 2021).

Food products obtained by extrusion are numerous and can be found in several sections of commercial spaces such as grocery stores, supermarkets and convenience stores, including, among others, products from cereals, confectionery, textured protein-based foods, and pet foods.

The versatility of extrusion is also reflected in the use of its products in the preparation of food rations for the army and camping, foods for specific diets and foods designed to satisfy nutritional needs in situations of natural disasters (EUFIC, 2002).

### 1.2.4 Evolution of the growth of the snacks and ready-to-eat products sector

The global snacks and ready-to-eat products sector have seen rapid growth over the past decades, driven by evolving consumer lifestyles and preferences **(Figure 4).** Initially dominated by basic packaged foods, the sector expanded as urbanization and working populations grew, particularly in the mid-20th century (Grand View Research, 2018). Companies like PepsiCo, Nestlé, and Kellogg's began diversifying into snack categories like savoury chips, frozen meals,

and instant breakfasts to cater to rising demand for convenience and variety (Grand View Research, 2018).

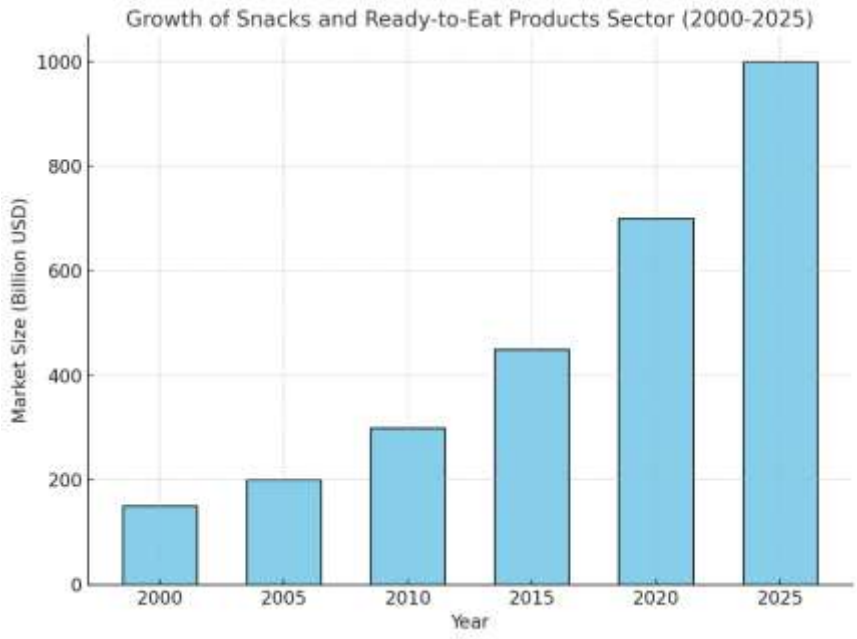


**Figure 4.** The growth of the snacks and ready-to-eat products sector (Grand View Research, 2018).

The pandemic accelerated sector growth as lockdowns boosted at-home snacking. Global snacks market sales grew by 7% in 2020 alone, with ready-to-eat frozen foods growing by 5%. Euromonitor reported that 55% of consumers increased snack consumption during the pandemic (Anne et al., 2022).

The Europe snacks market accounted for a revenue share of 25.41% in 2023 (Anne et al., 2022). There's a rising demand for natural, organic, and clean-label snacks as consumers become more health-conscious. Products free from artificial additives and preservatives and high in natural ingredients are preferred. Moreover, there is a rising trend of premiumization in the regional market. gourmet, artisanal, and high-quality ingredients are sought for a more indulgent snacking experience. The rise of plant-based diets is reshaping the snack landscape. As veganism and flexitarians grow in popularity, snacks derived from legumes, fruits, and vegetables are being embraced. Innovations in plant-based chips and bars have made it possible for consumers to enjoy savoury and sweet snacks without compromising their dietary choices. (Grand View Research, 2018).

The snacks and ready-to-eat markets in Portugal and Tunisia are experiencing notable growth, driven by evolving consumer preferences and lifestyle changes.

In Portugal, the snacks market is projected to grow at a compound annual growth rate (CAGR) of approximately 6.7% from 2024 to 2029, with an anticipated increase of USD 1.1 billion during this period. This growth is fuelled by rising health consciousness and demand for convenient food options. The ready-to-eat meals segment is also expanding, with volume expected to reach 220.08 million kilograms by 2030, reflecting a volume growth of 1.4%. (Grand View Research, 2018).

In Tunisia, the snacks market is anticipated to grow from USD 1,032.4 million in 2023 to USD 1,849.0 million by 2029, registering a strong CAGR of 10.2% over the period from 2024 to 2029. The ready-to-eat meals market is also on the rise, with revenue expected to reach USD 413.78 million in 2025 and an annual growth rate of 5.75% from 2025 to 2030. (Grand View Research, 2018).

Companies in both countries are leveraging digital marketing strategies, including social media campaigns and influencer partnerships, to enhance brand visibility and cater to the growing demand for health-focused and sustainable snack options. This dynamic landscape presents lucrative opportunities for businesses aiming to tap into the evolving consumer trends in Portugal and Tunisia

The snacks and ready-to-eat market's growth trajectory reflect a blend of cultural shifts, innovation, and changing consumer priorities. With health-focused and sustainable products gaining traction, the sector remains a dynamic and lucrative space for further exploration. (Grand View Research, 2018).

### 1.3 The particular case of legumes in the production of extruded products

Consumer demand for ready-to-eat foods is increasing due to the time-saving needs of the modern lifestyle. Extrusion cooking is a technique largely used to produce several ready-to-eat products, such as crisp expanded snacks (e.g.: pus, runs, collets, etc.), breakfast cereals, instant soups, meat analogues and sport foods (Moscicki, 2011; Offiah et al., 2018). Extruded foods can attract the consumer for their convenience, pleasant appearance and texture (Anton et al., 2009; Harper, 2019). The raw materials for extrusion cooking are mostly cereals, due to their good expansion characteristics. However, in addition to providing energy from starch, extruded foods could act as carriers of other nutrients, if enriched with other ingredients (Shah et al., 2018).

Legumes are a good source of proteins, starch, dietary fiber, vitamins and minerals, and are particularly important when the consumption of animal proteins is restricted due to limited adorability, or religious, dietary and ethical habits (Tharanathan et al., 2003; Yadav et al., 2013;

Ray et al., 2014). Furthermore, legumes are sustainable crops that are adaptable to marginal lands (De La Peña et al., 2011). In the past, only soybean was used for the development of extruded food products. In recent years, instead, several studies have considered the incorporation of other legumes (such as bean, lentil, pea, chickpea, and faba bean) to improve the nutritional value of extruded foods. Nutrient dense extruded multi-legume bars, mixed with whey protein concentrate, honey and palm oil, have been proposed to mitigate malnutrition in developing countries (Shah et al., 2018; Saadat et al., 2020).

Extrusion cooking technology is also known to reduce the levels of some anti-nutrients contained in legumes such as tannins, phytic acid, trypsin inhibitors and lectins (Gilani et al., 2012; Hegazy et al., 2017). In addition, extrusion cooking can increase the digestibility of starch and proteins (Patil et al., 2016).

Extrusion cooking therefore seems to be suitable for producing an array of ready-to-eat legume-added foods. This topic soon attracted the attention of researchers, and their interest increased over time. Indeed, over the past 10 years an increasing number of articles have been published containing the word combinations “extrusion” and “legume”, or “extrusion” and “pulse”, or “extrusion” and the name of a specific legume, as reported in the (Scopus scientific database, 2020) **(Figure 5A).** Among them, the largest number of studies were conducted on bean and pea, whereas faba bean was the least studied legume **(Figure 5B).** In addition, as a sign of the growing interest in legumes, the 68th session of the General Assembly of the (Organization of the United Nations declared, 2016) as the “International Year of Pulses”.

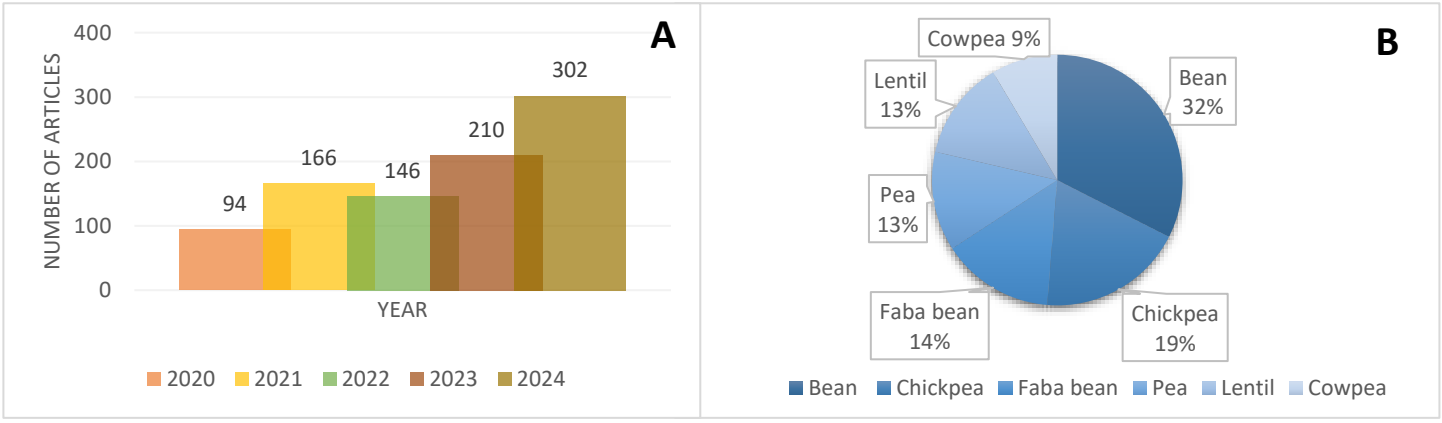


**Figure 5.** Number of articles regarding the extrusion cooking of legumes published from January 2011 to April 2020 (**A**) and percent distribution of articles according to legume type **(B)** (Elsevier; 2025).

## 2. Objectives

Legumes are a good source of protein, starch, dietary fiber, vitamins and minerals. Thus, the incorporation of legumes can improve the nutritional value of extruded foods. In this sense, this study aims to characterize and extrude chickpea flour combined with semoula and rice flours to produce ready-to-eat snacks **(Figure 6).**

Therefore, the specific objectives are:

i. Production of flours from chickpea, semoula, and rice;

ii. Physical characterization (color measurement) of the different ready-to eat snacks

iii. Nutritional characterization (fat, ash, protein, carbohydrate and energetic value) of the different ready-to-eat snacks obtained;

iv. Determination of chemical composition (namely total phenols, free sugars, fatty acids) of all samples under study.

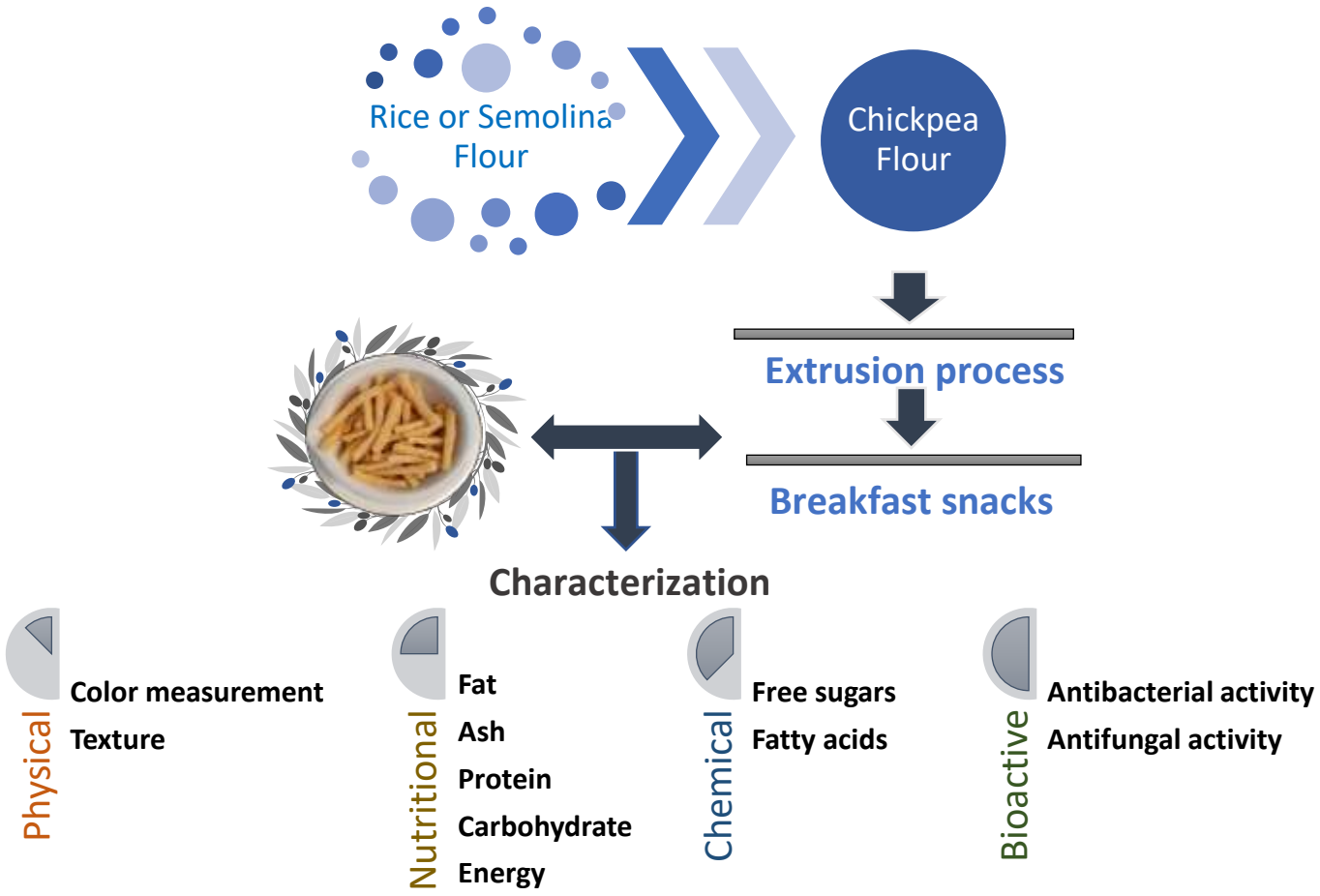


**Figure 6.** Diagram of the main objectives and procedures of this study

# 3. Material and Methods

The present study undertook the preparation and comprehensive analysis of plant-based flours derived from chickpeas, semolina, and rice. The primary objective was to optimize a formulation with a higher protein content and that guaranteed the desired physical properties for the consumer in order to obtain a new enriched ready-to-eat product.

## 3.1 Obtaining and preparing the flours

The raw materials utilized in this study comprised legumes, specifically chickpea flour, rice flour, and semolina flour. The selection of chickpeas, semolina, and rice was predicated on their widespread availability and nutritional significance, as well as their distinct characteristics that offered the potential for synergistic interactions in the creation of innovative and health-oriented food options, ultimately culminating in the development of a ready-to-eat snack. All flours were acquired in their desiccated state to ensure inherent stability and consistency throughout the experimentation. For detailed sourcing, the semolina flour **(Figure 7A)** and rice flour **(Figure 7B)** were acquired in *Continente Modelo*, SA (Bragança, Portugal).


**Figure 7. A:** Nutritional Composition of Commercial Maize Semolina (Espiga Brand). **B:** Nutritional Composition of Commercial Rice Flour (Espiga Brand).

In turn, the chickpea flour **(Figure 8)** was obtained from *Molendum Ingredients* S.L. (Zamora, Spain)**.**

Packaging - Harina de Garbanzo (Molendum)


**Nutritional Composition per 100g**

| Nutrient | Amount per 100g | Unit |
|---|---|---|
| Energy | 1640 | kJ |
| Protein | 21.0 | g |
| Carbohydrates | 60.0 | g |
| Sugars | 10.7 | g |
| Fat | 6.0 | g |
| Saturated Fat | 0.6 | g |
| Fiber | 17.0 | g |
| Salt | 0.06 | g |

**Figure 8.** Labeling and Nutritional Composition of Commercial Chickpea Flour (Molendum Ingredients)

Following raw material acquisition, distinct flour mixtures were meticulously prepared. Each unique sample was assigned a specific code to facilitate clear identification and robust traceability across all experimental procedures. The prepared mixtures and their corresponding codes were as follows:

GE: Chickpea flour (100%)

SE: Semolina flour (100%)

ARE: Rice flour (100%)

SGE: Semolina: Chickpea (50:50, *w/w*)

SBE: Semolina: Chickpea (60:40, *w/w*)

AGE: Rice: Chickpea (50:50, *w/w*)

GAE: Rice: Chickpea (60:40, *w/w*)

Upon preparation, both the individual powdered samples and their different mixtures were subsequently stored under stringent controlled conditions. Specifically, they were maintained in a cool, dry environment, meticulously protected from light, until further analysis was conducted. These careful storage conditions were diligently maintained to minimize

potential degradation or undesirable compositional changes in the flour samples, thereby ensuring the integrity and overall reliability of the experimental data derived from them.

### 3.2 Extrusion process

Extrusion tests was conducted on a Process 11 twin screw extruder from Thermo Scientific, Germany (11 mm barrel diameter, 400 mm twin screw length). The extruder has eight-cylinder zones with independent electrical resistance heaters (30-300ºC) mounted and temperatures was maintained by cooling water chiller (19ºC). The extrusion speed varies from 0 to 1000 rpm. The diameter of the die opening from 2 mm to 5 mm. The moisture content of the feed was controlled by injecting water into the extruder with a peristaltic pump (L/h). The flour was added to the barrel by a gravimetric feeder balance (kg/h). **(Figure 9).**


**Figure 9. A:** Control unit. **B:** Front view of twin-screw extruder. **C:** Die section with temperature and pressure sensors.

Different combinations of temperatures were tested in the last four zones of the barrel (120, 140, 160, 180ºC), as well as different rotation speeds of the double screws (300, 400 and 500 rpm). The amount of water and flour to be added were adjusted to obtain the best expansion of the extruded product.

The extrudates were dried, packed in polyethylene bags and stored for later analysis.

The moisture content of the extruded products was measured immediately, while further physical, biochemical and sensory analyses was carried out within 48 hours.

### 3.3 Determining the protein content of the products obtained

After the extrusion process, a critical analytical step was performed to determine the protein content of the resulting products. This ensures that the products meet the high-protein target.

The proteins content was evaluated using the official analysis methods (AOAC, 2016) by Kjeldahl method.

The determination of protein content involved a two-stage process: initial sample digestion using a BLOC DIGEST system. For the digestion phase, a precise 250 mg of each sample was weighed in duplicate into falcon tubes, which were subsequently transferred to MACRO tubes placed in a rack. A digested blank was prepared by adding 25 mL of water to the first MACRO tube of the initial rack, while subsequent tubes contained the samples. To each MACRO tube, two catalyst pellets were added, followed by the careful addition of 15 mL of sulfuric acid ($H_2SO_4$) within a fume hood. The rack containing the MACRO tubes was then placed into the BLOC DIGEST apparatus, with the electronic controller RAT-2 set to program 1 (400 °C for 70 minutes) to initiate the digestion process. During digestion, a vapor extractor was placed, and water supply was ensured.

Upon completion, signalled by an alarm, the vapor extractor was removed, and the rack with the MACRO tubes was carefully taken out of the BLOC DIGEST and allowed to cool. After that, 25 mL of distilled water was added. **(Figure10).**

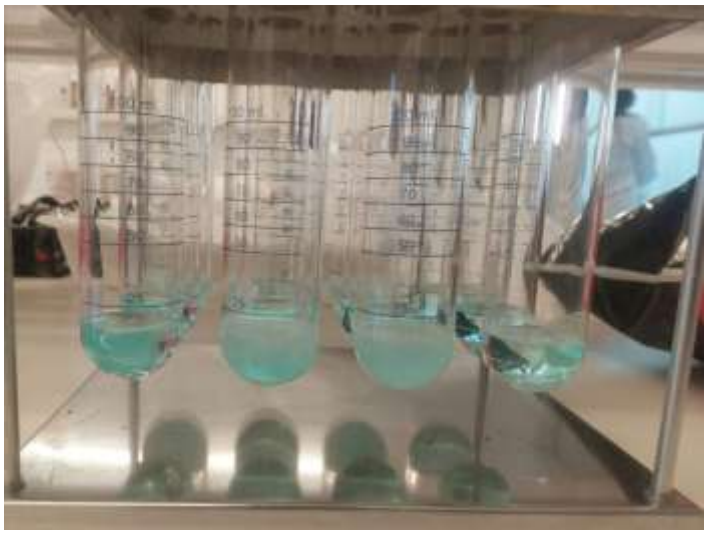

**Figure 10.** Post-Digestion Appearance of Samples.

Finally, the absorbance of the solutions was measured at 660 nm, with the blank utilized as the reference, and the protein percentage was calculated. The protein percentage is calculated using the **Equation 1:**

$$\% \, Protein = \frac{P2 \times 100 \times F}{P0}$$

**Equation *1* -** Determination of percentage of protein

### 3.4 Physical Characterization of the ready-to-eat snacks

#### 3.4.1 Colour measurement

Color measurements were performed in triplicate using a colorimeter (model CR-400, Konica Minolta Sensing Inc., Tokyo, Japan) using the method described by Rosso & Mercadante (2007). Prior to measurements, the instrument was meticulously calibrated against a standard white tile to ensure accuracy and consistency. This calibration step is crucial for obtaining reliable color data, establishing a baseline against which sample colors can be accurately compared **(Figure 11).**


**Figure 11.** Use of a Konica Minolta Colorimeter for Color Measurement of Sample Material

The color characteristics were quantified using the CIE *L*a*b** color space values. This widely accepted color model provides a comprehensive representation of color in three dimensions :

*L** represents lightness, ranging from 0 (perfect black) to 100 (perfect white).

*a** indicates the greenness-redness axis, with positive values signifying redness and negative values indicating greenness.

*b** represents the blueness-yellowness axis, where positive values denote yellowness and negative values signify blueness.

To evaluate the total color difference between samples, the Delta E (ΔE*) value was calculated. This metric provides a single numerical value that quantifies the perceived difference between two colors. The ΔE* was determined using the following **equation 2**, based on the method described by de Rosso & Mercadante (2007) :

$$\Delta E* = [(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]^{1/2}$$

**Equation *2* -** Calculation of the difference between two colors.

Where ΔL*, Δa*, and Δb* represent the differences in the *L**, *a**, and *b** values, respectively, between the two colors being compared. This comprehensive approach to color measurement and analysis ensures a robust and quantifiable assessment of color variations within the study.

### 3.4.2 Texture analysis

Texture profile analysis (TPA) was carried out. During the measurement phase, the instrument exhibited irregular force readings and unstable baselines, later attributed to a malfunction of the load cell calibration system. Several recalibrations attempt and repeat tests were performed; however, the signal instability persisted, affecting the reproducibility and accuracy of the measurements.

## 3.5 Nutritional Characterization of the ready-to-eat snacks

Based on the results obtained from the preliminary evaluations including protein content determination, color measurement, and visual texture assessment, the most promising formulations were identified and selected for subsequent analyses.

The nutritional evaluation of the ready-to-eat snacks [SGE: Semolina: Chickpea (50:50, *w/w*); AGE: Rice: Chickpea (50:50, *w/w*)] was determined according to official food analysis methodologies (AOAC, 2016). In this way, the concentrations of fat, protein, ash, carbohydrates, and the energy value were determined.

*Moisture :* The moisture content of the samples was precisely determined using an ADAM PMB moisture analyzer (AOAC, 2016). For each measurement, approximately 2 grams of the sample were accurately weighed and carefully placed onto the analyzer's weighing pan.

The instrument was programmed to heat the sample at a controlled temperature, specifically around 61°C. The moisture analyzer then automatically calculated the moisture content based on the difference between the initial and final weights of the sample **(Figure 12).**


**Figure 12.** Moisture Analyzer (Adam PMB 163) Used for Determination of Sample Moisture Content.

*Fat:* For the quantitative determination of lipid content, lipids were extracted using the standardized Soxhlet method with petroleum ether. A precisely known mass of samples, specifically 3 grams, was subjected to continuous extraction within a Soxhlet apparatus. Petroleum ether served as the chosen extraction solvent, with the process maintained at a consistent temperature of approximately 60 °C. The extraction was conducted for a duration of 7 hours **(Figure 13)**, after which the resulting solvent-lipid solution was evaporated to isolate the lipids. This entire procedure adheres to the established analytical standard, (AOAC 989.05).

**Figure 13.** Soxhlet extraction of lipids

*Proteins:* The quantification was conducted in accordance with the methodology previously described in Section 3.3, Determining the protein content of the products obtained.

*Ash :* The ash content of the solid samples was precisely determined through incineration in a muffle furnace using the AOAC procedures (AOAC 935.42), which was operated at a controlled temperature of 550 ± 10°C during 6 hours **(Figure 15).**

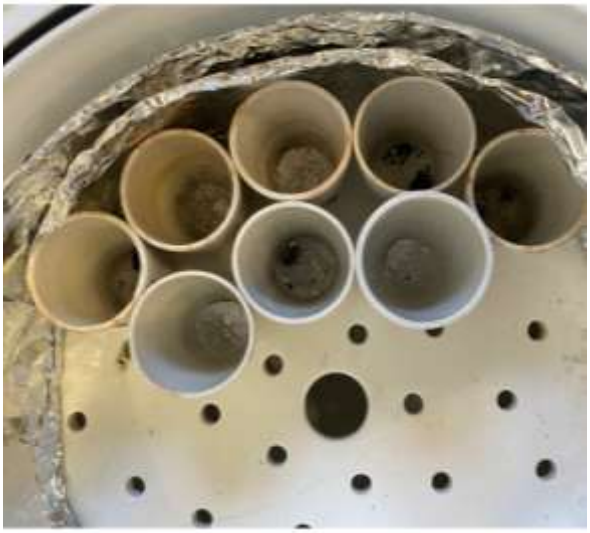

**Figure 15.** Evaluation of ash content.

Once cooled, the crucible, now holding the inorganic ash, was reweighed. The final ash content was then calculated as a percentage, specifically determined by the weight of the residue relative to the initial sample weight.

*Carbohydrates and energy :* Carbohydrates were calculated by difference according to **Equation 3**, and the energetic value was calculated as showed in **Equation 4.**

$$Carbohydrates = 100 - (g\ moisture + g\ proteins + g\ lipids + g\ ash)$$

**Equation *3*** - Calculation of carbohydrates

$$Energy\ (Kcal) = 4 \times (g\ proteins + g\ carbohydrates) + (9 \times g\ fats)$$

**Equation *4*** - Determination of total energy

### 3.6 Chemical composition of the ready-to-eat snacks

#### 3.6.1 Free sugars

Free sugars were determined by high performance liquid chromatography system coupled to a refractive index detector (HPLC-RI) as previously described by Barros et al., (2013).

The sample (1 g) was enriched with melezitose (used as an internal standard, 25mg/mL) and extracted with 40 mL of ethanol (80/20, *v/v*) in an 80 °C bath (Julabo, SW22; Seelbach, Germany) for 1 hour and 30 minutes, with agitation every 15 minutes. Subsequently, the supernatant obtained was centrifuged (K24OR refrigerated centrifuge, Centurion, West Sussex, United Kingdom) at 15,000 g for 10 minutes and transferred to a glass flask to evaporate the ethanolic fraction, using a rotary evaporator (Büchi R -210, Flawil, Switzerland) (60°C, reduced pressure). The aqueous phase was washed 3 times with diethyl ether (10 ml), then the rest was evaporated. To the dry residue obtained, water was added to make up a final volume of 5 mL and 1.5 mL of it was filtered (nylon filters - 0.2 µm, Whatman) into a vial, for later analysis of the profile in sugars in the HPLC system **(Figure 16)**.


**Figure 16.** Configuration of the HPLC System Comprising a Knauer Smartline 1000 Pump, Smartline Manager 5000 Degasser, and Jasco AS-2057 Auto-Sampler

The HPLC system is equipped with a pump (Knauer, Smartline 1000 System, Berlin, Germany), a degassing system (Smartline manager 5000), an autosampler (AS-2057 Jasco, Easton, Maryland, USA) and a detector refractive index (Knauer Smartline 2300). Chromatographic separation was achieved through a Eurospher 100-5 $NH_2$ column (4.6 x 250 mm, 5 mm, Knauer), which operated at a temperature of 30°C (7971 R Grace).

Acetonitrile/deionized water (70:30; *v/v*) was used as mobile phase at a flow rate of 1 ml/min. The Clarity 2.4 Software (DataApex) was used to identify the compounds, from which the relative retention times of the sample peaks were compared with known standards. The results were obtained by the internal standard method and expressed in grams of compound per 100 g of fresh mass.

### 3.6.2 Fatty acids

Fatty acids were determined by gas chromatography with flame ionization detection (GC-FID), as previously described by Pereira et al. (2012).

To the lipid extract previously obtained by Soxhlet extraction, 5 ml of a methanol/sulfuric acid/toluene solution were added, in a 2:1:1 (*v/v/v*) ratio, and the mixture remained in a bath (Julabo, SW22; Seelbach, Germany) at 50 °C (with 160 rpm agitation) for approximately 12 hours. After removing the tubes from the bath and, to enhance the phase separation, deionized water (3 mL) was added to the mixture and, later, to recover the fatty acid methyl esters (FAME), diethyl ether (3 mL), both vertexing steps.

After separating the phases, the supernatant was transferred to a vial, in which anhydrous sodium sulfate was previously added, to dehydrate the supernatant. Finally, it was filtered through nylon filters (0.2 µm; Whatman) into a vial **(Figure 17),** for further analysis in GC.

**Figure 17.** Preparation and labeling of vials for fatty acid derivatization analysis.

The fatty acid profile was obtained using a GC system (Model DANI GC 1000) equipped with a split/splitless injector, a flame ionization detector (FID, 260 °C) and a Zebron-Kame column (30 m x 0.25 mm ID x 0.20 μm df; Phenomenex, Lisbon, Portugal). The temperature program applied was as follows: initial temperature of 100 °C for 2 min; progressive temperature increases: 10 °C/min to 140 °C; 3°C/min to 190°C; 30 °C/min to 260 °C which was held for 2 min. The carrier gas used was hydrogen with a flow rate of 1.1 ml/min, measured at 100°C. Split injection (1:50) was performed at 250 °C, with 1 μL of the sample being injected.

The identification of fatty acids was based on the relative retention times of FAME peaks from samples with known standards. For processing the results, the CSW 1.7 software (DataApex 1.7, Prague, Czech Republic) was used and these were expressed as a relative percentage (%) for each fatty acid detected.

### 3.7 Evaluation of antimicrobial activity of the ready-to-eat snacks

For the evaluation of the antimicrobial activity, the lyophilized extracts obtained in section 3.5 were re-dissolved dimethyl sulfoxide (DMSO) (100 mg/mL).

#### 3.7.1 Antibacterial activity

For the evaluation of the antibacterial activity eight microorganisms were used : The Gram-positive bacteria: *Staphylococcus aureus* (ATCC 11632), *Bacillus cereus* (ATCC 14579), *Listeria monocytogenes* (NCTC 7973), as well as the following Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Enterobacter Cloacae* (ATCC 13047), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enterica* (ATCC 35664), and *Yersinia enterocolitica* (ATCC 27729) were used in order to determine potential antimicrobial activity of the samples **(Figure 18).**

**Figure 18.** Gram-Positive and Gram-Negative Bacteria used to evaluate antibacterial activity.

The microdilution method was used in order to determine the minimum inhibitory concentration (MIC) as well as the minimum bactericidal concentration (MBC) of the extracts.

The bacterial cultures were adjusted to a concentration of $1\times10^5$ CFU/mL using the spectrophotometer, which corresponds to a bacterial suspension obtained in a spectrophotometer at 625 nm. The absence of contamination as well as the verification of the validity of the inoculum was checked through the growth of the inoculum dilutions in a solid medium. The wells contained 100 µL of triptych soy broth (TSB), in which the dilutions of the hydroethanolic extracts were pipetted, and then 10 µL of inoculum was added. The microplates were maintained in the incubator during 24 hours at 37°C. To determine the MIC (minimal inhibitory concentration), iodonitrotetrazolium choloride (INT) (40 µL, 0.2 mg/mL) was added, then it was incubated at 37°C for 30 minutes. The MIC was identified as the lowest concentration that produced a significant inhibition (around 50%) of the growth of the bacteria comparing to the positive control.

### 3.7.2 Antifungal activity

For the evaluation of antifungal activity, two micromycetes were used: *Aspergillus fumigatus* (human isolate), *Aspergillus brasiliensis* (ATCC 16404)

The micromycetes were carried on malt agar (MA), and the cultures were stored at 4°C and sub-cultured monthly. The spores of the fungi were washed from the surface of the agar plates with 0.85% sterile saline solution that contain 0.1 % (*v/v*) of Tween 80. The spore suspension was adjusted with a sterile saline solution to a concentration of approximately $1.0\times10^5$ CFU/mL in a final volume of 100 µL per well. Inocula were stored at 4°C for subsequent use. The dilution of the inocula were grown on solid MA in order to verify the absence of contamination and the inoculum validity. The successive dilution technique in 96-well microplates were used to determine MIC.

The sample solution was added to the malt medium with the fungal inoculum then the microplates were incubated at 28°C for 72 hours. The lowest concentrations with no visible growth (using a binocular microscope) were defined as MIC. The minimum fungicidal concentrations (MFCs) were obtained by serial sub-cultures of 2 mL in microplates that contain 100 mL per well of malt broth, and subsequently incubated at 28°C for 72 hours. The lowest concentration with no visible growth was defined as the MFC, indicating 99.5% of loss of the original inoculum. DMSO 5% was used as negative control, and bifonazole and ketoconazole were used as positive control.

### 3.8 Statistical analysis

The assays mentioned in this study were performed in triplicate and duplicate, meanwhile, the results were expressed as mean ± standard deviation (SD). The statistical analysis of the data was performed to determine the significant differences between the different samples and was done through a one-way ANOVA analysis of variance, considering the different types of comparisons.

# 4. Results and Discussion

## 4.1 Extrusion process

The extrusion process was systematically investigated across two primary flour combinations: a) Rice:Chickpea and b)Semoula:Chickpea, each at two distinct ratios (50:50 and 60:40). The experimental conditions, including maximum and total output, water flow, screw speed, and a multi-zone temperature profile, were meticulously recorded to correlate with the observed product characteristics, defining the "best aspect" as a combination of desirable color, crunchy texture, homogeneous flow, and optimal particle diameter.

The results of the Rice:Chickpea flour are presented in **Table 5**.

For the Rice:Chickpea (50:50) blend, the most favorable product characteristics were consistently observed in samples AGE3 and AGE4. Both samples yielded extrudates with a white color, crunchy texture, homogeneous flow, and good particle diameter. These desirable attributes were achieved under consistent processing parameters, including a maximum output of 1.669 kg/h, total output of 1 kg/h, and a water flow of 400 mL/h. A key parameter for these successful outcomes was a screw speed of 600 rpm, coupled with a specific temperature profile across the barrel zones (Zona 2-8: 40-120°C) and a die temperature of 170°C for AGE3 and 180°C for AGE4.

Similarly, for the Rice:Chickpea (60:40) formulation, samples GAE2 and GAE3 demonstrated superior characteristics, presenting a white color, crunchy texture, homogeneous flow, and good particle diameter. These results were obtained at a maximum output of 2.007 kg/h, total output of 1.4 kg/h, and a water flow of 400 mL/h. While both samples maintained consistent temperatures across the initial zones (Zona 2-4: 40-80°C) and higher temperatures in later zones (Zona 8: 140°C, Die: 180°C), the optimal screw speeds were 700 rpm for GAE2 and 600 rpm for GAE3, indicating flexibility within a narrow range for achieving desired product quality in this specific blend.

The analysis of successful extrusion trials, specifically AGE3, AGE4 (50:50 Rice:Chickpea), and GAE2, GAE3 (60:40 Rice:Chickpea), demonstrated that acceptable product formation characterized by a white color, crunchy texture, and homogeneous flow was achieved using high screw speeds, namely 600 rpm or 700 rpm. These operational speeds overlap precisely with the range identified as optimal in the comparative study by Zahari et al. (2023), for producing high-moisture meat analogues (HMMA).

**Table 5.** Effect of Rice:Chickpea Blend Ratios and Process Conditions on Extruder Output and Physical Characteristics of the Product

| Sample | Ratio | Max Output (kg/h) | Total Output (kg/h) | Water Flow (mL/h) | Screw Speed (rpm) | T° Z2 | T° Z3 | T° Z4 | T° Z5 | T° Z6 | T° Z7 | T° Z8 | T° Die | Observations |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **AGE1 : Rice:Chickpea** | 50:50 | 1.669 | 1.0 | 400 | 400 | 40 | 60 | 80 | 100 | 110 | 110 | 120 | 160 | Non-homogeneous flow |
| **AGE2 : Rice:Chickpea** | 50:50 | 1.669 | 1.0 | 400 | 500 | 40 | 60 | 80 | 100 | 110 | 110 | 120 | 180 | Small particle diameter |
| **AGE3 : Rice:Chickpea** | 50:50 | 1.669 | 1.0 | 400 | 600 | 40 | 60 | 80 | 100 | 110 | 110 | 120 | 170 | White color, crunchy, homogeneous flow, good particle diameter |
| **AGE4 : Rice:Chickpea** | 50:50 | 1.669 | 1.0 | 400 | 600 | 40 | 60 | 80 | 100 | 110 | 110 | 120 | 180 | White color, crunchy, homogeneous flow, good particle diameter |
| **GAE1 : Rice:Chickpea** | 60:40 | 2.007 | 1.0 | 400 | 500 | 40 | 60 | 80 | 100 | 110 | 110 | 110 | 170 | Semi-humid, small particle diameter |
| **GAE2 : Rice:Chickpea** | 60:40 | 2.007 | 1.4 | 400 | 700 | 40 | 60 | 80 | 100 | 110 | 110 | 140 | 180 | White color, crunchy, homogeneous flow, good particle diameter |
| **GAE3 : Rice:Chickpea** | 60:40 | 2.007 | 1.4 | 400 | 600 | 40 | 60 | 80 | 110 | 110 | 110 | 140 | 180 | White color, crunchy, homogeneous flow, good particle diameter |
| **GAE4 : Rice:Chickpea** | 60:40 | 2.007 | 1.4 | 400 | 600 | 40 | 60 | 80 | 100 | 110 | 120 | 150 | 190 | White color, crunchy, homogeneous flow, small particle diameter |
| **GAE5 : Rice:Chickpea** | 60:40 | 2.007 | 1.4 | 400 | 700 | 40 | 60 | 80 | 100 | 100 | 100 | 160 | 200 | White color, crunchy, homogeneous flow, small particle diameter |

The comparison article recommended specific formulations utilizing hempseed protein concentrate (HPC) with wheat gluten (WG) or chickpea protein concentrate (CPC) extruded at either 600 rpm or 700 rpm to achieve good physicochemical and textural properties. This similarity in the required screw speed suggests that a comparable level of specific mechanical energy (SME) input was necessary in both systems to achieve sufficient melt plasticization, regardless of the differing product goals.

However, the product outcomes are fundamentally different, dictated by moisture and thermal profiles. While the goal of the current work was to achieve an expanded, crunchy texture, the comparative study explicitly utilized high-moisture extrusion (HME), maintaining moisture contents between 62.5% and 65.0%, to obtain a dense, fibrous, meat-like structure and prevent expansion.

Correspondingly, the maximum barrel temperature utilized in the comparative study was optimized at 130°C, significantly lower than the die temperatures used in the current successful trials (170°C or 180°C), which are necessary to induce the rapid pressure drop and vaporization required for the observed expansion and crunchy texture.

Furthermore, the consistent white color observed in the current extrudates contrasts with the HMMA products reported in the comparison, which exhibited darker hues (lower $L^*$ values, down to 50.66) that became more pronounced with increasing hempseed protein content.

This divergence confirms that although the mechanical shearing forces (screw speed) were shared, the difference in material composition (rice/chickpea versus hemp/gluten/chickpea) combined with the varying moisture and temperature conditions yielded two functionally distinct product categories: expanded snacks versus dense meat analogues.

The results of the Semoula:Chickpea flour are presented in **Table 6.**

**Table 6.** Effect of Semoula:Chickpea Blend Ratios and Process Conditions on Extruder Output and Physical Characteristics of the Product.

| Sample | Ratio | Max Output (kg/h) | Total Output (kg/h) | Water Flow (mL/h) | Screw Speed (rpm) | T° Z2 | T° Z3 | T° Z4 | T° Z5 | T° Z6 | T° Z7 | T° Z8 | T° Die | Observations |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SGE1 : Semoula:Chickpea** | 50:50 | 2.112 | 0.6 | 183 | 400 | 40 | 60 | 80 | 120 | 140 | 140 | 140 | 120 | Gold color, crunchy, homogeneous flow, medium particle diameter |
| **SGE2 : Semoula:Chickpea** | 50:50 | 2.112 | 0.7 | 250 | 450 | 40 | 60 | 80 | 140 | 160 | 160 | 160 | 140 | Semi-humid, non-homogeneous flow |
| **SGE3 : Semoula:Chickpea** | 50:50 | 2.112 | 1.0 | 305 | 500 | 40 | 60 | 80 | 140 | 140 | 140 | 140 | 140 | Semi-humid, non-homogeneous flow |
| **SGE4: Semoula:Chickpea** | 50:50 | 2.112 | 1.3 | 305 | 500 | 40 | 60 | 80 | 140 | 150 | 150 | 150 | 150 | Crunchy, non-homogeneous flow |
| **SBE1: Semoula:Chickpea** | 60:40 | 2.723 | 0.8 | 200 | 200 | 40 | 60 | 80 | 100 | 160 | 160 | 160 | 160 | Gold color, crunchy, non-homogeneous flow, medium particle diameter |
| **SBE2 : Semoula:Chickpea** | 60:40 | 2.723 | 0.8 | 200 | 150 | 40 | 60 | 80 | 100 | 170 | 170 | 170 | 170 | Gold color, crunchy, homogeneous flow, medium particle diameter |
| **SBE3 : Semoula:Chickpea** | 60:40 | 2.723 | 0.8 | 200 | 150 | 40 | 60 | 80 | 100 | 180 | 180 | 180 | 180 | Gold color, crunchy, homogeneous flow, small particle diameter |
| **SBE4 : Semoula:Chickpea** | 60:40 | 2.723 | 0.8 | 200 | 150 | 40 | 60 | 80 | 100 | 190 | 190 | 190 | 190 | Gold color, crunchy, homogeneous flow, small particle diameter |

The optimization trials for the Semoula:Chickpea blends clearly demonstrate that the specific ratio of raw materials dictates the necessary mechanical and thermal energy inputs required to produce extrudates with desirable attributes.

The optimal formulation for the 50:50 Semoula:Chickpea ratio (SGE1) yielded a gold color, crunchy texture, homogeneous flow, and medium particle diameter. Achieving this required a high screw speed of 400 rpm and a water flow of 183 mL/h, alongside high mid-zone temperatures (120–140°C). Conversely, samples representing the 60:40 Semoula:Chickpea ratio (SBE2, SBE3, and SBE4) maintained desirable physical qualities (gold color, crunchy texture, homogeneous flow) at a substantially lower screw speed of 150 rpm, utilizing a water flow of 200 mL/h. Furthermore, minute adjustments to the die temperature (from 170°C to 190°C) in the 60:40 blends effectively controlled the final particle size, shifting it from medium to small while sustaining overall product quality.

This necessity of tailoring process settings based on the input mix aligns strongly with findings in research concerning the optimization of extruded products made from complex blends, such as Alefew et al. (2024), who combined rice, lupin, and pumpkin flour. That study, which used a twin-screw extruder to assess blending ratio, barrel temperature, and feed moisture, similarly concluded that these independent variables significantly affected the functional and physical properties of the extruded product. The lupin-based research noted that a higher proportion of the protein/fiber-rich component (lupin) resulted in higher bulk density and notably reduced expansion ratio (ER), emphasizing that the composition of the blend directly dictates the resulting structure and physical outcome.

Moreover, while the rice-lupin-pumpkin study found an optimum condition at a constant screw speed of 150 rpm but varied barrel temperatures (115–155 °C) and feed moisture (14–20 %), the Semoula:Chickpea data highlights that shifting the primary raw material ratio necessitates large adjustments across multiple parameters, including screw speed (150 rpm versus 400 rpm). These comparative results confirm the fundamental principle that the unique chemical profiles of different raw material systems (e.g., starch, protein, fiber content) critically dictate the optimal parameters required for successful extrusion, whether one is targeting particle size control, as in the Semoula:Chickpea system, or optimal expansion characteristics, as in the rice-lupin-pumpkin system.

Among all tested conditions, sample SGE1 from the Semoula:Chickpea (50:50) blend and sample AGE4 from the Rice:Chickpea (50:50) blend emerged as exemplars of the "best aspect" extrudates. **(Figure 19).**

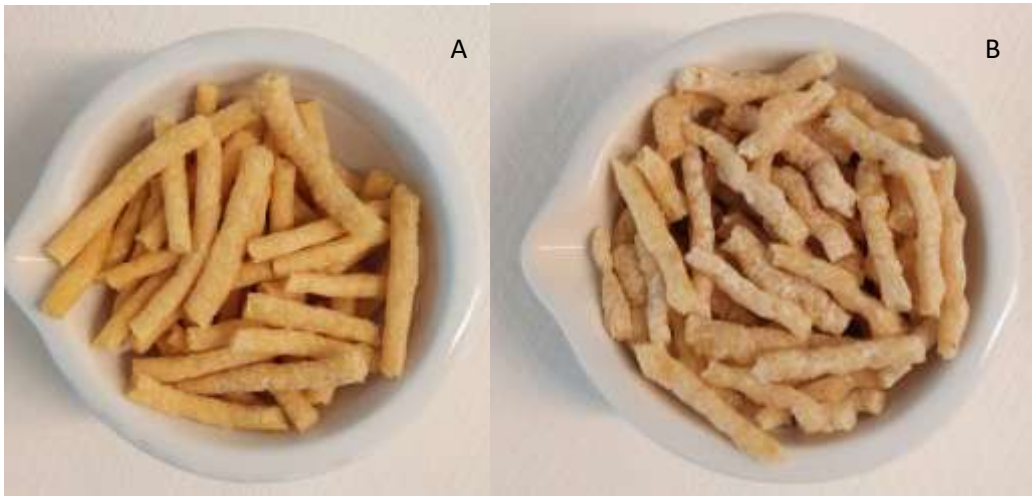


**Figure 19.** Extruded products of **A)** Semoula:Chickpea (50:50) and B)Rice:Chickpea (50:50).

SGE1 consistently demonstrated a gold color, crunchy texture, homogeneous flow, and medium particle diameter. These desirable characteristics were achieved under specific conditions: a maximum output of 2.112 kg/h, total output of 0.6 kg/h, water flow of 183 mL/h, a screw speed of 400 rpm, and a unique temperature profile (Zona2-4: 40-80°C, Zona5-8: 120-140°C, and a relatively lower die temperature of 120°C).

Similarly, AGE4 represented the optimal outcome for the Rice:Chickpea (50:50) blend, yielding extrudates with a white color, crunchy texture, homogeneous flow, and good particle diameter. Its production involved a maximum output of 1.669 kg/h, total output of 1 kg/h, water flow of 400 mL/h, a screw speed of 600 rpm, and a consistent temperature profile across multiple zones (Zona2-8: 40-120°C) with a die temperature of 180°C.

These findings underscore the significant impact of raw material composition on dictating the precise extrusion parameters required for optimal product quality. The distinct processing windows for SGE1 and AGE4 highlight the need for tailored approaches when extruding different flour blends to consistently achieve superior physical and textural properties.

### 4.2 Determining the protein content of the products obtained

The analysis of crude protein content for the composite flours, calculated using a conversion factor of 6.25, confirmed the achievement of enhanced nutritional profiles across all formulations. **(Table 7).**

**Table 7.** Crude Protein Content of Composite Semolina:Chickpea and Rice:Chickpea Flours (g/100 g DW)

| | Code | % Protein *6.25 (g/100 g DW) |
|---|---|---|
| **Semolina : Chickpea (50:50)** | SGE 1 | 12.6 ± 0.1 |
| | SGE 2 | 11.3 ± 0.1 |
| | SGE 3 | 11.5 ± 0.9 |
| | SGE 4 | 11.92 ± 0.05 |
| **Semolina : Chickpea (60:40)** | SBE 1 | 10.3 ± 0.3 |
| | SBE 2 | 10.8 ± 0.1 |
| | SBE 3 | 10.7 ± 0.05 |
| | SBE 4 | 10.2 ± 0.5 |
| **Rice : Chickpea (50:50)** | AGE 1 | 12.4 ± 0.7 |
| | AGE 2 | 12.6 ± 0.4 |
| | AGE 3 | 12.03 ± 0.94 |
| | AGE 4 | 13.39 ± 0.05 |
| **Rice : Chickpea (60:40)** | GAE 1 | 10.7 ± 0.5 |
| | GAE 2 | 11.5 ± 0.5 |
| | GAE 3 | 10.4 ± 0.3 |
| | GAE 4 | 11.3 ± 0.6 |
| | GAE 5 | 10.9 ± 0.4 |

SGE: Semolina: Chickpea (50:50, *w/w*); AGE: Rice: Chickpea (50:50, *w/w*). The results are presented: mean ± sd

The measured protein levels ranged from a minimum of 10.2 g/100 g DW (SBE 4) to a maximum of 13.39 g/100 g DW (AGE 4). Specifically, the Semolina:Chickpea blends (50:50) showed values of 12.6 g/100 g DW (SGE 1), 11.3 g/100 g DW (SGE 2), 11.5 g/100 g DW (SGE 3), and 11.92 g/100 g DW (SGE 4). The Semolina:Chickpea (60:40) blends were slightly lower, recording 10.3 g/100 g DW (SBE 1), 10.8 g/100 g DW (SBE 2), 10.7 g/100 g DW (SBE 3), and 10.2 g/100 g DW (SBE 4). The Rice:Chickpea (50:50) samples generally demonstrated the highest protein content: 12.4 g/100 g DW (AGE 1), 12.6 g/100 g DW (AGE 2), 12.03 g/100 g DW (AGE 3), and 13.39 g/100 g DW (AGE 4). Lastly, the Rice:Chickpea (60:40) GAE samples exhibited protein contents of 10.7 g/100 g DW (GAE 1), 11.5 g/100 g DW (GAE 2), 10.4 g/100 g DW (GAE 3), 11.3 g/100 g DW (GAE 4), and 10.9 g/100 g DW (GAE 5).

These composite flour results align with strategies that utilize pulses to fortify cereal diets. For comparison, the study by Liu et al. (2025) reported the native protein content of the

primary raw materials: raw rice flour (RF-RM) contained a low concentration of (4.89 ± 0.14)% protein, while raw chickpea flour (CF-RM) provided substantially higher content at (18.07 ± 0.25)%. Generally, pulses possess roughly double the protein content of cereals. Therefore, the obtained composite blend protein levels (10.2–13.39 g/100 g DW) successfully demonstrate improved protein quality relative to the high-starch cereal flours alone.

Further comparison with the findings of Liu et al. (2025), highlights the impact of thermal processing, such as the screw extrusion (SE) technique implicitly used to produce these instant powders. Liu et al. (2025) investigated the basic nutrients of single flours subjected to processing and found that the screw extrusion treatment intensified alterations, resulting in the lowest protein concentrations compared to raw or dry-heated materials. For instance, raw chickpea flour (CF-RM) content of (18.07 ± 0.25%) decreased to (16.87 ± 0.36%) after screw extrusion (CF-SE). Similarly, extruded rice flour (RF-SE) showed a protein content of (4.25 ± 0.44)%, a reduction from its raw state of (4.89 ± 0.14%). This declining trend in crude protein concentration following heat treatment has also been documented by Sopiwnyk et al. (2020). This decrease is primarily attributed to protein denaturation and the enhancement of interactions between starch and protein chains under elevated temperatures and shear forces, leading to the creation of starch-protein complexes that result in a measured reduction in protein content.

Among all results obtained, sample SGE1 from the Semoula:Chickpea (50:50) blend and sample AGE4 from the Rice:Chickpea (50:50) blend emerged as exemplars of the "highest protein content" extrudates.

## 4.3 Physical Characterization of the ready-to-eat snacks

### 4.3.1 Colour measurement

The colorimetric evaluation of the ready-to-eat snacks provided crucial insights into the visual distinctions between the SGE (50:50) and AGE (50:50) formulations, as clearly demonstrated by their respective *L**, *a**, and *b** parameters. **(Table 8).**

**Table 8.** Colour measurement of the ready-to-eat snacks

| ***Colour measurement*** | **SGE (Semolina : Chickpea 50:50)** | **AGE (Rice : Chickpea 50:50)** |
|---|---|---|
| *L** | 79.9 ± 0.7 | 78.4 ± 0.5 |
| *a** | 1.18 ± 0.04 | - 0.5 ± 0.1 |
| *b** | 50.9 ± 1.6 | 36 ± 1 |
| *ΔL** | - 13 ± 1 | - 14.8 ± 0.5 |
| *Δa** | 0.73 ± 0.04 | - 0.9 ± 0.1 |
| *Δb** | 47 ± 2 | 32 ± 1 |
| *ΔE** | 49 ± 2 | 35 ± 0.8 |

SGE: Semolina: Chickpea (50:50, *w/w*); AGE: Rice: Chickpea (50:50, *w/w*). The results are presented: mean ± sd

Specifically, the SGE sample registered a marginally greater lightness (*L** = 79.9 ± 0.7) in comparison to the AGE counterpart (*L** = 78.4 ± 0.5), thereby indicating a visibly lighter overall appearance for the semolina-based product. In terms of chromaticity, the SGE formulation exhibited a positive *a** value (1.18 ± 0.04), which is indicative of a subtle reddish tint, whereas the AGE formulation displayed a negative *a** value (-0.5 ± 0.1), suggesting a faint greenish cast. A more pronounced divergence between the two formulations was observed in their *b** values, where SGE presented a significantly stronger yellow component (50.9 ± 1.6) when compared to AGE (36 ± 1), underscoring a heightened yellow pigmentation in the semolina-derived product**.**

Furthermore, the calculated total color differences (ΔE*) between the initial raw materials and their respective final processed products were substantial for both formulations, registering 49 ± 2 for SGE and 35 ± 0.8 for AGE, a magnitude of change that profoundly signifies considerable color transformation throughout the entire processing stage.

Moreover, the analysis included ΔL*, Δa*, Δb*, and ΔE* values for each snack type, which typically represent the change or total color difference from a reference point. For the SGE snack, the ΔL* was -13 ± 1, Δa* was 0.73 ± 0.04, and Δb* was 47 ± 2. The total color

difference (ΔE*) for SGE was 49 ± 2. For the AGE snack, the ΔL* was -14.8 ± 0.5, , Δa* was -0.9 ± 0.1, and Δb* was 32 ± 1. The ΔE* for AGE was 35 ± 0.8.

The elevated Δb* and ΔE* values particularly observed in SGE imply that semolina's intrinsic pigments, such as carotenoids, likely played a significant role in either preserving or intensifying yellowness during the extrusion process**.** Conversely, the rice-based formulation might have experienced greater pigment degradation or received less chromatic contribution from its foundational cereal component during processing. Such discrepancies in color attributes could additionally stem from variations in Maillard reaction intensities and starch-protein interactions within the distinct cereal-legume matrices, as these factors are well-known to influence browning and overall color stability in food products.

Collectively, these findings underscore the significant impact that formulation type has on the visual characteristics of ready-to-eat snacks**,** with semolina-based products consistently exhibiting a brighter and more intensely yellow appearance compared to their rice-based equivalents, a difference with potential ramifications for consumer perception and market attractiveness.

### 4.3.2 Texture analysis

Texture analysis was conducted to determine the mechanical properties of the samples. However, during testing, the instrument exhibited irregular force readings and unstable baselines, later attributed to a malfunction of the load cell calibration. As a result, the measurements showed high variability and poor reproducibility across replicates.

This limitation highlights the importance of instrument verification prior to data acquisition and suggests that further analysis using a fully functional and recalibrated texture analyzer is necessary to confirm these preliminary observations.

### 4.4 Nutritional Characterization of the ready-to-eat snacks

The nutritional profiles of the two developed ready-to-eat snacks, SGE (Semolina: Chickpea, 50:50, *w/w*) and AGE (Rice: Chickpea, 50:50, *w/w*), were thoroughly characterized, and the results are presented in **Table 9**. This characterization encompassed key parameters such as humidity, fat, protein, ash, and carbohydrate content, as well as energy values.

**Table 9.** Nutritional Characterization of the ready-to-eat snacks

| | **SGE (Semolina : Chickpea 50:50)** | | **AGE (Rice : Chickpea 50:50)** | |
|---|---|---|---|---|
| | | *p-value* | | *p-value* |
| **Humidity (%)** | 3.9 ± 0.1 | <0.01 | 3.6 ± 0.1 | <0.01 |
| **Fat (g/100 g fw)** | 0.048 ± 0.003 | <0.01 | 0.04 ± 0.03 | <0.01 |
| **Proteins (g/100 g dw)** | 12.6 ± 0.1 | <0.01 | 13.39 ± 0.01 | <0.01 |
| **Ash (g/100 g fw)** | 0.12 ± 0.04 | <0.01 | 0.12 ± 0.03 | <0.01 |
| **Carbohydrates (g/100 g fw)** | 83,3± 0.3 | <0.01 | 82.8 ± 0.3 | <0.01 |
| **Energy (Kcal/100 g)** | 384 ± 1 | <0.01 | 385 ± 1 | <0.01 |
| **Energy (Kj/100 g)** | 1607 ± 4 | <0.02 | 1612 ± 4 | <0.02 |

SGE: Semolina: Chickpea (50:50, *w/w*); AGE: Rice: Chickpea (50:50, *w/w*). The results are presented: mean ± sd.

The humidity content was found to be 3.9 ± 0.1% for SGE and 3.6 ± 0.1% for AGE. Both values are relatively low, which is desirable for ready-to-eat snacks as it contributes to their stability and extended shelf-life by inhibiting microbial growth and reducing water activity. The values for both samples indicates a significant difference in humidity between the two formulations. The slightly lower humidity in AGE might contribute to a marginally greater stability or crispness compared to SGE, although both are within a suitable range for dry snack products.

The fat content was observed to be very low for both snack types. SGE contained 0.048 ± 0.003 g/100 g fw**,** while AGE had 0.04 ± 0.03 g/100 g fw**.** This low-fat profile makes these snacks a potentially healthier alternative to many conventional snacks, aligning with current dietary recommendations to reduce saturated and unhealthy fat intake. The values for both also suggests a significant difference in fat content between SGE and AGE, with AGE being slightly lower in fat.

The protein content, expressed on a dry weight basis, showed that AGE had a higher protein content than SGE. Specifically, SGE contained 12.6 ± 0.1 g/100 g dw of protein, whereas AGE contained 13.39 ± 0.01 g/100 g dw**.** The higher protein content in AGE compared to SGE is a notable finding, suggesting that the rice-chickpea combination might offer a more protein-rich snack option. The values for both formulations indicates a significant difference in their protein levels. This high protein content, derived from ingredients like chickpea, could contribute to increased satiety and muscle maintenance, enhancing the nutritional value of these snacks.

The ash content, representing the total mineral content, was relatively similar between the two snacks. SGE had 0.12 ± 0.04 g/100 g fw, and AGE had 0.12 ± 0.03 g/100 g fw. While both values are low, the values for both indicates a significant difference, with AGE possessing a marginally higher mineral content. This suggests that the rice-chickpea blend might offer a slightly richer source of dietary minerals compared to the Semolina:Chickpea blend.

Carbohydrates constituted the major macronutrient in both snacks, as expected for grain and legume-based products. SGE contained 83.3 ± 0.3 g/100 g fw carbohydrates, and AGE contained 82.8 ± 0.3 g/100 g fw. Both values are very high, confirming their primary role as energy providers. Although the difference is slight, the values for both indicates a significant difference between the two formulations. SGE exhibited a slightly higher carbohydrate content compared to AGE.

The energy content for both snacks was determined and found to be quite similar. In terms of kilocalories, SGE provided 383 ± 1 Kcal/100 g, while AGE provided 385 ± 1 Kcal/100 g. Similarly, in kilojoules, SGE had 1607 ± 4 Kj/100 g**,** and AGE had 1612 ± 4 Kj/100 g**.** Both values for energy measurements, indicated a significant, albeit very small, difference in energy content between the two snacks, with AGE being marginally higher. These energy densities are characteristic of dry, low-moisture snack products, providing a concentrated source of energy.

These results were discussed in the context of findings reported by Pena et al. (2025), who characterized ready-to-eat expanded whole grain bean snacks (Black Bean [BB], Red Bean [RB], Carioca Bean [CB], and Cowpea [CW]). Comparing the present study's cereal-pulse blends (SGE and AGE) with the whole bean snacks revealed a notable difference in macronutrient distribution: the bean snacks showed significantly higher protein contents, ranging from 21.53 ± 1.28 g/100 g (CB) to a maximum of 27.84 ± 0.71 g/100 g (CW). This

protein range substantially exceeds the values observed in the SGE and AGE formulations, likely reflecting the high percentage of cereal components in our snacks. Correspondingly, the whole bean snacks exhibited lower carbohydrate concentrations, ranging from 48.44 ± 0.05 g/100 g to 56.79 ± 0.51 g/100 g, contrasting sharply with the dominant carbohydrate profile of SGE and AGE. Although both types of snacks were low in fat, the bean snacks generally contained slightly higher lipid levels (0.22 ± 0.03 g/100 g to 0.49 ± 0.02 g/100 g) compared to the nearly negligible levels in SGE and AGE. Ultimately, while the SGE and AGE samples contained less protein and more carbohydrates than the bean-based snacks, they exhibited higher overall caloric density (approx. 384–385 Kcal/100 g) compared to the caloric range of the bean snacks (297.46 to 342.87 kcal/100 g).

The nutritional characterization reveals that both SGE and AGE are low-fat, high-carbohydrate, and moderately high-protein ready-to-eat snacks. The consistently observed values across all measured parameters for both SGE and AGE indicates that the differences in their nutritional composition are significant. This difference highlights that the choice of cereal component (semolina versus rice) measurably impacts the final nutritional profile of the snack, even when combined with the same proportion of chickpea. Specifically, the AGE formulation generally presented slightly lower humidity and fat, along with higher protein and marginally higher ash and energy content compared to SGE. These findings provide valuable insights for product development, indicating that specific ingredient choices can lead to distinct nutritional advantages.

## 4.5 Chemical composition of the ready-to-eat snacks

### 4.5.1 Free sugars

The quantitative assessment of free sugars within the developed ready-to-eat snack formulations, specifically the SGE and AGE variants, elucidated a remarkably constrained saccharide profile, with sucrose emerging as the sole quantifiable free sugar and melezitose notably absent from detection. **Table 10**. This focused analytical outcome holds particular significance when considered against the objective of formulating protein-enriched, nutritionally balanced convenience foods.

**Table 10.** Composition of free sugars of the ready-to-eat snacks.

| ***Free Sugars (g/100 g fw)*** | **SGE (Semolina : Chickpea 50:50)** | | **AGE (Rice : Chickpea 50:50)** | |
|---|---|---|---|---|
| | | *p-value* | | *p-value* |
| *Sucrose* | 0.77 ± 0.01 | <0.01 | 0.53 ± 0.03 | <0.01 |
| *Total sugars* | 0.77 ± 0.01 | <0.01 | 0.53 ± 0.03 | <0.01 |

SGE: Semolina: Chickpea (50:50, *w/w*); AGE: Rice: Chickpea (50:50, *w/w*). The results are presented: mean ± sd

In the SGE formulation, composed of semolina and chickpea in a 50:50 weight-to-weight ratio, the sucrose content was precisely determined at 0.77 ± 0.01 g/100 g fresh weight (fw). Conversely, the AGE formulation, based on a rice and chickpea blend in an equivalent ratio, presented a lower sucrose concentration of 0.53 ± 0.03 g/100 g fw. The disparity in sucrose levels between the two formulations is posited to originate from the inherent carbohydrate matrices of their primary cereal components; semolina typically harbors a greater abundance of disaccharides compared to rice, and variations in starch hydrolysis during processing may further contribute to these observed differences.

From a nutritional standpoint, the comparatively modest free sugar content identified in both products is advantageous, especially when aligning with the principal aim of developing protein-enriched snacks. Minimizing extraneous free sugars is crucial for enhancing the overall nutritional integrity of a snack, particularly one designed to deliver significant protein. This outcome resonates strongly with contemporary public health directives advocating for reduced free sugar intake. The data thus suggest that both the SGE and AGE formulations stand as viable candidates for consumers seeking convenient, protein-enriched snack alternatives that concurrently offer a controlled sugar profile.

According the study, made by Sreerama et al. (2012), that investigated the nutrient and antinutrient composition of cowpea, horse gram, and chickpea flours reported the presence and quantity of several sugar-related components, primarily focusing on flatulence-forming oligosaccharides. These oligosaccharides, including raffinose, stachyose, and verbascose, were detected in all three legume flours. For instance, chickpea flour contained the highest amount of total α-galactosides (34.9 mg/g), while horse gram had the lowest content (26.8 mg/g).

### 4.5.2 Fatty acids

The fatty acid (FA) analysis conducted on the two formulated products, SGE (Semolina: Chickpea 50:50) and AGE (Rice: Chickpea 50:50), revealed distinct lipid profiles. The results, presented in detail in **Table 11**, demonstrate significant differences in the distribution of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids between the two samples.

**Table 11.** Fatty acids profile of SGE (Semolina: Chickpea 50:50) and AGE (Rice: Chickpea 50:50)

| | **SGE (Semolina : Chickpea 50:50)** | | **AGE (Rice : Chickpea 50:50)** | |
|---|---|---|---|---|
| | **Fatty acids (%)** | | | |
| | | ***p-value*** | | ***p-value*** |
| C16:0 | 45.3 ± 0.4 | <0.01 | 14.37 ± 0.02 | <0.01 |
| C18:0 | 10.0 ± 0.5 | <0.01 | 3.183 ± 0.004 | <0.01 |
| C18:1n9 | 40.1 ± 0.4 | <0.01 | 34.006 ± 0.05 | <0.01 |
| C18:2n6 | 4.6 ± 0.3 | <0.01 | 48.4 ± 0.1 | <0.01 |
| **SFA** | 55.3 ± 0.1 | <0.01 | 17.6 ± 0.05 | <0.01 |
| **MUFA** | 40.1 ± 0.2 | <0.01 | 34.0 ± 0.1 | <0.01 |
| **PUFA** | 4.6 ± 0.2 | <0.01 | 48.4 ± 0.1 | <0.01 |

SGE: Semolina: Chickpea (50:50, *w/w*); AGE: Rice: Chickpea (50:50, *w/w*). The results are presented: mean ± sd

The SGE product exhibited a lipid profile dominated by Saturated Fatty Acids (SFA), recorded at 55.3 ± 0.1%, followed closely by Monounsaturated Fatty Acids (MUFA) at 40.1 ± 0.2%. Conversely, the SGE product showed a low Polyunsaturated Fatty Acid (PUFA) content (4.6 ± 0.2%). The most abundant individual fatty acid detected in SGE was palmitic acid (C16:0), registering 45.3 ± 0.4%. This was followed by oleic acid (C18:1n9) at 40.1 ± 0.3% and stearic acid (C18:0) at 10.0 ± 0.5%.

The high concentration of C16:0 observed in SGE is consistent with findings in the study conducted by Dias et al. (2015). Palmitic acid is often noted as the predominant fatty acid in processed snack foods and biscuits, with reported percentages ranging up to 50.64% in some samples. Furthermore, the overall high SFA content in SGE (55.3%) aligns with observations that certain convenience foods, such as biscuits analyzed in comparative studies, can exhibit high SFA levels, sometimes exceeding 60%. The elevated levels of SFAs, particularly palmitic acid, raise public health concerns. A high intake of saturated fat contributes to the development of chronic diseases, such as coronary heart disease. Specifically, palmitic acid levels have been linked to C-reactive protein expression in aortic endothelial cells, which may contribute to endothelial dysfunction.

In contrast, the AGE product presented a profile significantly skewed toward PUFA (48.4 ± 0.1%). The AGE sample contained the lowest SFA content (17.6 ± 0.05%) among the two preparations. The MUFA content in AGE was 34 ± 0.1%. Linoleic acid (C18:2n6) was the most prominent fatty acid in AGE, recorded at 48.4 ± 0.07%. This concentration significantly exceeds the typical range observed for this common PUFA in biscuits, which generally ranges from 8.51% to 31.18%. The SFA content in AGE was primarily derived from palmitic acid (C16:0), which measured 14.372 ± 0.02%. This value is notably lower than the minimum C16:0 content reported in sweet biscuits (22.73%) in the study conducted by Dias et al. (2015). Oleic acid (C18:1n9) constituted the primary MUFA at 34.006 ± 0.05%, a concentration typical of those reported for high-fat biscuits.

The dominance of PUFAs, especially linoleic acid, in AGE is nutritionally beneficial. Dias et al. (2015), indicate that increased linoleic acid (C18:2n6) is associated with a reduced risk of cardiovascular diseases. This high PUFA concentration may suggest the use of vegetable oils, such as soya oil, in the preparation of the base components, which are known sources of linoleic acid. The AGE product's relatively low SFA content is favorable, contrasting with the general trend of high SFA found in many market biscuits and processed foods, which often result from the replacement of partially hydrogenated fats with high-SFA vegetable fats like palm oil.

A favorable finding across both formulations was the relative absence of trans fatty acids (TFA) in SGE and AGE. This contrasts positively with results found in salty snacks and specific biscuits analyzed in the reference literature. In these comparative study, TFAs, often present as elaidic acid isomers (C18:1), which are formed during hydrogenation, were detected at levels

as high as 7.94% in salty snacks. Although some biscuits show low TFA concentrations (0.86% of total fatty acids), the near absence in SGE and AGE suggests adherence to industry initiatives aimed at reducing the use of hydrogenated vegetable fat in response to knowledge about the deleterious health effects of TFAs. High TFA, particularly in convenience foods like salty snacks, is a concern as it has been associated with adverse effects such as increased low-density lipoprotein cholesterol (LDL).

## 4.6 Evaluation of antimicrobial activity of the ready-to-eat snacks

### 4.6.1 Antibacterial activity

The antimicrobial activity of the AGE and SGE extracts was evaluated against a panel of Gram-negative (*Enterobacter cloacae, Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica, and Yersinia enterocolitica*) and Gram-positive (*Bacillus cereus, Listeria monocytogenes, and Staphylococcus aureus*) foodborne pathogens, utilizing a maximum tested concentration of 10 mg/mL. Overall, both AGE and SGE demonstrated limited inhibitory or bactericidal effects, as the majority of tested strains including *E. coli, P. aeruginosa, S. enterica, Y. enterocolitica*, *L. monocytogenes*, and *S. aureus* exhibited resistance with both Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values exceeding the maximum concentration tested (>10 mg/mL). Only the Gram-positive bacterium *B. cereus* showed weak susceptibility to AGE, yielding an MIC of 10 mg/mL, though a full bactericidal effect was not confirmed (MBC > 10 mg/mL). **(Table 12).**

**Table 12.** Antibacterial activity (MIC and MBC, mg/mL) of SGE (Semolina: Chickpea 50:50) and AGE (Rice: Chickpea 50:50)

| | | | | | Positive Control | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AGE | | SGE | | Streptomicin 1mg/mL | | Methicilin 1mg/mL | | Ampicillin 10mg/mL | |
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| ***Gram-negative bacteria*** | | | | | | | | | | |
| *Enterobacter Cloacae* | >10 | >10 | >10 | >10 | 0.007 | 0.007 | n.t. | n.t | n.t. | n.t. |
| *Escherichia coli* | >10 | >10 | >10 | >10 | 0.01 | 0.01 | n.t. | n.t. | n.t. | n.t. |
| *Pseudomonas aeruginosa* | >10 | >10 | >10 | >10 | 0.06 | 0.06 | n.t. | n.t. | n.t. | n.t. |
| *Salmonella enterica* | >10 | >10 | >10 | >10 | 0.007 | 0.007 | n.t. | n.t. | n.t. | n.t. |
| *Yersinia enterocolitica* | >10 | >10 | >10 | >10 | 0.007 | 0.007 | n.t. | n.t. | n.t. | n.t. |
| ***Gram-positive bacteria*** | | | | | | | | | | |
| *Bacillus cereus* | 10 | >10 | >10 | >10 | 0.007 | 0.007 | n.t. | n.t. | n.t. | n.t. |
| *Listeria monocytogenes* | >10 | >10 | >10 | >10 | 0.007 | 0.007 | n.t. | n.t. | 0.25 | 0.5 |
| *Staphylococcus aureus* | >10 | >10 | >10 | >10 | 0.007 | 0.007 | 0.007 | 0.007 | 0.25 | 0.5 |

*MIC- minimum inhibitory concentration; MBC – minimum bactericidal concentration

These findings regarding the limited efficacy of AGE and SGE contrast sharply with the potent results reported by Kaningini et al. (2024), who evaluated the antibacterial properties of green synthesized zinc oxide nanoparticles of chickpea (ZnO NPs). Kaningini et al. reported that their ZnO NPs displayed moderate activity against certain tested microbes, with the Gram-negative *E. coli* being highly susceptible (MIC 0.16 µg/mL) and the Gram-positive *S. aureus* also showing inhibition (MIC 0.31 µg/mL). The authors further noted that Gram-negative *E. coli* strains were more susceptible to the nanoparticles; however, they observed resistance for *Salmonella typhimurium* and *B. cereus* (MIC > 2.5 µg/mL). The substantial difference in antimicrobial efficacy is evident when comparing the low MIC values observed for ZnO NPs by Kaningini et al. (2024) against *E. coli* and *S. aureus* to the complete lack of activity observed for AGE and SGE against the same species in the present study, even at concentrations up to 10 mg/mL.

### 4.6.2 Antifungal activity

The antifungal activity of AGE and SGE was effectively quantified using the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) against two pathogenic *Aspergillus* species, *Aspergillus brasiliensis* and *Aspergillus fumigatus*. Both AGE and SGE exhibited fungistatic activity, with MIC values ranging from 5 mg/mL to 10 mg/mL, but failed to achieve fungicidal activity, as the MFC exceded the maximum tested concentration of 10 mg/mL. **(Table 13).**

**Table 13.** Antifungal activity (MIC and MFC, mg/mL) of SGE (Semolina: Chickpea 50:50) and AGE (Rice: Chickpea 50:50)

| | *Aspergillus brasiliensis* (MIC/MFC) | *Aspergillus fumigatus* (MIC/MFC) |
|---|---|---|
| AGE | 5/>10 | 5/>10 |
| SGE | 5/>10 | 10/>10 |
| Ketoconazole | 0.06/0.125 | 0.5/1 |

MIC: Minimal Inhibitory Concentration; MFC: Minimal Fungicidal Concentration

Specifically, AGE displayed an MIC of 5 mg/mL against both *A. brasiliensis* and *A. fumigatus* (MFC >10 mg/mL for both), while SGE showed comparable inhibition (MIC 5 mg/mL) against *A. brasiliensis* and required 10 mg/mL for the MIC against *A. fumigatus* (MFC >10 mg/mL in both cases). These results indicate that relatively high concentrations of the crude extracts are necessary to inhibit fungal growth, especially when contrasting them with the synthetic control, Ketoconazole, which demonstrated markedly higher potency (MIC/MFC of 0.06/0.125 mg/mL against *A. brasiliensis* and 0.5/1 mg/mL against *A. fumigatus*). The required inhibitory concentrations of AGE and SGE (5–10 mg/mL) align with findings reported for other complex, natural antifungal preparations, suggesting the presence of a mixture of compounds, potentially acting synergistically or competitively.

These findings confirm the presence of antifungal bioactivity in both chickpea-based extruded snacks, which can be attributed to legume-derived proteins and peptides known for their defense roles against fungal pathogens. Similar results were previously reported by Rizzello et al. (2017), who observed that hydrolysates obtained from mixed legume flours (pea, lentil, and faba bean) exhibited strong antifungal activity against Penicillium roqueforti and

Aspergillus parasiticus, with inhibition rates exceeding 85% at peptide concentrations of 6–25 mg/mL. The antifungal activity in their study was associated with native legume proteins such as defensins, vicilins, and lipid transfer proteins, and with short peptides released during enzymatic hydrolysis. Likewise, Özçelik et al. (2010) demonstrated that methanolic extracts of Cicer arietinum (chickpea) seeds exhibited notable antifungal activity against Candida albicans with MIC values as low as 8 µg/mL, which they attributed to peptides such as cicerin and arietin possessing strong membrane-disruptive properties.

Compared with these studies, the relatively higher MIC values observed in AGE and SGE (≥ 5 mg/mL) suggest that the extrusion process may have partially denatured or reduced the availability of active antifungal peptides. Moreover, the lower activity observed in SGE relative to AGE may be related to compositional differences between semolina and rice matrices, where the higher starch and lower lipid content of semolina could limit peptide accessibility or diffusion during testing.

Overall, the antifungal potential of AGE and SGE confirms that chickpea inclusion contributes bioactive compounds capability of inhibiting fungal growth, albeit at moderate concentrations. Future optimization of processing conditions, such as controlled enzymatic hydrolysis or reduced thermal degradation during extrusion, could further enhance the antifungal efficacy of such legume-based snacks.

# 5. Conclusion

This study successfully developed and comprehensively characterized nutritionally beneficial ready-to-eat snacks derived from extruded leguminous flour (Chickpea, Semolina, and Rice flour), affirming the viability of leveraging extrusion technology to address consumer demand for convenient and healthy options while promoting legume consumption.

The primary objective to extrude chickpea flour combined with semolina (SGE) and rice (AGE) flours to produce a high protein snacks was accomplished through meticulous process optimization.

Specific formulations, notably AGE4 (Rice:Chickpea 50:50) and SGE1 (Semolina:Chickpea 50:50), emerged as exemplars, exhibiting highly desirable physical attributes, including a crunchy texture, homogeneous flow, and white/gold coloration.

Nutritional characterization revealed that both snack types are low-fat, high-carbohydrate, and high-protein options. The AGE formulation, in particular, demonstrated a statistically significantly higher protein content (13.39 ± 0.01 g/100 g dw) compared to SGE (12.58 ± 0.10 g/100 g dw), suggesting that the rice-chickpea combination offers a more protein-rich snack.

Furthermore, both blends exhibited very low fat and low humidity levels, favorable characteristics that enhance shelf-life and provide potential health benefits. Chemical analysis revealed a modest, controlled-sugar profile, with sucrose identified as the sole quantifiable free sugar, aligning favorably with public health recommendations for reduced sugar intake. The fatty acid profile of AGE was nutritionally beneficial, dominated by Polyunsaturated Fatty Acids (PUFA: 48.4 ± 0.1%), contrasting with the Saturated Fatty Acid (SFA)-dominated profile of SGE (55.3 ± 0.1%). While extracts from the snacks showed limited antibacterial activity (MIC generally >10 mg/mL), they did exhibit fungistatic activity against *Aspergillus* species, with MIC values ranging from 5 mg/mL to 10 mg/mL.

In conclusion, this research confirms that the choice of plant matrix significantly impacts the final nutritional profile and required extrusion parameters, positioning these low-fat, moderately high-protein snacks as viable, health-oriented alternatives to conventional snack products.

# 6. References


**A**

Abeykoon, C., Li, K., McAfee, M., Martin, P. J., & Irwin, G. W. (2011). Extruder melt temperature control with fuzzy logic. IFAC Proceedings Volumes, 44(1), 8577-8582. . https://doi.org/10.3182/20110828-6-IT-1002.01576.

Alefew, Y. D., Tiruneh, A. T., & Yehuala, T. F. (2024). Optimization of extrusion conditions for development of high quality rice-lupin-pumpkin based extruded snack food. Heliyon, 10(24). https://doi.org/10.1016/j.heliyon.2024.e40913.

Anton, A. A., Fulcher, R. G., & Arntfield, S. D. (2009). Physical and nutritional impact of fortification of corn starch-based extruded snacks with common bean (Phaseolus vulgaris L.) flour: Effects of bean addition and extrusion cooking. Food chemistry, 113(4), 989-996. https://doi.org/10.1016/j.foodchem.2008.08.050.

AOAC. (2016). Dr. Latimer, George W, Jr. (ed.), Official Methods of Analysis of AOAC INTERNATIONAL, 22nd Edition (New York, 2023; online edn, AOAC Publications, 4 Jan. 2023), https://doi.org/10.1093/9780197610145.001.0001, accessed 17 Jan. 2025.

Ayilara, M.S., Abberton, M., Oyatomi, O.A., Odeyemi, O., and Babalola, O.O. (2022). Potentials of underutilized legumes in food security. Front. Soil Sci. 2:1020193. doi: 10.3389/fsoil.2022.1020193.

**B**

Backus, R.G., Boshold, R.F., Johanisson, T.G. (2014). Drawing, extruding and upsetting. In: Wick C, Benedict JT, Veilleux RF, editors. Tool and manufacturing engineering handbook, vol 2. 4th ed. Dearborn, MI: Society of Manufacturing Engineers. p. 11–13.

Barahuie, F., Dorniani, D., Saifullah, B., Gothai, S., Hussein, M. Z., Pandurangan, A. K., & Norhaizan, M. E. (2017). Sustained release of anticancer agent phytic acid from its chitosan-coated magnetic nanoparticles for drug-delivery system. International journal of nanomedicine, 2361-2372. https://doi.org/10.2147/IJN.S126245.

Barros, L., Pereira, E., Calhelha, R. C., Dueñas, M., Carvalho, A. M., Santos-Buelga, C., & Ferreira, I. C. (2013). Bioactivity and chemical characterization in hydrophilic and lipophilic compounds of Chenopodium ambrosioides L. Journal of Functional Foods, 5(4), 1732-1740. https://doi.org/10.1016/j.jff.2013.07.019.

Basheer-Salimia, R., Aloweidat, M.Y., Al- Salimiya, M.A., Hamdan, Y.A.S. and Sayara, T.A.S. (2021). Comparative Study of Five Legume Species under Drought Conditions. Legume Research - An International Journal. DOI: 10.18805/LR-577.

Brennan, C., Brennan, M., Derbyshire, E., & Tiwari, B. K. (2011). Effects of extrusion on the polyphenols, vitamins and antioxidant activity of foods. Trends in Food Science & Technology, 22(10), 570-575. https://doi.org/10.1016/j.tifs.2011.05.007

Bruneau, A., de Queiroz, L.P., Ringelberg, J.J., (2024). Advances in Legume Systematics 14. Classification of Caesalpinioideae. Part 2: Higher-level classification. PhytoKeys. 2024; 240:1-552. Published 2024 Apr 3. doi:10.3897/phytokeys.240.101716.

**C**

Carlo, G. R., Michela, V., Stefano, B., Valerio, G., Marco G. (2017). Hydrolysate from a mixture of legume flours with antifungal activity as an ingredient for prolonging the shelf-life of wheat bread, Food Microbiology, Volume 64, Pages 72-82, ISSN 0740-0020, https://doi.org/10.1016/j.fm.2016.12.003.

Chokshi, R., & Zia, H. (2004). Hot-melt extrusion technique: a review., 3–16 https://doi.org/10.22037/ijpr.2010.290

Choton, S., Gupta, N., Bandral, J. D., Anjum, N., & Choudary, A. (2020). Extrusion technology and its application in food processing: A review. The Pharma Innovation Journal, 9(2), 162-168. https://doi.org/10.22271/tpi.2020.v9.i2d.4367.

**D**

Dahl, W. J., Foster, L. M., & Tyler, R. T. (2012). Review of the health benefits of peas (Pisum sativum L.). British Journal of Nutrition, 108(S1), S3-S10. doi:10.1017/S0007114512000852.

Deenanath, E. D., & Egal, A. (2017). Food Extrusion Technology: Initiatives to Address Food and Nutrition Insecurity in South Africa. Journal of Pharmacy and Nutrition Sciences, 7(3), 116–123. https://doi.org/10.6000/1927-5951.2017.07.03.7.

De la Peña, T.C., Pueyo, J.J. (2011). Legumes in the reclamation of marginal soils, from cultivar and inoculant selection to transgenic approaches. Agron. Sustain. Dev. 32, 65–91. https://doi.org/10.1007/s13593-011-0024-2.

**E**

European Journal of Public Health, Vol. 33, Supplement 2, (2023). Our Food, Our Health, Our Earth: A Sustainable Future for Humanity Dublin, Ireland 8–11 November 2023. https://doi.org/10.1093/eurpub/ckad160.fm001.

**F**

Fan, P. H., Zang, M. T., & Xing, J. (2015). Oligosaccharides composition in eight food legumes species as detected by high-resolution mass spectrometry. Journal of the Science of Food and Agriculture, 95(11), 2228-2236. https://doi.org/10.1002/jsfa.6940.

Flávia, S. L. D., Maria E. A. P., Maria G. T. C., Maria L. M. L., Vera L. V. M. (2015). Fatty acid profile of biscuits and salty snacks consumed by Brazilian college students, Food Chemistry, Volume 171, Pages 351-355, ISSN 0308-8146, https://doi.org/10.1016/j.foodchem.2014.08.133.

Francielle, B. P., Andressa, A. S., Jaqueline, M. V. T., Monique, B. S., Carlos, W. P. C., Hércia, S. D. M., Bárbara, P. S., (2025). Physicochemical, nutritional, and sensory properties of ready-to-eat whole grain bean snacks, Food Research International, Volume 217, 116754, ISSN 0963-9969, https://doi.org/10.1016/j.foodres.2025.116754.

**G**

Gamel, T. H., Abdel-Aal, E. S. M., Ames, N. P., Henderson, K., Prothon, F., Kongraksawech, T., & Tosh, S. M. (2015). AACCI approved methods technical committee report: A new AACCI approved method (32-24.01) for measuring viscosity of β-glucan in cereal products using the rapid visco analyzer. Cereal Foods World, 60(6), 279-283. http://dx.doi.org/10.1094/CFW-60-6-0279.

Ganesan, K., Xu, B. (2017). Polyphenol-Rich Lentils and Their Health Promoting Effects. International Journal Molecular Science. 2017;18(11):2390. doi:10.3390/ijms18112390.

Gilani, G. S., Xiao, C. W., & Cockell, K. A. (2012). Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. British Journal of Nutrition, 108(S2), S315-S332.doi: 10.1017/S0007114512002371.

Grand View Research, (2018). Snacks Market Size, Share & Trends Analysis Report By Product (Frozen & Refrigerated, Fruit, Bakery, Savory, Confectionery, Dairy), By Packaging, By Distribution Channel, By Region, And Segment Forecasts, 2024 – 2030

Gupta, P., Singh, R., Malhotra, S., Boora, K. S., & Singal, H. R. (2010). Characterization of seed storage proteins in high protein genotypes of cowpea [Vigna unguiculata (L.) Walp.]. Physiology and Molecular Biology of Plants, 16, 53-58. https://doi.org/10.1007/s12298-010-0007-9.

Guzmán-Maldonado, S. H., Acosta-Gallegos, J., & Paredes-López, O. (2000). Protein and mineral content of a novel collection of wild and weedy common bean (Phaseolus vulgaris L). Journal of the Science of Food and Agriculture, 80(13), 1874-1881. https://doi.org/10.1002/1097-0010(200010)80:13%3C1874::AID-JSFA722%3E3.0.CO;2-X

**H**

Harper, J.M. (2019). Extrusion of Foods, 1st ed.; CRC Press: Boca Raton, FL, USA,; p. 220. Hegazy, H.S.; El-Bedawey, A.E.A.; Rahma, E.H.; Gaafar, A.M. E_(2017). Extrusion process on nutritional, functional properties and antioxidant activity of germinated chickpea incorporated corn extrudates. American. Journal. Food Science. Nutrtion. Res, 4, 59–66. https://doi.org/10.1201/9780429290428.

Horax, R., Hettiarachchy, N. S., Chen, P., & Jalaluddin, M. (2004). Preparation and characterization of protein isolate from cowpea (Vigna unguiculata L. Walp.). Journal of food science, 69(2), fct114-fct118. https://doi.org/10.1111/j.1365-2621.2004.tb15500.x

**I**

Izalin, Z., Simona, R., Cecilia, A., Karolina, Ö., Marilyn, R., Jeanette, P. (2023), High moisture meat analogues from hemp – The effect of co-extrusion with wheat gluten and chickpea proteins on the textural properties and sensorial attributes, LWT, Volume 189, 115494. ISSN 0023-6438, https://doi.org/10.1016/j.lwt.2023.115494.

Ibáñez, M. A., De Blas, C., Cámara, L., & Mateos, G. G. (2020). Chemical composition, protein quality and nutritive value of commercial soybean meals produced from beans from different countries: A meta-analytical study. Animal Feed Science and Technology, 267, 114531. https://doi.org/10.1016/j.anifeedsci.2020.114531.

**J**

Jukanti, A. K., Gaur, P. M., Gowda, C. L. L., & Chibbar, R. N. (2012). Nutritional quality and health benefits of chickpea (Cicer arietinum L.): a review. British Journal of Nutrition, 108(S1), S11–S26. doi:10.1017/S0007114512000797.

**K**

Kaiwen, L., Xiaolin, A., Pengfei, J., Baoshang, F., Libo, Q., Chengrong, W., Shang, S., (2025). Effects of dry heating and extrusion on physicochemical properties, in vitro digestibility and instant properties of rice, chickpea, and oat flour, Food Bioscience, Volume 69, 106929, ISSN 2212-4292, https://doi.org/10.1016/j.fbio.2025.106929.

Kaningini, A. G., Motlhalamme, T., More, G. K., Azizi, S., Mohale, K. C., & Maaza, M. (2024). Evaluation of the antimicrobial activity and chickpea growth stimulation effects of green synthesized zinc oxide nanoparticles. Plant Stress, 12, 100442. https://doi.org/10.1016/j.stress.2024.100442.

Kim, H., Caulfield, L.E., Garcia-Larsen, V., Steffen, L.M., Coresh, J., Rebholz, C.M. (2018). Plant-Based Diets Are Associated With a Lower Risk of Incident Cardiovascular Disease, Cardiovascular Disease Mortality, and All-Cause Mortality in a General Population of Middle-Aged Adults. Journal of the American Heart Association. 2018 Aug 20;8(16):e012865. doi: 10.1161/JAHA.119.012865. Epub 2018 Aug 7. PMID: 31387433; PMCID: PMC6759882.

Kostic, M., Smiljkovi´c, M., Petrovi´c, J., Glamo˘clija, J., Barros, L., Ferreira, I. C. F. R., Sokovi´c, M. (2017). Chemical, nutritive composition and a wide range of bioactive properties of honey mushroom: Armillaria mellea (Vahl: Fr.) Kummer. Food and Function, 8(9), 3239–3249. https://doi.org/10.1039/c7fo00887b

Kumar, N., Hazra, K.K., Nath, C.P., Praharaj, C.S., Singh, U. (2018). Grain Legumes for Resource Conservation and Agricultural Sustainability in South Asia. In: Meena, R., Das, A., Yadav, G., Lal, R. (eds) Legumes for Soil Health and Sustainable Management. Springer, Singapore. https://doi.org/10.1007/978-981-13-0253-4_3.

Kumar, N., (2024). Grain legumes: crops for novel food and nutritional security. (2024). Journal of Food Legumes, 35(2), 79-80. https://doi.org/10.59797/journaloffoodlegumes.v35i2.339.

**L**

Lohani, U.C., Muthukumarappan, K. (2017). Process optimization for antioxidant enriched sorghum flour and apple pomace based extrudates using liquid CO2 assisted extrusion. LWT 86, 544–554. doi: 10.1016/j.lwt.2017.08.034 .

**M**

Madurapperumage, A., Tang, L., Thavarajah, P. (2021). Chickpea (Cicer arietinum L.) as a Source of Essential Fatty Acids - A Biofortification Approach. Front Plant Science. 2021; 12:734980.

Maniruzzaman, M., Boateng, J. S., Snowden, M. J., & Douroumis, D. (2012). A Review of Hot-Melt Extrusion: Process Technology to Pharmaceutical Products. ISRN Pharm. 2012, 1–9. doi:10.3389/fpls.2021.734980.

Marinangeli, C.P.F., Curran, J., Barr, S.I., Slavin, J., Puri, S., Swaminathan, S., Tapsell, L. and Patterson, C.A. (2017). Enhancing nutrition with pulses: defining a recommended serving size for adults. Nutrition Reviews 75: 990-1006. https://doi.org/10.1093/nutrit/nux058.

Menis-Henrique, M.E.C., Janzantti, N.S., Monteiro, M., Conti-Silva, A.C. (2020). Physical and sensory characteristics of cheese-flavored expanded snacks obtained using butyric acid and cysteine as aroma precursors: effects of extrusion temperature and sunflower oil content. LWT 122 (1–24), 109001. doi: 10.1016/j.lwt.2019.109001 .

Moscicki, L. (2011). Extrusion-Cooking Techniques: Applications, Theory and Sustainability, 1st ed.; Wiley-VCH: Weinheim, Germany, p. 234.

Mullins, A.P., Arjmandi, B.H. (2021). Health Benefits of Plant-Based Nutrition: Focus on Beans in Cardiometabolic Diseases. Nutrients. ;13(2):519. Published 2021 Feb 5. doi:10.3390/nu13020519.

**N**

Nachal, N., Moses, J.A., Karthik, P., Anandharamakrishnan, C. (2019). Applica- tions of 3-D printing in food processing. Food Engineering. Review. 11 (3), 123–141. doi: 10.1007/s12393-019-09199-8 .

Nhu, Q. T., Larry, M.Y., Allyssa, D., and Margaret, R.D. (2024). A mixture of grass–legume cover crop species may ameliorate water stress in a changing climate, AoB PLANTS, Volume 16, Issue 4, plae039, https://doi.org/10.1093/aobpla/plae039.

NPGA. (2016). 2015 Pulse Quality Survey. Online publication. North- ern Pulse Growers Association. www.northernpulse.com/uploads/7/ 2015USPulseQualitySurvey.pdf

**O**

Offiah, V., Kontogiorgos, V., Falade, K.O. (2018). Extrusion processing of raw food materials and by-products: A review. Critical Review Food Science Nutrition. 2019;59(18):2979-2998. doi: 10.1080/10408398.2018.1480007.

Organization of the United Nations. (2016). 68th Session, Resolution Adopted by the General Assembly on 20 December 2013 [on the Report of the Second Committee (A/68/444)] 68/International Year of Pulses, 2016. 2014. Available online : https://www.un.org/en/sections/observances/international-years/index.html.

Ottoboni, M., Spranghers, T., Pinotti, L., Baldi, A., DeJaeghered, W., Eeckhoute, M., (2017). Inclusion of HermetiaIllucens larvae or prepupae in an experimental extruded feed: process optimization and impact on in vitro digestibility. Italian. Journal. Of Animal. Science. 17, 1–10. doi: 10.1080/1828051X.2017.1372698.

Özçelik, B., Kan, A., Kartal, M. U. R. A. T., Özdemir, Z. A., & Özgen, S. (2010). In vitro antimicrobial activities of Cicer arietinum L (Chickpea). Tropical Journal of Pharmaceutical Research, 9(5). https://doi.org/10.4314/tjpr.v9i5.68386.

**P**

Paraman, I., Sharif, M.K., Supriyadi, S., Rizvi, S.S., (2015). Agro-food industry by- products into value-added extruded foods. Food Bioproduction Process. 96, 78–85. doi: 10.1016/j.fbp.2015.07.003.

Paredes, M., Becerra, V., & Tay, J. (2009). Inorganic nutritional composition of common bean (Phaseolus vulgaris L.) genotypes race Chile. Chilean Journal of Agricultural Research, 69(4), 486-95. http://hdl.handle.net/1807/45773.

Patterson, C. A., Curran, J., & Der, T. (2017). Effect of processing on antinutrient compounds in pulses. Cereal Chemistry, 94(1), 2-10. https://doi.org/10.1094/CCHEM-05-16-0144-FI.

Patil, S. S., Brennan, M. A., Mason, S. L., & Brennan, C. S. (2016). The Effects of Fortification of Legumes and Extrusion on the Protein Digestibility of Wheat Based Snack. Foods, 5(2), 26. https://doi.org/10.3390/foods5020026.

Pereira, E., Barros, L., Martins, A., & Ferreira, I. C. (2012). Towards chemical and nutritional inventory of Portuguese wild edible mushrooms in different habitats. Food Chemistry, 130(2), 394–403. https://doi.org/10.1016/j.foodchem.2011.07.057.

Pismag, R.Y., Polo, M.P., Hoyos, J.L., Bravo, J.E., Roa, D.F. (2024). Effect of extrusion cooking on the chemical and nutritional properties of instant flours: a review. F1000Res. 2024; 12:1356. doi:10.12688/f1000research.140748.1.

**R**

Ramachandra, H. G., & Thejaswini, M. L. (2015). Extrusion technology: A novel method of food processing. International Journal of Innovative Science, Engineering & Technology, 2(4), 358-369.

Ray, H., Bett, K.E., Tar'An, B., Vandenberg, A., Thavarajah, D., Warkentin, T. (2014). Mineral micronutrient content of cultivars of field pea, chickpea, common bean, and lentil grown in Saskatchewan, Canada. Crop Science. 54, 1698–1708. https://doi.org/10.2135/cropsci2013.08.0568.

Riaz, M.N., (2019). Food extruders. In: Kutz M., editor. Handbook of Farm, Dairy and Food Machinery Engineering. 2nd ed. Elsiever; Amsterdam, The Netherlands. pp. 483–497. https://doi.org/10.1016/B978-0-12-385881-8.00016-1

Rosso, V. V., & Mercadante, A. Z. (2007). Identification and quantification of carotenoids, by HPLC-PDA-MS/MS, from Amazonian fruits. Journal of Agricultural and Food Chemistry, 55(13), 5062-5072. http://dx.doi.org/10.1021/jf0705421.

**S**

Saadat, S., Akhtar, A., Ismail, T., Sharif, M., Shabbir, U., Ahmad, N., & Ali, A. (2020). Multilegume bar prepared from extruded legumes flour to address protein energy malnutrition. Italian journal of food science, 32(1). Https://doi.org/10.14674/ijfs-1559.

Scopus Database. (2020). Document Search. Elsevier B.V.: Amsterdam, The Netherlands. Availableonline:https://www.scopus.com/search/form.uri?zone=TopNavBar&origin=sbrowse&display=basic (accessed on 20 April 2020).

Sreerama, Y. N., Sashikala, V. B., Pratape, V. M., & Singh, V. (2012). Nutrients and antinutrients in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their flour functionality. Food Chemistry, 131(2), 462-468. https://doi.org/10.1016/j.foodchem.2011.09.008.

Shah, F. U. H., Sharif, M. K., Bashir, S., & Ahsan, F. (2019). Role of healthy extruded snacks to mitigate malnutrition. Food Reviews International, 35(4), 299-323. https://doi.org/10.1080/87559129.2018.1542534.

Singh, S., Kudapa, H., Garg, V., & Varshney, R. K. (2019). Comprehensive analysis and identification of drought-responsive candidate NAC genes in three semi-arid tropics (SAT) legume crops. BMC genomics, 22(1), 289. https://doi.org/10.1186/s12864-021-07602-5.

Spréa, R. M., Fernandes, Â., Calhelha, R. C., Pereira, C., Pires, T. C., Alves, M. J., ... & Ferreira, I. C. (2020). Chemical and bioactive characterization of the aromatic plant Levisticum officinale WDJ Koch: A comprehensive study. Food & function, 11(2), 1292-1303. doi:10.1039/C9FO02841B.

Sopiwnyk, E., Bourr´e, L., Young, G., Borsuk, Y., Lagass´e, S., Boyd, L., Malcolmson, L. (2020). Flour and bread making properties of whole and split yellow peas treated with dry and steam heat used as premilling treatment. Cereal Chemistry, 97(6), 1290–1302. https://doi.org/10.1002/cche.10357

**T**

Tharanathan, R. N., & Mahadevamma, S. (2003). Grain legumes—a boon to human nutrition. Trends in Food Science & Technology, 14(12), 507-518. https://doi.org/10.1016/j.tifs.2003.07.002.

**U**

United States Department of Agriculture (USDA) ; (2016). "Feeding the Future With Pulse Crops" was published in the February 2016 issue of AgResearch Magazine.

**W**

Wallace, T. C., Murray, R., & Zelman, K. M. (2016). The Nutritional Value and Health Benefits of Chickpeas and Hummus. Nutrients, 8(12), 766. https://doi.org/10.3390/nu8120766.

Wang, N., & Daun, J. K. (2004). Effect of variety and crude protein content on nutrients and certain antinutrients in field peas (Pisum sativum). Journal of the Science of Food and Agriculture, 84(9), 1021-1029. https://doi.org/10.1002/jsfa.1742.

Wang, N., Hatcher, D. W., Tyler, R. T., Toews, R., & Gawalko, E. J. (2010). Effect of cooking on the composition of beans (Phaseolus vulgaris L.) and chickpeas (Cicer arietinum L.). Food Research International, 43(2), 589-594. https://doi.org/10.1016/j.foodres.2009.07.012.

Wolfe, K., Wu, X., & Liu, R. H. (2003). Antioxidant activity of apple peels. Journal of agricultural and food chemistry, 51(3), 609-614. https://doi.org/10.1021/jf020782a.

**Y**

Yadav, D.N., Anand, T., Chhikara, N., Singh, A.K. (2013). Co-extrusion of pearl millet-whey protein concentrate for expanded snacks. International. Journal of. Food Science. Technology. 49, 840–846. https://doi.org/10.1111/ijfs.12373

Yu, L. (2011). Extrusion processing of protein rich food formulations. McGill University (Canada). https://escholarship.mcgill.ca/downloads/qn59q8444

**Z**

Zia-Ul-Haq, M., Ahmad, M., Iqbal, S., Ahmad, S., & Ali, H. (2007a). Characterization and compositional studies of oil from seeds of desi chickpea (Cicer arietinum L.) cultivars grown in Pakistan. Journal of the American Oil Chemists' Society, 84(12), 1143-1148. https://doi.org/10.1007/s11746-007-1136-3.

Zia-Ul-Haq, M., Ahmad, S., Shad, M. A., Iqbal, S., Qayum, M., Ahmad, M., Luthria, D. L., and Amarowicz, R. (2011). Compositional studies of lentil (Lens culinaris Medik.) cultivars commonly grown in Pakistan. Pakistanian. Journal. Bot. 43:1563-1567.

Zia-Ul-Haq, M., Ahmad, S., Amarowicz, R., & Ercisli, S. (2013). Compositional studies of some pea (Pisum sativum L.) seed cultivars commonly consumed in Pakistan. Italian Journal of Food Science/Rivista Italiana di Scienza degli Alimenti, 25(3).https://link.gale.com/apps/doc/A354661748/AONE?u=anon~c7b59681&sid=googleScholar&xid=97c3b303.

Zhou, W., Yu, J., Lin, J., & Dean, T. A. (2020). Effects of die land length and geometry on curvature and effective strain of profiles produced by a novel sideways extrusion process. Journal of Materials Processing Technology, 282, 116682. https://doi.org/10.1016/j.jmatprotec.2020.116682.