Preparation of Plant-Based Beverage from Chickpea: A Study on Process Optimization and Nutritional Evaluation

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Table of Content

Abstract

Chickpea (*Cicer arietinum*), a versatile legume rich in proteins, dietary fibers, and essential nutrients (vitamins and minerals like iron, magnesium and folate), has gained attention as a potential ingredient for the development of plant-based beverages.

This study began by exploring the various process parameters for extraction of chickpea beverage preparation. **Objective 1** was to develop an optimized protocol that ensures maximum extraction of desirable components while minimizing undesirable compounds. Using response surface methodology, selected process parameters were optimized for maximizing protein content, carbohydrate content, and total solid content (for maximizing nutritional content) and minimizing specific LOX (lipoxygenase) activity to reduce beany/off flavour of the beverage. The predicted optimal conditions obtained included a water-to-chickpea ratio of 7.13:1, extraction temperature of 77.9°C, and extraction time of 51 minutes. Under these conditions, the beverage contained 4.12 g/100ml of protein, 5.78 g/100ml of total carbohydrates, 10.57 g/100ml of total solids, and had a specific LOX activity of 0.0084 units/mg of protein. These parameters help minimize the beany flavor while preserving the beverage's nutritional properties.

Furthermore, in **Objective 2,** the nutritional composition of the chickpea-based beverages was assessed in detail, considering macronutrients, micronutrients, and bioactive compounds. The evaluation encompasses proximate analysis, amino acid profiling, mineral content determination, and rheology of the beverage. The results provide insights into the nutritional potential of chickpea-based beverages and their suitability as functional food products. Sensory evaluation is also an integral part of this research, as consumer acceptability plays a crucial role in determining the market viability of the developed beverage. Panellists were recruited to assess the sensory attributes, such as taste, aroma, texture, and overall acceptability, using standardized sensory

evaluation techniques. Their feedback was analyzed to assess the sensory characteristics of chickpea-based beverages, emphasizing their distinctiveness and consumer appeal. The chickpea beverage stood out with its high protein content (5.59±0.17%), surpassing soy, oats, rice, and almond milk. Its PDCAAS score of 0.89 highlighted its quality protein source status. Sensory evaluation showcased strong consumer appeal, earning positive ratings for flavor, taste, and aftertaste. These results indicate its potential for market success, appealing to health-conscious consumers seeking nutritious plant-based options.

In recent years, novel technologies such as ultrasound and microwave have gained attention as alternative approaches to producing plant-based beverages, challenging conventional methods. In **Objective 3**, process parameters for Microwave and Ultrasound assisted processing were optimized to maximize the protein content of the processed chickpea beverage.

Other beverage characteristics, like protein solubility, In vitro protein digestibility (IVPD) and Trypsin inhibitor (TI) activity, were also studied at optimum conditions and compared with the conventional treatment. Both microwave and ultrasound processing significantly increased protein content in chickpea beverage, with microwave enhancing it by 47.78% and ultrasound by 21.22% compared to conventional methods. Improved characteristics, such as protein solubility, in vitro protein digestibility, and reduced trypsin inhibitor activity, were observed at optimized conditions. Response Surface Methodology (RSM) determined optimal conditions for microwave (65.8°C, 6.7 minutes) and ultrasound (50% duty cycle, 13.5 minutes) processing to maximize protein extraction.

Following the preparation of chickpea-based beverages, prepared by three optimized processes, a comparative storage stability study was conducted to assess their shelf life. In **Objective 4**, parameters such as microbial growth, pH, color changes, and total soluble solids were monitored

periodically over a defined storage period. This investigation helped in determining the beverage's stability and suitability for commercial production, aiding in the development of appropriate packaging and storage recommendations. In addition, the retention of antioxidant and bioactive compounds in chickpea-based beverages was also evaluated throughout the storage period. For this, total phenolic content, total flavonoid content, and antioxidant activity measurement were completed. The results indicated that storage studies conducted over 21 days for optimized chickpea beverage, prepared using conventional, ultrasound, and microwave processing methods, demonstrated that ultrasound and microwave processing surpassed conventional processing in preserving bioactive compounds. Ultrasound increased phenolic compounds by 27.11% and flavonoids by 29.16%, while microwave processing boosted phenolic compounds by 47.32% and flavonoids by 58.34%. Both techniques effectively maintained the total phenolic content (TPC) during storage, with ultrasound at 18.71% and microwave at 17.79%.

In **Objective 5**, the by-product produced during the preparation of chickpea beverages i.e Chickpea okara was studied for its physicochemical, functional, and microstructural properties to explore its potential applications as a food ingredient for value addition and biomass upcycling. The results showed significant variations in the composition and properties of okara flours across processing methods, with each method having distinct effects. Consequently, no single "best" method was identified, as all yielded unique and interesting properties. This research offers valuable insights into the impact of processing methods on chickpea okara flour, emphasizing the importance of method selection based on specific flour properties and applications.

Overall, this thesis contributes to the understanding of the preparation of beverages from chickpeas, shedding light on the optimization of processing parameters, nutritional composition, and sensory attributes, along with exploring microwave and ultrasound technologies to enhance

extraction of protein and their effect on the nutritional and storage attributes of these beverages. The findings of this study can aid in the development of innovative, nutritious, and sustainable plant-based beverage options, catering to the growing consumer demand for healthier and sustainable alternatives.

Résumé

Le pois chiche (*Cicer arietinum*), est une légumineuse polyvalente riche en protéines, en fibres alimentaires et en nutriments essentiels, qui a attiré notre attention en tant qu'ingrédient intéressant pour le développement de boissons à base de plantes.

L'objectif 1 s'est concentré sur le développement d'un protocole optimisé qui assure une extraction maximale des composants souhaitables tout en minimisant les composés indésirables. À l'aide de la méthodologie de surface de réponse, les paramètres d'extraction ont été optimisés pour maximiser les teneurs en protéines, en glucides, totale en solides et minimiser l'activité LOX (lipoxygénase) spécifique.

De plus, dans **l'objectif 2**, la composition nutritionnelle et la qualité fonctionnelle des boissons ont été évaluées, en tenant compte des macronutriments, des micronutriments et des composés bioactifs. L'évaluation a tenu compte de l'analyse immédiate, le profilage des acides aminés, la teneur en minéraux et la rhéologie de la boisson. Les résultats donnent un aperçu du potentiel nutritionnel des boissons à base de pois chiches et de leur pertinence en tant que produits alimentaires fonctionnels. L'évaluation sensorielle fait partie intégrante de cette recherche, car l'acceptabilité par le consommateur joue un rôle crucial dans la viabilité commerciale de la boisson développée. Des panélistes ont été recrutés pour évaluer les attributs sensoriels, tels que le goût, l'arôme, la texture et l'acceptabilité globale. Leurs commentaires ont été analysés pour évaluer les caractéristiques sensorielles des boissons à base de pois chiches, en soulignant leur caractère distinctif et leur attrait pour le consommateur.

Ces dernières années, de nouvelles technologies telles que les ultrasons et les micro-ondes ont attiré l'attention en tant qu'approches alternatives pour l'extraction.

Pour **l'objectif 3**, les paramètres de production assistée par micro-ondes et ultrasons ont été optimisés pour maximiser la teneur en protéines de la boisson de pois chiche.

D'autres caractéristiques, telles que la solubilité des protéines, la digestibilité des protéines in vitro (IVPD) et l'activité de l'inhibiteur de la trypsine (TI), ont également été étudiées dans des conditions optimales et comparées au procédé conventionnel de production d'une boisson à base de légumineuses.

Suite à la préparation de boissons à base de pois chiches, préparées selon trois procédés optimisés, une étude comparative de stabilité pendant l'entreposage a été menée pour évaluer la durée de conservation. Pour **l'objectif 4**, la croissance microbienne, le pH, les changements de couleur et les solides solubles totaux ont été évalués périodiquement sur une période définie. Cette enquête a aidé à déterminer la stabilité de la boisson et la possibilité d'envisager une production commerciale, aidant à l'élaboration de recommandations d'emballage et de stockage appropriées. De plus, la rétention des composés antioxydants et bioactifs dans les boissons à base de pois chiche a été évaluée tout au long de la période de stockage. Pour cela, la teneur totale en phénols, la teneur totale en flavonoïdes et la mesure de l'activité antioxydante ont été établies.

Pour **l'objectif 5**, le résidu de production issu de la préparation des boissons de pois chiche, connu sous le nom d'okara de pois chiche, a été étudié pour ses propriétés physicochimiques, fonctionnelles et microstructurales afin d'explorer ses applications potentielles en tant qu'ingrédient alimentaire pour la valorisation et le recyclage de cette biomasse résiduelle.

Dans l'ensemble, cette thèse contribue à la compréhension de la préparation de boissons végétales à base de pois chiche, en mettant en lumière l'optimisation des paramètres de traitement, de la composition nutritionnelle et des attributs sensoriels, ainsi qu'en explorant les technologies micro-

ondes et ultrasons pour améliorer l'extraction et son effet sur la qualité nutritionnelle et les attributs de manutention de ces boissons.

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Contribution to Knowledge

The following are significant contributions to the knowledge:

1. The optimization of several extraction parameters using Response Surface Methodology (RSM) for the preparation of a chickpea-based beverage provides valuable insights into an effective technique to obtain the optimal yield of nutrients from chickpeas. This optimization approach explores the impact of various extraction parameters and their interactions on the beverage's physicochemical and nutritional properties, leading to an improved understanding of the extraction process.
2. The comprehensive quality evaluation, nutritional assessment and sensory studies of the optimized chickpea beverage helped in evaluating the beverage's chemical composition, nutritional content, and sensory attributes. This study provides a holistic understanding of the produced beverage’s overall quality and consumer acceptance. This contribution is vital as it bridges the gap between laboratory-scale optimization and real-world applicability, providing valuable data for the development of commercially viable and marketable chickpea beverages.
3. Investigation into the effect of microwave and ultrasound extraction parameters on protein quantity and quality parameters of the chickpea beverage contributes to the application of innovative processing techniques for plant-based beverage production. Understanding the impact of microwave and ultrasound extraction on the beverage's protein content and overall quality offers novel insights into the potential of these non-thermal technologies for enhancing the nutritional value and safety of plant-based beverages, which is an emerging area of interest in the food industry.

4. The storage studies of the chickpea beverage, which examined the effects on the physicochemical and functional properties, and microbial analysis, address a critical research gap concerning the shelf life and stability of plant-based beverages. Investigating the changes that occur during storage provides essential information for the development of effective preservation strategies, ensuring the beverage maintains its quality and nutritional value over time.
5. Lastly, the investigations of the physicochemical, microscopical, and functional properties of chickpea okara flour extracted by different processing methods provide insights into the valorization of by-products from chickpea-based beverage production. This study sheds light on the potential of chickpea okara flour as a valuable ingredient for food formulations, thus contributing to sustainable food production by reducing food waste and improving the nutritional composition of various food products.

Contribution of Authors

This thesis consists of two literature-review manuscripts (Chapter 2 and Chapter 3) and five first-author research-based manuscripts (Chapter 4, Chapter 5, Chapter 6, Chapter 7, Chapter 8) and the contributions of all authors to each manuscript are listed below.

Chapter 2: Plant-based beverages: Current Scenario and key challenges – A review (to be submitted)

Authors: Neha Sharma, Valérie Orsat

Contributions: Conceptualization, Methodology-development, Methodology-application, Investigation, Writing-original draft, Neha Sharma; Writing-review and editing, Neha Sharma, Valérie Orsat, Funding acquisition- Valérie Orsat; Supervision, Valérie Orsat

Chapter 3: Rise of plant-based beverages: A consumer-driven perspective (to be submitted)

Authors: Neha Sharma, Valérie Orsat, Laurette Dubé

Contributions: Conceptualization, Methodology-development, Methodology-application, Investigation, Writing-original draft, Neha Sharma; Writing-review and editing, Neha Sharma, Valérie Orsat, Funding acquisition- Valérie Orsat; Supervision, Valérie Orsat, Laurette Dubé

Chapter 4: Optimization of extraction parameters for preparation of *Cicer arietinum*-based beverage using Response Surface Methodology (published in Journal of Food Processing and Preservation; doi.org/10.1111/jfpp.16428)

Authors: Neha Sharma, Valérie Orsat

Contributions: Conceptualization, Methodology-development, Methodology-application, Investigation, Writing-original draft, Neha Sharma; Writing-review and editing, Neha Sharma, Valérie Orsat, Funding acquisition- Valérie Orsat; Supervision, Valérie Orsat.

Chapter 5: Quality evaluation and comparative physicochemical and sensory studies of optimized chickpea beverage (to be submitted)

Authors: Neha Sharma, Valérie Orsat, Laurette Dubé

Contributions: Conceptualization, Methodology-development, Methodology-application, Investigation, Writing-original draft, Neha Sharma; Writing-review and editing, Neha Sharma, Valérie Orsat, Funding acquisition- Valérie Orsat; Supervision, Valérie Orsat, Laurette Dubé

Chapter 6: Effect of Microwave and Ultrasound extraction on protein and quality parameters of chickpea beverage (to be submitted)

Authors: Neha Sharma, Valérie Orsat

Contributions: Conceptualization, Methodology-development, Methodology-application, Investigation, Writing-original draft, Neha Sharma; Writing-review and editing, Neha Sharma, Valérie Orsat, Funding acquisition- Valérie Orsat; Supervision, Valérie Orsat.

Chapter 7: Storage studies of chickpea beverage: Effects on the physicochemical and functional properties and microbial analysis (to be submitted)

Authors: Neha Sharma, Valérie Orsat

Contributions: Conceptualization, Methodology-development, Methodology-application, Investigation, Writing-original draft, Neha Sharma; Writing-review and editing, Neha Sharma, Valérie Orsat, Funding acquisition- Valérie Orsat; Supervision, Valérie Orsat.

Chapter 8: Physicochemical, microscopical and functional properties of chickpea okara flour extracted by different processing methods (to be submitted)

Authors: Neha Sharma, Valérie Orsat

Contributions: Conceptualization, Methodology-development, Methodology-application, Investigation, Writing-original draft, Neha Sharma; Writing-review and editing, Neha Sharma, Valérie Orsat, Funding acquisition- Valérie Orsat; Supervision, Valérie Orsat.

List of Tables

List of Figures

List of Abbreviations

LOX	Lipoxygenase
IVPD	Invitro Protein digestibility
TI	Trypsin Inhibitor
TIA	Trypsin inhibitor activity
MRSA	Methcillin-resistant Staphylococcus aureus
LDL	Low-density lipoprotein
CAGR	Compound annual growth rate
FDA	Food & Drug Administration
SFA	Saturated fatty acid
UHT	Ultra high temperature
UHPH	Ultra high-pressure homogenization
PUFA	Polyunsaturated fatty acid
ALDH	Aldehyde dehydrogenase
RSM	Response surface methodology
PEF	Pulse electric field
HPP	High-pressure processing
LCA	Life cycle analysis
PDCASS	Protein digestibility corrected amino acid score
FMPS	Flavourings with modulating properties
AI	Artificial Intelligence

ANFs	Anti nutrition factors
CCD	Central composite design
BCA	Bicinchoninic acid
DF	Dilution factor
ANOVA	Analysis of variance
MTBSTFA	N-methyl-N-tert-butyldimethylsilyl trifluoroacetamide
AA	Amino acid
BSA	Bovine serum albumin
MAE	Microwave-assisted extraction
UAE	Ultrasound-assisted extraction
CFU	Colony forming unit
FC	Folin Ciocalteu
GAE	Gallic acid equivalent
TFC	Total flavonoid content
TPC	Total phenolic content
QE	Quercetin equivalent
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric ion reducing antioxidant power assay
TPTZ	2,4,6-tripyridyls-triazine
WHC	Water holding capacity
OHC	Oil holding capacity
EAI	Emulsifying activity index

EA	Emulsifying activity
ESI	Emulsifying stability index
PS	Protein solubility
SEM	Scanning electron microscopy
TDF	Total dietary fibre
CP	Conventional processing
MWP	Microwave processing
USP	Ultrasound processing

Chapter 1: Introduction

In recent years, there has been a notable surge in the popularity of plant-based beverages and plant-based nutrition driven by their perceived health claims and positive environmental impact. These beverages serve as a practical substitute for conventional dairy products, effectively meeting the requirements of individuals with lactose intolerance, dietary limitations, or those desiring plant-based choices. Chickpeas, scientifically known as *Cicer arietinum*, is a leguminous crop widely cultivated and consumed across the globe. They are renowned for their high protein content, dietary fiber, and various essential nutrients such as folate, iron, and magnesium. Chickpeas mainly come in two varieties, namely, kabuli and desi types. In general, the surface of the kabuli chickpea has a beige coat and it is a large seed without edges, while the desi chickpea has a dark-coloured coat cover with smaller and rough seeds.

Typically, chickpeas are deemed ready for harvesting when most of the plants have transitioned to a yellow hue, and a significant portion of the pods have reached maturity. However, it is crucial to exercise caution, as chickpeas are remarkably sensitive to late-season frosts, which can have a detrimental impact on the seeds. Following a successful harvest, the focus shifts to post-harvest practices and storage. Chickpeas, like many legumes, must be properly cleaned and dried to reduce moisture content, which is essential for preventing spoilage and maintaining the quality of the crop during storage. Adequate storage facilities should be in place to protect the chickpeas from environmental factors such as moisture and pests. Properly stored chickpeas can have a longer shelf life and retain their nutritional value. The key chickpea-producing nations, notably India, Turkey, Pakistan, Australia, and Ethiopia, play a pivotal role in this agricultural landscape. Together, these countries contribute to approximately 75% of the world's total chickpea

production. Among them, India stands out as the foremost producer, single-handedly accounting for about 60% of the world's chickpea output. Canada is ranked at the ninth position globally in chickpea production, contributing approximately 1.76% of the world's total chickpea output. The country dedicates over 155,800 hectares of land to chickpea cultivation, achieving a commendable yield of 16,142 kilograms per hectare. Saskatchewan stands out as the primary hub for chickpea production in Canada. This province has earned its reputation for offering highly favorable conditions that have propelled it to the forefront of chickpea cultivation in the country. Saskatchewan's distinct advantage lies in its arid climate and exceptionally fertile soils, creating an environment that is exceptionally well-suited for chickpea farming.

In the world of plant-based options, chickpeas have become a noteworthy candidate, drawing significant attention as a promising ingredient for making healthy drinks. Additionally, chickpeas offer a variety of beneficial properties that make them a great choice for adding to beverage recipes. The extraction of proteins and other bioactive compounds from chickpeas offers an opportunity to develop plant-based beverages that not only mimic or improve the sensory attributes of traditional dairy products but can also provide additional health benefits.

The production of chickpea-based beverages involves several processing steps, including soaking, grinding, extraction, and formulation. Each of these steps can significantly impact the final product quality, nutritional composition, and sensory attributes. Therefore, process optimization becomes crucial to achieve desirable characteristics and maximize the utilization of chickpeas' nutritional potential. Through careful investigation and experimentation, it is possible to identify the optimal conditions for processing, leading to the development of a high-quality, chickpea beverage with improved nutritional value and sensory acceptability.

1.1 Hypothesis and Implication

Hypothesis: In comparing chickpea-based beverages with other popular plant-based milk options in the market, we anticipate that the optimization of process parameters for chickpea beverage preparation, coupled with a comprehensive analysis of its nutritional properties and sensory quality, will demonstrate its competitive position among plant-based alternatives. Furthermore, we expect to uncover distinct variations in the properties of chickpea beverages and storage stability when subjected to thermal (microwave) or non-thermal (ultrasound) processing methods for beverage preparation, which may contribute to its unique positioning and consumer preference within the plant-based milk market.

The study aims to validate above mentioned hypothesis through comprehensive experimentation, analysis, and evaluation of the nutritional composition and sensory attributes of chickpea-based beverages. By addressing the existing research gaps and uncovering the effects of novel processing techniques, this study seeks to contribute valuable knowledge to the field of plant-based beverage preparation, paving the way for the development of high-quality and nutritionally rich chickpea beverages. The study will provide insights into the potential benefits and drawbacks of processing, allowing for the identification of optimal conditions that enhance the nutritional quality of the beverage.

1.2. Overall objective

The overall objective of this study is to prepare a plant-based beverage from chickpeas and evaluate its nutritional and sensory properties. Further, other than conventional processing (using water bath, microwave and ultrasound have also been explored for chickpea nutrient extraction and subsequent storage studies of the prepared beverage.

In addition to the beverage, the study will also focus on the upcycling potential of the by-product obtained from the chickpea extraction process, known as chickpea okara.

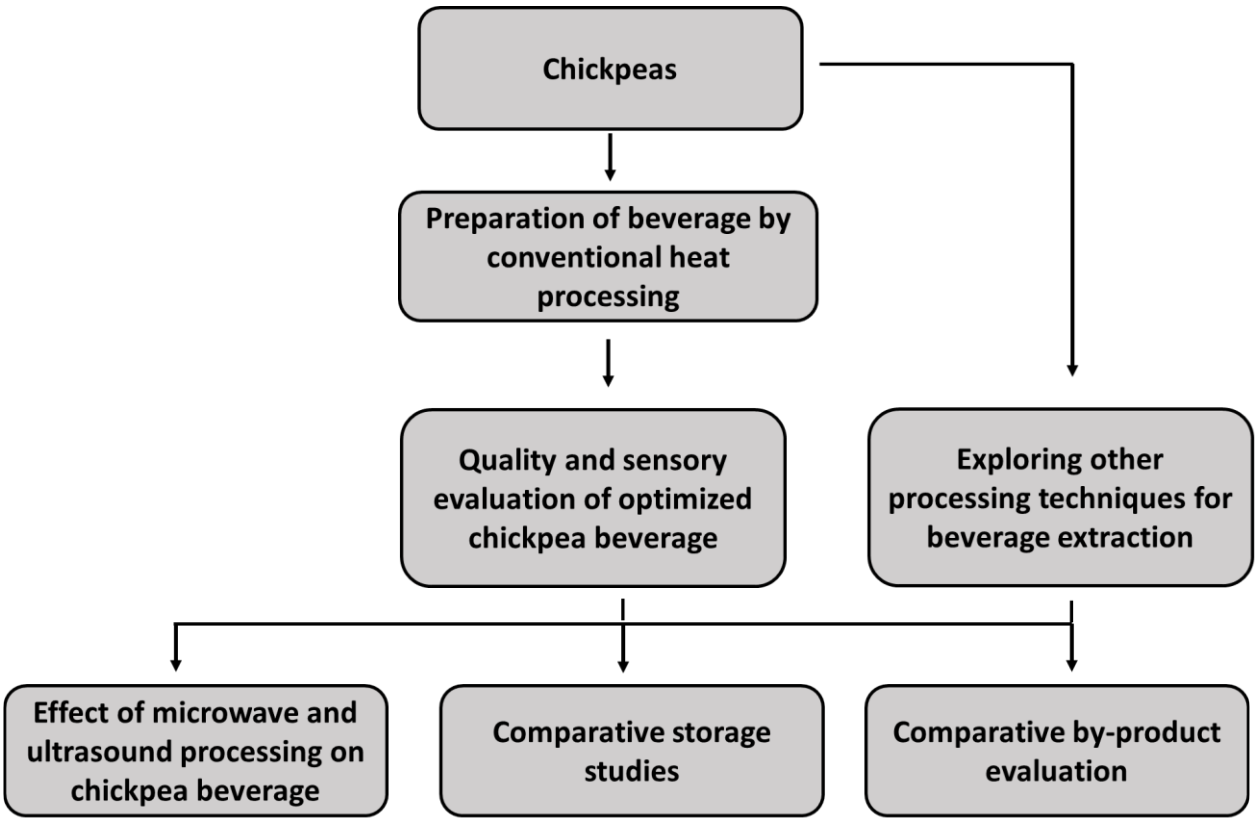

Figure 1-1: The schematic representation of the study objectives

1.3 Specific objectives

i. To optimize the extraction parameters for the preparation of chickpea-based beverages using Response Surface Methodology.

ii. To conduct physicochemical characterization, quality evaluation and sensory studies of optimized chickpea beverage obtained from objective 1.

iii. To study the effect of microwave and ultrasound extraction on protein and quality parameters of chickpea beverage.

iv. To carry out comparative storage studies on chickpea beverages including the processing effects on physicochemical properties, microbial analysis and functional properties.

v. Characterization of the chickpea okara flour, the by-product of chickpea beverage processing, obtained from different processing methods (conventional, microwave and ultrasound).

Literature Review – Part 1

Chapter 2: Plant-based beverages: Current Scenario and key challenges – A review

Abstract

Bovine milk has been an indispensable part of the human diet for ages. But the consumption of plant-based beverages has gained significant attention in recent years due to the increasing demand for non-dairy and non-animal protein sources owing to lactose intolerance, allergies, and diverse lifestyles. This review article provides a comprehensive overview of the current scenario of plant-based beverages, including their market trends, bioactive compounds and health benefits. The article discusses the types of plant-based beverages available, including soy, almond, oat, rice, and other less-known beverages, and their nutritional properties. Additionally, the review highlights the key challenges associated with the processing of plant-based beverages, such as physical stability, off-flavour, antinutrient content and nutrition imbalance. The article concludes by emphasizing the need for further research and development in plant-based beverages to overcome the current challenges and meet the growing demand for sustainable and healthy food options.

Keywords: plant-based beverages, bioactive compounds, health benefits, off-flavor

2.1 Introduction

Plant-based "milk" also termed "dairy alternatives" are dominating the market in the beverage category and have undergone a rapid transformation in recent years. Increasing demand for plant-based beverages has opened new dimensions in this field promoting more research and development with new products added now and then. Not only plant-based beverages but plant-based foods, in general, are making a statement for themselves in the market and among

consumers. Internet and social media have significantly contributed to consumers' increased knowledge about health and nutrition. Consumers may quickly acquire information on a variety of health-related issues, such as diet, exercise, and illness prevention, due to the large quantity of information accessible online and can make more informed decisions for themselves. This has contributed to consumers becoming increasingly aware of the various health and environmental concerns associated with animal-based foods. Animal-derived foods are often associated with several public health risks and concerns like the use of antibiotics which contributes to the spread of antibiotic-resistant bacteria posing a risk to human health in terms of morbidity, death, and rising health costs (Mehndiratta & Bhalla, 2014). For example, the emergence of ciprofloxacin resistance is due to the utilization of enrofloxacin in poultry farming (Organization, 2000). Similarly, the spread of MRSA (methicillin-resistant *Staphylococcus aureus*) has been reported in pork processing industries leading to infections and therapeutic failure in humans (Song et al., 2011). In addition to this, animals, livestock and poultry are often administered with hormones during growth and production which may have long-term toxicological implications on human health (Malekinejad & Rezabakhsh, 2015). Furthermore concerns about sustainability and environmental consciousness have inspired consumers globally to make choices favourable to the planet thus increasing demand for plant-based food and beverages (Hemler & Hu, 2019). A review study by (Nelson et al., 2016) showed that multiple forms of modelling/simulations, life cycle analyses, and land use studies consistently demonstrated that diets rich in animal-based meals were more detrimental to the environment than diets high in plant-based foods. Additionally, animal-based food production demands substantial land, water, and other resources, resulting in deforestation, soil degradation, and water pollution (Djekic et al., 2014; Theurl et al., 2020). Also, animal-based foods, particularly meat and dairy products, are high in saturated fat and cholesterol,

which have been linked to an increased risk of heart diseases and other chronic health conditions due to increased low-density lipoprotein cholesterol (LDL) levels (Lordan et al., 2018). Studies have suggested an increased risk of type 2 diabetes, cardiovascular disease and colorectal cancer from long-term increased consumption of red meat (Richi et al., 2015).

In recent years, the expansion of the market for plant-based beverages has been fueled by the rising popularity of plant-based diets and the need for more sustainable and ecologically friendly food alternatives. Plant-based beverages not only cater to the needs of consumers suffering from lactose intolerance and looking for some alternative to conventional milk but have proven beneficial for people suffering from cow milk allergy, hypercholesterolemia, having calorie concerns and looking for lifestyle changes like switching to vegan diets (Sethi et al., 2016). As a result of consumer demand, the market is already flooded with different types of plant-based beverages including soy, rice, coconut, oats and almond-based beverages with different flavors and compositions (Fructuoso et al., 2021). But there are still some key challenges associated with these beverages that need to be addressed by the researchers to enhance acceptance of these products by the masses. Firstly, many consumers have concerns about the flavor and non-familiarity with the taste when they are looking to switch to plant-based beverages from conventional milk (Giacalone et al., 2022). Secondly, researchers aspire to develop and focus on novel processing technologies to reduce energy consumption for achieving economic and environmental sustainability in beverage production (Penha et al., 2021). Therefore, a thorough study and understanding of the molecular and physicochemical properties of plant-based constituents are required to develop products with more acceptable physicochemical and organoleptic properties while exploring and adopting green technologies at the same time.

This review will explore various properties and nutritional profiles, preparation of currently available plant-based beverages in the market, health benefits and novel technologies being explored by the researchers to improve their nutritional and sensory profile as well as the planet's health.

2.2 Current market, types, nutritional profile and health benefits of plant-based beverages

2.2.1 Plant-based beverages market size and share

Plant-based beverages have reached mainstream markets and are gaining a lot of momentum among consumers in the healthy and functional category. Consumers' desire to explore healthy plant foods and beverages is reflected in global market trend projections showing extensive and sustained growth in this category. Plant-based milk has emerged as a focused category and primary growth driver in the plant-based food industry and continues to grow due to ingredient innovation, retail space and diversity offered to consumers. Recent projections published by Fairfield market research have shown an estimated growth of more than 12% during 2019-2026, with revenue surpassing US$34.8 Billion by the end of 2026 (Fairfeild, 2022). Market insights of The Vegan Society "The growing Plant milk market" states that Almond milk and soy milk each accounted for nearly 40% of the worldwide market value of plant-based milk in 2019. The remaining 20% consisted of rice, oats, hazelnut, coconut, and pea milk. These worldwide percentages are expected to stay relatively stable by 2025, with almonds gaining a few percentage points on soy (Society, 2022). Another report by Persistence Market research predicted that the market for plant-based milk would rise at a CAGR (Compound Annual growth rate) of 5.9%, with revenue increasing from US$ 14,934.3 Million to about US$ 36,176.4 Million by 2033 (Research, 2023). This

continuous entry and growth of plant-based beverages into the fluid milk markets seem to have contributed to the ongoing drop in per capita cow milk consumption which decreased by more than 25 percent from 2000 to 2018 and still shows a declining trend (Khanal & Lopez, 2021; Vanga & Raghavan, 2018).

2.2.2 Types and nutritional profile

Plant-based alternatives to dairy products can vary in their nutritional content depending on the ingredients used to make them. However, many plant-based alternatives are fortified with vitamins and minerals to make up for any potential deficiencies**.** These products are typically made from nuts, seeds, or grains, such as soy, almond, and oat. Plant-based milk can offer a variety of nutritional benefits, including reduced saturated fat, cholesterol, and lactose. Some widely accepted plant-based beverage and their general preparation methods are described in subsequent sections. The nutritional profile of some commercially popular plant-based beverages and their comparison with bovine milk is shown in per 240 mL Table 2-1.

Table 2-1: Nutritional composition of plant-based milk and cow milk (Collard & McCormick, 2021; Munekata et al., 2020; Singhal et al., 2017)

Type of plant-based milk alternative	Calories (kcal)	Protein(g)	Lipids (g)	Total Carbohydrates (g)
Cow's milk	61	7.69	3.25	4.8
Soy milk	80	7	4	4
Almond milk	40	1	3	2

Oats Milk	80	2.5	4	24
Rice milk	130	1	2	27

A) Soy milk

Soymilk is the most popular plant-based milk used around the world which was first launched in Hong Kong in 1940 (Mäkinen et al., 2016). Over the years, soy milk has emerged as a great substitute for traditional milk for consumers willing to switch to plant-based milk. Soybean is considered a superfood containing the highest amount of protein among cereals and legumes (Liu & Liu, 1997). Soy is also found to be rich in vitamins, phospholipids, monounsaturated and polyunsaturated fatty acids, several important minerals, dietary fibre and isoflavones (Messina & Messina, 2000). The traditional method of soymilk preparation consists of steps like soaking, grinding and separating the sediments by filtration. The liquid obtained is further heated to obtain proper consistency and viscosity similar to cow's milk. The traditional method has been modified by different researchers adjusting it to the requirements of the customers and improving the quality and nutritional value of the final product (Nelson et al., 1976). Although soy milk or soy beverage is highly nutritious and packed with numerous health benefits, its use is still limited hindering mass acceptance. This is mainly attributed to the off-flavour or beany odour associated with soy milk. The beany flavour is mainly attributed to the combination of low molecular weight compounds formed by the action of lipoxygenase enzymes and unsaturated fatty acid (Torres-Penaranda & Reitmeier, 2001). Due to its popularity, the market offers a vast selection of versions. Available types include light, dairy-like, and rich soy milk based on solids content, sweetened, original, and flavoured soy milk based on fortification, and regular, enhanced, and blended soy milk based on formulation (Liu, 2004).

B) Rice milk

Rice is a staple food item for over half of the world's population, particularly in Southeast Asia (Paul et al., 2020). Rice consists mostly of carbohydrates (9.4-14.2 g/100 mL) and has a low protein level (0.8-0.2 g/ 100 mL) (Jiang et al., 2016; Plengsaengsri et al., 2019). It is deficient in threonine and lysine, but abundant in ferulic acid, sinapic acid, and p-coumaric acid (Guo et al., 2021; Shao & Bao, 2015). Iron, a vital element, is present in rice, with more than 85 percent of it concentrated in the bran (Bhosale & Vijayalakshmi, 2015) which is eliminated during the rice-milk processing process. Therefore, it is necessary to add iron to rice milk to improve its nutritional content. Rice also lacks calcium and vitamins, therefore consumption of rice milk as a bovine milk substitute without proper fortification may result in malnutrition, particularly in newborns and toddlers (Chalupa-Krebzdak et al., 2018).

However, in recent times, the focus of industries has shifted to the production of Rice Bran Milk (RBM) due to the underutilization of rice bran and its high nutrient content (Issara & Rawdkuen, 2018). Research has shown that rice milk has a lower total soluble solids value and viscosity compared to soy milk (Issara & Rawdkuen, 2014), but its broad acceptance as a hypoallergenic cereal has contributed to its popularity in the milk analog market (Michelet et al., 2017). The general process of rice milk preparation includes mixing milled broken rice or rice flour with water followed by homogenizing to a particle size range of 5-20 μm followed by heating to ensure food safety and extend shelf life (Plengsaengsri et al., 2019). Rice is found to be high in starch resulting in poor emulsion stability of these beverages therefore enzymatic hydrolysis of starch by α and β amylase and glucosidase is often done during commercial preparation of the beverage (Sethi et al., 2016).

C) Oats Milk

Oats and oats-based food products have recently gained a lot of popularity and attention due to their high nutrition value, phytochemicals and high dietary fibre content (Deswal et al., 2014a). Oats are an excellent source of high-quality protein with a balanced profile of amino acids and contain β-glucan, a bioactive molecule (Liu et al., 2010). β-glucan, a soluble fibre forms a viscous solution in the human digestive tract, resulting in decreased postprandial glucose and insulin responses and increased insulin sensitivity in both diabetic and nondiabetic patients (Hanai et al., 1997). The Food and Drug Administration (FDA) has recognized that a daily intake of 3 g of β-glucan reduces the risk of coronary heart disease (Julie M, 2001). In addition to this, the consumption of β-glucan has proven beneficial in reducing blood cholesterol, and blood pressure while it also possesses anti-cancer properties in the case of colon cancer (Bode & Dong, 2009; Butt et al., 2008; Rasane et al., 2015). Oat milk has become a popular alternative to dairy milk in recent years not only due to health claims and nutraceutical benefits associated with it but also due to its mild flavor and creamy texture (X. Wang et al., 2022). Oat milk is primarily made from a mixture of water and oat flour, which undergoes several steps to produce the final product. Initially, the oat flour and water are combined to create a slurry. Enzymatic hydrolysis is then used to break down the starch, after which the mixture is filtered, subjected to heat treatment, and homogenized. Finally, the product is heated further to enhance its microbiological stability (Mäkinen et al., 2016). The abundance of fiber and protein present in oat milk can promote a feeling of fullness, potentially aiding in the maintenance of a healthy weight and adherence to dietary guidelines (Bridges, 2018).

D) Almond Milk

Due to their potential health advantages, dried fruits and nuts have emerged as nutritious dietary options. Almonds are the most widely eaten nut, and they are an excellent source of protein (25%), especially amandin (Sathe et al., 2002). Almonds are a rich source of vitamin E and manganese. The physiologically active form of vitamin E, alpha-tocopherol, is a powerful antioxidant that provides vital protection against free-radical reactions (Burton & Ingold, 1989). Other minerals found in abundance in almonds include calcium, magnesium, selenium, potassium, zinc, phosphorus, and copper (Sethi et al., 2016). Almonds are found to be low in saturated fatty acids (SFA) and high in unsaturated fatty acids (91–94% of fats include oleic acid and linoleic acid) thus beneficial in reducing the risk of cardiovascular disease (Jung et al., 2018). In addition to the advantages mentioned above, almonds may also have prebiotic qualities owing to the presence of pectic compounds including arabinose in their cell walls. These chemicals have been shown to lower blood cholesterol and triglyceride levels, boosting the health benefits of almond intake (Barreca et al., 2020). Almond milk is made by blending ground almonds, water, and emulsifiers to create an oil-in-water emulsion (Silva et al., 2020). Commercial almond milk and other vegetable-based drinks undergo heat processing, such as ultra-high-temperature (UHT) treatment for food safety and shelf life stability (Vazquez-Landaverde et al., 2005). However, heat treatment may also result in undesirable chemical changes, such as the loss of vitamins and amino acids, browning processes, and the oxidation of lipids, which leads to the creation of undesirable tastes, thus proper optimization of process parameters is required (Maghsoudlou et al., 2016).

E) Other plant-based beverages

Peanut is classified among the nuts (actually a legume) that are known to have the highest antioxidant content in plant foods and thus play a major role in helping to prevent cardiovascular

diseases (Nepote et al., 2005). Peanuts have a high concentration of proteins ranging between 25-29% and approximately 20-23% carbohydrates (Latif et al., 2013). When compared to tree nuts like almonds, walnuts, and pecans, peanuts have almost the same health benefits but at a comparatively very low price, attractive to consumers (Bao et al., 2013). The potential for the use of milk imitation products from peanuts is very high in developing countries that suffer from a major problem of protein-calorie malnutrition (Galvez, 1990). Peanut milk is mainly prepared either by wet grinding of dehulled whole peanuts or utilizing peanut flour for formulating a water extract (Diarra et al., 2005). Being rich in lipids, additional processing of peanuts is required to maintain the stability and textural characteristics of the final product (Sethi et al., 2016). Cowpea is another popular legume mainly cultivated in semi-arid areas of Africa, Asia, Europe, and America. Researchers have prepared cowpea milk mainly as a fermented beverage and infant formula (Sanni et al., 1999). Cowpea milk in African countries, especially Nigeria, has been tested mainly as an infant food to supplement breast milk, and it has been suggested that it is low in energy as compared to soymilk (Akinyele & Abudu, 1990). To make cowpea milk more nutritious, the methods of extraction need to be modified and other fat sources need to be added to increase the overall energy content.

Legumes like pigeon pea (*Cajanus cajan*) and lupin have also been used to prepare milk beverages or add them to other milk-based products to enhance their techno-functional properties. Cheese analogue from soya milk, prepared by incorporating an optimum amount of pigeon pea milk, has been reported to enhance the shelf life as well as the nutritional properties of the cheese (Verma et al., 2005). Lupin milk prepared by fortification with 0.1% D-L methionine yielded a product with a protein ratio comparable to casein, i.e most common protein found in cow milk (Camacho, 1988).

Fortified milk obtained in this way was also spray-dried for better storage and distribution targeting lupin-producing countries suffering from a problem of malnutrition (Hall et al., 2005).

2.2.3 Health benefits and bioactive compounds

Numerous health advantages make plant-based drinks a good alternative to conventional dairy milk. These beverages can be lower in saturated fats, rich in fibre, and include health-promoting vitamins and minerals (Sethi et al., 2016). For instance, soymilk is a rich source of isoflavones (a well-known phytoestrogen) and beneficial monounsaturated and polyunsaturated fatty acids. The preventive effect of isoflavones against cancer, cardiovascular disease, and osteoporosis is established and recognized (Omoni & Aluko, 2005). Plant-based beverages are also high in fibre, which promotes healthy digestion and bowel movements. Almond milk and oat milk, are naturally high in fibre, which helps regulate digestion and can alleviate symptoms of constipation (Sethi et al., 2016). Plant-based beverages also have a lower glycemic index than traditional dairy milk, making them an excellent option for people with diabetes or those at risk of developing type 2 diabetes (Shkembi & Huppertz, 2023). Bioactive compounds and health benefits offered by some selected plant beverages are summarized in Table 2-2.

Table 2-2: Functional components of plant-based milk alternatives and their health benefits

Type of plant-based milk alternative	Functional/bioactive compounds	Health benefits	Selected studies
Soy Milk	Isoflavones, Phytosterol, α-tocopherol	• Protective effects against breast, prostate and colon cancer	(Omoni & Aluko, 2005)

		• Decrease risk of cardiovascular disease and osteoporosis	(Fukui et al., 2002)
Almond milk	α-tocopherol, flavonoids and phytosterols	• Antioxidant properties i.e protecting against free-radical reactions, • Improves gastrointestinal health	(Maria & Victoria, 2018) (Sethi et al., 2016) (Decloedt et al., 2017)
Oats milk	β-glucan, phytosterols	• Delay the time of gastric emptying • increases gastrointestinal transit time which is associated with their reduced blood glucose level. • Reduction of total and LDL cholesterol • Body weight management • Anti-cancer properties	(Truswell, 2002) (Deswal et al., 2014a) (Sethi et al., 2016)

Rice milk	Phytosterols, especially βsitosterol and γ-oryzanol	• Reduces cholesterol levels • Reduces hypertension • Anti-diabetic, anti-inflammatory and anti-oxidative effects	(Faccin et al., 2009) (Biswas et al., 2011) (Sethi et al., 2016)
Sesame milk	Lignans (sesamin, sesamolin, and sesaminol)	• Antioxidative, hypocholesterolemic, anticarcinogenic, antitumor, and antiviral activities	(Namiki, 2007) (Fitrotin et al., 2015)
Peanut milk	Phenolic compounds	• Preventive function against oxidative damage and illnesses such as coronary heart disease, stroke, and several malignancies	(Diarra et al., 2005) (Settaluri et al., 2012) (Wien et al., 2014)

2.3 General processing steps in preparation and treatments

Plant-based beverages are prepared generally by following more or less some common steps i.e. crushing the raw plant material into water followed by extraction, heat treatment and filtration with some modifications depending upon the type of beverage, disintegration method, heat treatment conditions and homogenization procedure (Sethi et al., 2016). A schematic representation of common steps that are followed for preparing plant-based beverages is shown in Figure 2-1. Before the extraction process, raw materials often undergo a pre-treatment. Some of these processes are

dehulling, blanching, defatting, soaking and other such processes depending on the raw material or final product requirements (Gajdoš Kljusurić et al., 2015). The soaking process is done either in water (rice, oats and cashew) or in an aqueous solution of 0.2%–2% sodium bicarbonate (alkaline soaking) (Reyes-Jurado et al., 2021). Alkaline soaking is recommended in the case of legume-based beverages to get rid of the beany flavours. Soaking results in swelling and softening of the outer shell of the plant materials thus increasing the extraction yield (Kizzie-Hayford et al., 2016). Blanching (hot water immersion) is done when skin removal (almonds, peanuts) is required but it also aids in the elimination of off flavours to a certain extent by inactivating trypsin inhibitors and lipoxygenases (Maestri et al., 2000; Yuan et al., 2008). Different pre-treatments are recommended for different beverages for making the extraction process more effective and make the final product even more acceptable.

Extraction can be done by wet milling or dry milling (if soaking is not involved) which is the most common procedure following soaking to disintegrate plant materials into smaller pieces. The concentration of the final product extracted depends upon several factors including the pH of the extraction solution, temperature of extraction, quantity of water and extraction rate (Kizzie-Hayford et al., 2016). Extraction yield can be improved by modification of the pH, optimizing the milling temperature and adding enzymes. The solubility of protein, fat and polysaccharides is enhanced by these methods resulting in increased total soluble solids in the final beverages (Penha et al., 2021). Enzyme addition, i.e. alpha and beta-amylase, prevents the gelatinization of starch and hydrolyzes it during the extraction process (Mäkinen et al., 2016). Once extraction is completed, solid residues are separated from the liquid by a process of filtration. The filtered liquid is further formulated into a beverage by adding ingredients like sweeteners, stabilizers, flavouring agents, salts, vitamins and minerals (Reyes-Jurado et al., 2021). As the beverage contain particles

with different ranges of sizes, homogenization is done to evenly distribute the particles, stabilizing the emulsion and obtaining a smoother texture (Vogelsang-O'Dwyer et al., 2022). Before storage and packaging, heat treatment is done to kill pathogenic bacteria and inactivate enzymes responsible for spoilage (Cruz et al., 2007). To prolong the shelf life of beverages, pasteurization at ultra-high temperature (UHT) and ultra-high pressure homogenization (UHPH) can be explored (Ferragut et al., 2015). The time and temperature of processing should be accurately optimized as a high-temperature treatment for a prolonged time may result in aggregation of the particles, denaturation of the protein, and loss of flavor and vitamins affecting adversely the nutritional and sensory qualities of the product (Poliseli-Scopel et al., 2014). After the heat treatment is concluded, beverages are packed aseptically for storage and distribution. In addition to packaging in liquid form, these beverages can also be processed into their powdered form using spray drying or drum drying which can be reconstituted into liquid form before use (Romulo, 2022).

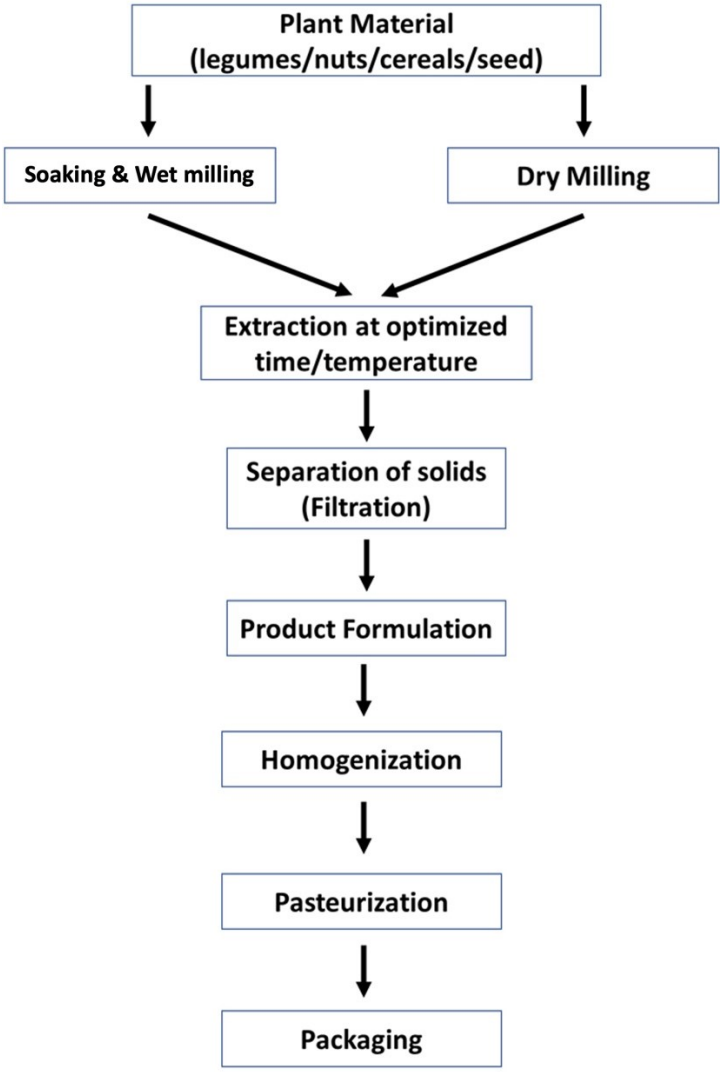

Figure 1-1: General steps in plant-based beverages preparation

2.4 Key challenges in plant-based beverage processing and technologies to address them

There are still a lot of challenges associated with plant-based beverage processing which need to be addressed to enhance mass acceptance and enhance the nutritional value and sensory properties of these milk alternatives. Some of the key challenges are nutrient imbalances, especially in the case of the quality of protein (i.e. amino acid composition) in comparison to bovine milk, lack of physical stability, presence of beany- and nutty- flavors and presence of antinutrients (Ramesh et

al., 2022). These challenges and technologies used to address them are discussed below in subsequent sections. Selected studies and their findings are summarized in Table 2-3.

2.4.1 Improving stability

The stability of plant-based beverages depends on several factors like particle size of the dispersed phase (fat globules, proteins and starch granules, etc.), protein solubility and grinding technique used making it difficult to store for longer intervals due to the sedimentation of these particles (Paul et al., 2020). The presence of these big particles also affects negatively the mouth feel of these plant-based beverages. Particle size reduction can be used to improve the stability of these beverages. Earlier use of a colloidal mill was a popular method to reduce the size of the dispersed phase while preparing soy and peanut milk but the product needed to be further sterilized at higher temperatures leading to the loss of vital components (Dhiman & Prabhakar, 2021). An alternative technology to thermal processing, ultra-high pressure homogenization (UHPH), has shown promising results in reducing the size of the colloidal particles with the simultaneous destruction of microorganisms due to the processing at very high-pressures (Poliseli-Scopel et al., 2012). An application of 200 and 300 MPa on soymilk significantly improved the physical stability with considerably reduced microbial load thus increasing its shelf life (Cruz et al., 2007). Another nonthermal technique known as ultrasound processing has been under investigation to study dispersed phase size reduction. The effect of ultrasound waves on the droplet size of coconut milk was studied for ultrasound power levels ranging from 2.5 to 7.0 W and exposure time from 5 to 25 minutes which suggested a significant decrease in droplet diameter with increasing power and exposure time (Iswarin & Permadi, 2012). The presence of high starch and high-fat content also adversely affects stability. Rice and oat milk have low emulsion stability due to the presence of

high starch content. In such cases, manufacturers generally use α- and β-amylase or glucosidase for starch hydrolysis and to prevent gelatinization (Silva et al., 2020). Similarly, peanut milk which has high-fat content can have its stability improved by adding emulsifiers like alginates, gums, and gelatin (Paul et al., 2020). Other than these, new technologies like high hydrostatic pressure, pulse electric field, and hydrodynamic cavitation and their specific studies are shown in Table 2-3.

2.4.2 Off-flavor removal

Depending on the plant source or raw material used, milk alternatives have some characteristic flavor and mouthfeel. For instance, legume-based milk beverages like soymilk, cowpea milk, lupin, and peanut milk have a beany flavor associated with them, to various levels of intensity, while almond milk has a sweet nutty flavor, making these alternate milk beverages somewhat unpleasant and less acceptable to some sections of consumers (Reyes-Jurado et al., 2021). Plant-based beverage, especially soymilk, has an off flavour associated with the presence of lipoxygenases (LOX) enzyme and polyunsaturated fatty acids (PUFA). LOX and PUFA, which remain separated inside the plant cells, come in contact with each other during the soaking and grinding (in the presence of water) operations of beverage preparation and react to generate oxidation products and volatile compounds (Yang et al., 2016). For instance, medium chain aldehydes and alcohols, i.e. n-hexanal and n-hexanol, are produced from the unsaturated fatty acids and the reaction is catalyzed by lipoxygenase enzyme during soymilk processing (Mizutani & Hashimoto, 2004). Although heat inactivation is commonly employed to inactivate lipoxygenase, its application as a promising technique has been hampered by undesired protein modifications and the loss of other thermolabile nutrients (Davies et al., 1987). Aldehyde dehydrogenase (ALDH) is the enzyme that catalyzes the irreversible conversion of aldehydes into their

corresponding acids; hence, it has been employed to remove off flavour from soybean extracts and soy milk (Chiba et al., 1979). A hydrolytic enzyme preparation Celluclast^{TM} (1.2% for 3 hours) has been used and has shown considerable reduction in the beany flavor of soymilk (Rosenthal et al., 2003). Genistein and daidzein are the main isoflavones in soybean and they are responsible and contribute significantly to its beany flavour (Matsuura et al., 1989). During the processing of soybean, for the production of soy milk, the concentration of these isoflavones gets increased by the action of β-glucosidases. The addition of glucono-δ-lactone to the soaking water, resulted in the production of soymilk with lower off-flavours as glucono-δ-lactone is a competitive inhibitor of β-glucosidases, thus inhibiting the release of isoflavones (Matsuura & Obata, 1993). Development of beany flavor is also prevented by grinding the beans in boiling water also known as the "boiling-water grind" method. This method results in rapid heat-inactivation of the lipoxygenase (Bourne, 1976). It has been discovered that deodorization and the use of artificial or natural flavorings and sugar can boost general acceptability and hide or mask off-flavors (Reyes-Jurado et al., 2021).

2.4.3 Removal of antinutrients

Antinutrients are compounds such as protease inhibitors, phytate, lectins, trypsin inhibitors etc., which interfere with the absorption of nutrients in the body. The presence of these antinutrients not only affects the bioavailability of the nutrients but also influences the sensory profile negatively (Munekata et al., 2020). Trypsin inhibitors, the most common antinutrients found in soybeans and other legumes decrease dietary protein absorption and reduce digestion by forming complexes that are indigestible despite the presence of abundant digestive enzymes (Avilés-Gaxiola et al., 2018).

These anti-nutrients are susceptible to denaturation and heat inactivation, although heat treatments that target to inactivate trypsin inhibitors may result in the loss of thermo-sensitive essential nutrients and denaturation of proteins (Smith, 1972). Therefore, proper optimization of the time–temperature combination in thermal processing is required for the processing of plant beverages to retain their nutritional value. A comparative study to assess the effects of conventional, steam injection, blanching, and UHT (Ultra-High Temperature) processing methods to inactivate soymilk trypsin inhibitor (Yuan et al., 2008). Conventional and steam injection to 100 °C for 20 minutes resulted in 13% residual trypsin inhibitor activity (TIA), whereas blanching inactivated 25–50% of raw soy milk's TIAs. On the other hand, UHT treatment with an increase in temperature and duration resulted in a product with around 10% residual TIA (Yuan et al., 2008). Phytates, are another group of antinutrients, which are storage forms of phosphorus and inositol in cereals, pulses, nuts and seeds (López-Moreno et al., 2022). In the acidic pH of the stomach, phytates may form soluble complexes with divalent cations such as zinc, iron, and calcium, so reducing the bioavailability of these nutrients in the digestive system (Schlemmer et al., 2009). Phytates are thermally stable and do not degrade after cooking but can be reduced effectively with the help of phytase enzymes. Sprouting, a non-chemical and nonthermal technique results in the *de novo* synthesis of phytase, hence increasing mineral bio-accessibility and bioavailability (Murugkar, 2014). The degree of phytate reduction varies concerning the sprouting conditions (pH, time and temperature), cereal/legume species, cultivar, and native phytase activity (Elliott et al., 2022). Fortunately, the majority of antinutrients are removed to acceptable levels through dehulling, soaking, fermenting or thermal treatment. A combination of these treatments may prove to be more effective in eliminating antinutrients to a greater extent (Reyes-Jurado et al., 2021). Selected studies and techniques used to eliminate antinutrients are listed in Table 2-3.

2.4.4 Nutritional imbalance and sensory attributes

In terms of nutritional content, plant-based beverages vary from one to another to a great extent depending upon the source material. Thus, mixing two or more plant-based milk to create a product with balanced nutrition, like cow's milk, is a great opportunity to investigate. A chocolate-flavored peanut-soy beverage was prepared using response surface methodology based on sensory parameters and it was found that samples prepared using soy protein isolates were more palatable than those prepared with soy flour (Deshpande et al., 2008). Thus suggesting, that protein isolate not only elevates nutrition value but can improve sensory properties too. Similarly,

simulated milk consisting of soybean flour, sesame seed flour, and coconut meal with the goals of improving the nutritional value and palatability was granted a patent (US patent 3386833) (Johnson, 1968). The developed product had a relatively high proportion of the two essential amino acids lysine and methionine, a much higher calcium content than that of cow's milk and conventional soy milk, and approximately six times as much iron as found in bovine milk. In addition, this product also exhibited a good number of natural antioxidants found in sesame seeds. Researchers have also combined plant-based milk with cow milk and discovered that blended plant-based milk tastes better than plant-based milk alone (Singh & Bains, 1988).

Improving sensory attributes and nutrition imbalance in plant-based beverages can be a complex process that requires a combination of various strategies. Choosing the right combination of plant-based ingredients, optimizing the process parameters, adding functional ingredients and masking strong and off flavors can help improve the sensory attributes as well as the nutritional content of milk ingredients.

Table 2-3: Key challenges and technological interventions to address them for increased acceptability of plant-based milk alternatives.

Type of plant-based milk alternative	Key Challenge	Treatment	Treatment conditions	Main Finding	References
Soymilk	Stability improvement	High-pressure homogenization (HPH) or Ultra high-pressure homogenization (UHPH)	UHPH (300 MPa)	Treated soymilk exhibited less particle settling resulting in more stability during the storage (30 or 60 days at 5°C) compared with soymilk heat treated.	(Cruz et al., 2007)
Almond milk			HPH at 172 MPa (2–4 s) and heating treatment at 85°C for 30 min	The physical stability of the almond milk alternative was improved since treatments formed big oil droplet-protein aggregates which were embedded in a continuous protein	(Bernat et al., 2015)

			matrix to avoid phase separation after 28 days of storage at 4°C	
Almond milk		UHPH at 350 MPa, 85°C	Particle size was reduced till nano emulsion resulted in higher stability	(Vickers, 2017)
Oats milk	Enzymatic hydrolysis of starch by alpha and beta amylase	2.1 % (*w*/*w*) enzyme concentration	Optimized results by RSM, maximizing stability due to starch disintegration	(Deswal et al., 2014a)
Soymilk	High Hydrostatic Pressure	500 MPa at 83.7°C and 650 MPa at 77.4°C	Treatments reduced the settling of suspended particles compared with samples treated with a thermal process	(Manassero et al., 2016)
Peanut milk	Hydrodynamic Cavitation	via venturi throat diameter of 5 mm at 10 bars	The treatment reduced the fat particle size leading to a more homogenized and stable peanut milk alternative.	(Egerton, 2007)

Soymilk	Off-flavor removal	Pulsed electric field (PEF)	PEF, 20-40 kV/cm, 0-1000 μs)	The residual activity of lipoxygenase decreased with the increment of PEF time and pulsed electric strength. Maximum inactivation of the enzyme (88%) was achieved	(Li et al., 2013)
Soymilk		Hot grinding	Hot grinding of seeds at 80.5°C and UHT using vacuum evaporation	Removal of volatile compounds such as hexanal, 2-pentylfuran, and (E, E)- 2,4-nonadienal	(Zhang et al., 2012)
Soymilk		Ultra-high pressure homogenization	200 MPa, 75°C	Reduced beany and grass flavors in soymilk evaluated by sensory analysis compared with pasteurized soymilk	(Poliseli-Scopel, 2013)
Soymilk		High hydrostatic pressure	614MPa, 85.5°C	The treatment completely inhibited lipoxygenase	(Manassero et al., 2016)

	Antinutrient removal				
Soymilk		Thermal treatments	Conventional and by microwave processing (2450 MHz, 1000 W)	The residual activity of the trypsin inhibitor was 1% for conventional heating treatment (100°C, 30 min) and 3% for microwave heating process (100°C, 8 min).	(Vagadia et al., 2018)
		Microwave-assisted extraction	Power levels: 540, 675 or 810 W Temperature: 70, 80 or 90°C and 140, 160 or 180 rpm	Trypsin Inhibitor Activity was reduced up to 3.24% at 810 W, 90°C and 140 rpm	(Varghese & Pare, 2019)
Soymilk		Ohmic heating	220 V, 50 Hz	4 to 15 min of ohmic heating inactivated TIA by 22% to 87%	(Lu et al., 2015)

Soymilk	Fermentation	Fermentation pre-treatment at 25°C	Phytic acid was reduced by 15%, 27% or 32% in cooked soymilk prepared from soybeans fermented for 28, 50 or 72 h respectively	(Jiang et al., 2013)

2.5 Future research and conclusion

The consumption of plant-based beverages has expanded dramatically in recent years. Despite the abundance of information on this category of beverages, consumers still have concerns about its consumption due to a lack of familiarity with the taste of plant ingredients as a replacement for bovine milk. The research could focus on identifying and developing new plant-based ingredients that are rich in nutrients and have desirable taste and texture characteristics which consumers are more accustomed to. This could involve exploring underutilized crops or using biotechnology to modify existing crops. Also, to create a nutritionally complete and widely accepted plant-based milk, thorough research on processing, technology interventions, and fortification methods is necessary. Non-thermal innovative technologies like high-pressure homogenization, high-pressure processing (HPP), cold plasma, ultrasound and pulsed electric fields can be explored, especially on a large scale, to address the challenges of processing plant-based milk to develop low-cost, nutrient-rich alternatives which are more consumer-centric.

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Literature Review – Part 2

Chapter 3: Rise of plant-based beverages: A consumer-driven perspective

Abstract

The demand for plant-based beverages is increasing due to increased public awareness as valuable alternatives to dairy milk. There are a variety of plant-based beverages present in the market, such as soy milk, almond milk, rice milk, oat milk, etc. which overcome some of the caveats in traditional dairy milk (lactose intolerance, environmental footprint, etc.). However, the success of these plant-based beverages depends not only on the characteristic properties of these beverages (such as organoleptically pleasant) but also on in-depth analysis and a well-developed market strategy, which starts with a thorough understanding of consumer behaviour. Understanding consumer psychology is very important in predicting the decision to buy or not. To understand consumer behaviour, a study of their motivations and barriers to consumption is required. The present paper summarises the factors that might affect consumer behaviour and other strategies leading to interest from new customers and gaining the loyalty of already existing customers. In addition, the paper also explores the challenges and opportunities within the plant-based beverage industry.

Keywords: Lactose intolerance, environmental footprints, consumer behaviour

3.1 Introduction

The recent decade has seen an unprecedented leap in the number of consumers willing to switch to plant-based nutrition. Many factors are considered responsible for this shift, including personal health, environmental concerns, and animal welfare. One of the primary and most important factors is the potential health benefits of a plant-based diet, which can reduce the risk of lifestyle

diseases such as coronary heart disease, type 2 diabetes, and certain cancers (Sethi et al., 2016). These foods not only are found to be a rich source of fibre, vitamins, and minerals but can also promote overall well-being, as they are low in saturated fat and do not contain cholesterol (Craig & Fresán, 2021). In addition to this, increasing awareness of the environmental impact of animal agriculture, which contributes to greenhouse gas emissions, deforestation, and water pollution, has also influenced the choice of consumers to some extent (McClements et al., 2019). Finally, ethical considerations such as animal welfare are another factor that has influenced consumer attitudes towards the consumption of animal-based food products. This trend is expected to continue as more people realize the benefits of plant-based diets. Consumers now have more knowledge about the concerns associated with dairy consumption such as hormone use (to increase milk production), allergies (lactose intolerance, milk protein allergy) and perceived harmful profile (saturated fats and antibiotic use). In addition to this, the popularity of plant-based beverages has also been fuelled by the increasing availability of tasty and convenient options. Innovation in the development of new products is a key concept to keep new food products thriving in the market; therefore, food scientists and technologists are exploring a variety of plant-sourced materials that may have a more familiar taste for consumers, to make their switch to plant-based diets easier, thus increasing market demand for such new food products. The plant-based beverage market is one of the fastest-growing sectors with a market size worth USD 27.90 billion in 2022 (Precedence, 2023). The plant-based beverage market accounted for 7.4% of the overall milk market share in 2020 while by 2023, this percentage is projected to increase significantly to 18.5% (Schiano et al., 2020). Meanwhile, the traditional/bovine milk market is undergoing a declining trend, which may contribute, to some extent, to the ever-growing demand for plant-based beverages (Kuchler, 2022). This review highlights the main drivers that motivate consumers to choose plant-based beverages

and the consumer-centric barriers they phase while transitioning on what they are consuming. Furthermore, how these barriers are addressed and overcome is also discussed.

3.2 Major drivers and motivators for consumers behind the popularity of plant-based beverages

3.2.1 Health conditions and consumer awareness of health

The health and nutritional claims associated with plant-based beverages are a primary driver and motivation behind their increasing demand and market share. The present age of the internet and the large amount of digital knowledge available to consumers worldwide has made them more aware than ever about the food and nutrition they put into their bodies. The current trends of conscious eating have offered a great innovation platform for scientists to work on. Plant-based eating is a major trend which has gained popularity in recent years and has given rise to the ever-expanding plant-based beverage market with positive functional, healthy, and nutritional claims. Around 65% of the world population suffers from the problem of lactose tolerance and in East Asia, this number is almost 70-100 % (Aydar et al., 2020). Previously, the majority of consumers switching to plant-based beverages were those who suffered from lactose intolerance and bovine milk allergy, but now other reasons like hypercholesteremia, weight management, and lifestyles like veganism are also making them explore new beverage options. In addition to this, plant-based beverages have been shown to improve blood cholesterol levels with regular consumption, ie, lowering plasma cholesterol and low-density lipoproteins after 4 weeks of consumption of soymilk/oat milk (Önning et al., 1998). The bioactive and functional compounds present in these beverages, such as isoflavones and antioxidants, are effective in preventing cardiovascular disease, prostate cancer, and osteoporosis (Patisaul & Jefferson, 2010). In addition to this, a plant-based diet rich in soybeans and nuts can reduce the risk of neurodegenerative disorders such as

Alzheimer's and Parkinson's disease (Pistollato et al., 2018; Sarni & Baroni, 2019). Although these beverages are low in saturated fat, some of them have a high caloric value due to the presence of sugar (Mäkinen et al., 2016). In addition, plant proteins are considered to have poor digestibility and restrict the diversity in amino acid composition, thus having a lower protein nutritional value (Friedman, 1996). Furthermore, the presence of antinutrients in plant products restricts the bioavailability of vitamins and minerals (Dubey & Patel, 2018). The problem of nutritional deficiency is addressed by proper and optimum fortification of these beverages and the bioavailability of minerals can be increased through processes such as germination and fermentation (Sarangapany et al., 2022). A recent study on the relationship between the qualities of non-dairy beverages and how people decide to buy them found that health-related nutritional factors are the most important factors in how much people are willing to pay for plant-based alternatives (DeKinder-Smith, 2020). Consumers know a lot about the nutritional benefits of dairy alternatives, such as how many calories, protein, fat, and vitamins A and D they have, and are willing to pay more for beverages that have these benefits.

3.2.2 Planet health, sustainability, and animal welfare

According to the Food and Agriculture Organisation (FAO), sustainable food can be defined as food that protects and respects biodiversity and ecosystems, is culturally acceptable, economically fair, accessible, cheap, safe, nutritious, healthy, and makes the best use of natural and human resources (Willett et al., 2019). Diets that are considered sustainable and more environmentally appropriate are composed of more plant-based foods and less animal-based food (Frehner et al., 2022; Poore & Nemecek, 2018; Wellesley et al., 2015). In recent years, some customer groups have become more aware of the effects of food production and seem to have started a shift toward

sustainability, which is reflected in their purchasing patterns and the increase in the demand for plant-based food (Biesbroek et al., 2019). To evaluate the impact of food on the environment a life cycle analysis (LCA) is performed which consists of evaluating how much energy, water, land, greenhouse gas emissions, pollution, and fossil fuels are used during agricultural production, storage, shipping, sale, and use.

In a recent study, information was collected from different sources and compared about the life cycle of dairy and plant-based beverages (Grant & Hicks, 2018). The researchers found that dairy milk had the biggest general effect on the environment, scoring highest in most of the areas they looked at, as shown in Table 3-1. But surprisingly, plant-based milk had a higher total amount of energy used and a higher chance of contributing to global warming than cow milk. However, these observations regarding potential environmental impacts completely depend on the type of assumptions made in the analysis. For example, in another study (Poore & Nemecek, 2018), it was reported that cow milk had more greenhouse gas emissions, eutrophication, land use and water use than all plant-based milk. However, there were significant disparities between the various types of plant milk. Specifically, almond and rice milk used significantly more water than soy and oat milk, while soy and oat milks used more land. Furthermore, rice milk had a significantly higher degree of eutrophication than other plant-based milks. These results suggest that transitioning from dairy milk to plant-based milk may have significant environmental benefits, but the extent and character of these benefits depend on the type of milk substitute chosen.

Table 3-1: Impact on the environment of different plant-based beverages calculated per litre of beverage produced (McClements et al., 2019; Poore & Nemecek, 2018).

Beverage Type	Land Use m^2 per kg	Greenhouse Gas (Emissions $kgCO_2eq$ per litre)	Freshwater Use (Litres per litre)	Eutrophication (Kg PO_4 per litre)
Dairy milk	8.95	3.15	628.80	10.65
Rice milk	0.34	1.18	269.81	4.69
Oat milk	0.76	0.90	48.24	1.62
Almond milk	0.50	0.70	361.46	1.50
Soy milk	0.66	0.98	27.80	1.06

Other than this, consumers may also choose plant-based food products out of concern for animal welfare or because they dislike certain sensory characteristics of dairy/animal products (Ploll & Stern, 2020). Both policy and media have a role to play in informing and motivating consumers to change their dietary habits. In addition to individual factors such as attitudes, knowledge, values, and motivations, the adoption of sustainable diets is also influenced by social and cultural factors, including social norms and identity. But in the present scenario, consumers need more knowledge, education and awareness of the environmental impact of animal agriculture and how switching to plant-based alternatives can reduce their carbon footprint and make more informed choices for themselves and the planet (Allen et al., 2018; Stoll-Kleemann & Schmidt, 2017).

3.2.3 Constant innovation and major companies joining the trend

The growing popularity of plant-based beverages can also be attributed to constant innovation in the industry and major companies joining the plant-based trend. Consumers are motivated to choose plant-based beverages because they are considered a healthier and more sustainable alternative to traditional dairy products. As a result, many companies are investing in the development and marketing of plant-based beverages to meet this demand. The availability and accessibility of these products in grocery stores and restaurants also play a role in their increasing popularity among consumers. Traditionally, companies conduct focus group research and surveys during the new product development process to gain market insights and ensure the product's potential (Leerapong, 2013). But these methods do not always reveal the real needs and desires of consumers or fulfil their expectations. To address this caveat, more and more companies are opting for consumer-centric research and keeping consumer behaviour at the centre of their product innovation (Choy & Park, 2016). Consumer behavioural studies and the study of consumer purchasing data have opened up a whole new dimension for various sectors to target the right consumers with the right products. In addition to consumer differentiation, consumer behaviour studies also provide useful data for predicting future market trends and open opportunities for innovating in the development of new customer-centric products (Grunert, 2005; Perez-Cueto, 2020). This customer-centric research has led to the undeniable popularity of plant-based beverages as the demand for healthier and more sustainable food options is increasing.

To meet this demand, food companies have invested in research and development to create plant-based beverages that are not only nutritious but also taste good. Companies are also using consumer feedback to improve their products, packaging, and marketing strategies to better meet consumers' needs and preferences. The plant-based dairy segment is experiencing rapid growth

both in retail and food service menus. Plant-based dairy is being used in a variety of dishes and sauces and is particularly prevalent in fast casual and mid-size restaurants offering health-focused options. Oat milk is a standout performer with over 400% growth in menu penetration in the last four years (Good, 2023). While consumers are increasingly preferring milk alternatives, their presence on menus is still relatively low, indicating an opportunity for operators to meet this unmet demand. Plant-based cheese options have also seen growth, particularly American and cheddar-style cheeses, but nontraditional options like ricotta and cream cheese are also gaining traction. Since dairy products are a popular ingredient in food service, the plant-based dairy segment presents a significant opportunity for future growth.

Joining the growing plant-based trend are many big food company names now exploring dairy alternatives with more consumer-focused R&D and business collaborations for expanding their plant-based portfolios.

PepsiCo is one such company that announced the introduction of its plant-based beverage line, "Drinkfinity," which features a variety of flavours created with fruit and plant-based ingredients (Reynolds, 2018). Similarly, Danone has introduced plant-based beverage options under its Silk brand, including oat milk, almond milk, and coconut milk (Fox, 2017). Beyond offering traditional plant-based milk alternatives, companies are exploring innovative ingredients and flavours as well. For example, Coca-Cola has introduced a line of premium tea beverages called "Gold Peak" that includes flavours such as "Vanilla Chai Latte" and "Almond Toffee" (Watrous, 2022). Nestle has also launched a range of plant-based coffee creamers under its Coffee-mate brand, using almond and oat milk as a base in caramel and French vanilla flavour (Kanowsky, 2022). In addition to these new product launches, there is also significant investment in research and development to

further innovate within the plant-based beverage market. The use of artificial intelligence (AI) to innovate within plant-based dairy products has become increasingly popular among food companies. By utilising machine learning algorithms, companies can analyze consumer preferences, market trends, and ingredient formulations to create innovative and delicious dairy alternatives. An example of this is NotCo, a Chilean company that produces plant-based alternatives to a variety of foods, including milk and animal products using artificial intelligence (Adams, 2021). AI can help identify the optimal combination of plant-based ingredients that mimic the taste, texture, and nutritional profile of traditional dairy products at the molecular level. It can also improve the production process, reduce costs, and minimize waste by optimizing resource use and reducing the need for trial-and-error experimentation.

3.3 Barriers to the consumption of plant-based beverages

3.3.1 Food neophobia

Food neophobia is the fear or reluctance to explore new or novel food products. This instinct has evolved as a survival mechanism to prevent us from imbibing potentially hazardous or noxious substances. However, in modern times, neophobia can inhibit our willingness to try new and unfamiliar foods, including plant-based beverages (Langfield, 2021). Although plant-based beverages have become increasingly popular in recent years, despite the increasing awareness of the benefits of plant-based diets, some people still struggle to incorporate these beverages into their diets due to unfamiliar ingredients and novel taste profiles (Faccio & Guiotto Nai Fovino, 2019). Some consumers may find it difficult to associate the concept of consuming grains, legumes, and nuts in the form of milk, which may lead to a reluctance to try plant-based beverages made from these ingredients. It is suggested that informative and clear labelling can help reduce

food neophobia by providing consumers with a better understanding of the ingredients, nutritional content, and potential health benefits of the product (Alcorta et al., 2021). When labels are clear and easy to read, consumers can make informed decisions about what they are purchasing and consuming, which can help reduce anxiety and increase their willingness to try new foods. In addition to this, the familiarisation strategy can be considered, i.e., by allowing consumers to sample the plant-based beverages so that they can become more accustomed to their flavour and texture.

3.3.2 Incomplete nutrition

Incomplete nutrition is one of the primary concerns of choosing plant-based beverages over dairy milk. Almond and rice milk are typically limited in protein, whereas coconut milk is high in saturated fat (Escobar-Sáez et al., 2022). These beverages can also lack certain vitamins and minerals, such as calcium and vitamin D, that are present in dairy milk (McClements et al., 2019). Some popular plant-based beverages and the nutritional deficiencies associated with them, if not fortified, are listed in Table 3-2.

Table 3-2: Nutritional deficiency in popular plant-based beverages before fortification (Sethi et al., 2016)

Type of beverage	Nutritional Deficiency
Soy	Calcium, Vitamin D
Oats	Protein, Calcium, Vitamin D
Rice	Low in protein and fat, high in carbohydrates

Almond	Low in protein, calcium, and vitamin D
Coconut	No protein, rich in saturated fats

Considering nutrition, not only the quantity but also the evaluation of quality is of utmost importance. To evaluate the protein quality of foodstuffs, the Protein Digestibility Corrected Amino Acid Score (PDCAAS) is used frequently and is calculated by multiplying the amino acid score of a protein source by its true digestibility (Marinangeli & House, 2017). Typically, all plant proteins have lower PDCAAS scores compared to milk protein (having adjusted PDCASS score of 1) i.e. they may not provide all the essential amino acids necessary for optimal human nutrition in the right proportions (Drewnowski et al., 2021).

In addition to this, plant-based foods have limited bioavailability of certain nutrients due to the presence of antinutrients such as phytate, polyphenols, and oxalate, which significantly lowers their nutritional value (Hotz & Gibson, 2007). Although the problem can be addressed by using different processing methods like thermal or mechanical with cooking and fermentation, proper optimization of factors affecting these processes should be done to prevent further nutrient losses (Rousseau et al., 2020).

3.3.3 Sensory attributes

The sensory properties and attributes of plant-based beverages pose a major barrier to consumer acceptance. These beverages, especially soymilk, have off flavours such as a beany or grassy taste due to the activity of lipoxygenase enzymes associated with them (Mäkinen et al., 2016). According to a study in which acceptability based on the sensory attributes of lactose-free cow's

milk and soy milk was compared between adults and children in the United States, cow's milk was the preferred option (Palacios et al., 2010). Although new technologies and innovations have greatly improved the sensory quality of plant-based beverages, consumers still tend to have low expectations due to their previous negative experiences (Haas et al., 2019). As this product category is spending efforts, like never before, on addressing taste and texture issues at molecular levels, understanding the chemistry of flavour components and advanced sensory testing techniques has proved a game changer and helped in enhancing product acceptability.

3.3.4 Trends in consumer behaviour: emotional associations and decision making

It is a known fact that emotions play a crucial role for consumers when making purchase decisions. In addition to rational considerations such as health and price, purchase decisions are also driven by past consumption-related emotions (Desmet & Schifferstein, 2008; Thomson & Coates, 2021). Similarly, consumers who switch to plant-based beverages may be distracted by their emotions related to past emotions and experiences with bovine milk. To capitalize on these emotional associations, businesses are placing a greater emphasis on developing emotional connections with consumers. This may involve establishing marketing campaigns that elicit positive emotions, such as pleasure or nostalgia, or developing products that appeal to the emotional requirements and desires of consumers (Jaeger & Giacalone, 2021). In addition to this, situational appropriateness i.e. the extent to which a product fits the specific situation or context is a strong predictor of consumer choices for food and beverages (Giacalone & Jaeger, 2019).

Food and beverage companies can take these aspects of consumer behaviour into account to better understand consumer needs and develop products that can resonate with their choices and preferences (Costa & Jongen, 2006; Jaeger & Porcherot, 2017).

3.3.5 The high price of plant-based beverages

The high cost of plant-based beverages can act as a significant barrier to consumers who are considering purchasing them (Acquah et al., 2023). Retail prices of all types of plant beverages are almost double compared to dairy milk, due to the high cost of raw materials, their sourcing, and expensive blending and packaging procedures (Poinski, 2021). In addition to raw materials, additional ingredients such as stabilizers, emulsifiers, flavours, sweeteners (to mimic taste and texture), vitamins, and minerals enhance nutritional content up to the level of dairy milk. In the Mintec Insight Series report authored by (Marcel GoldenberǨ 2021), a comprehensive analysis was presented of the main factors that contribute to the high cost of plant-based beverages. The report outlines several key factors, including the utilization of expensive premium tetra-pack packaging materials, substantial capital investments required for establishing processing plants, and less efficient logistics operations that require superior end-to-end supply management and lead to additional expenses. Another crucial aspect that contributes to the higher costs of plant-based beverages is the investment required in marketing and innovation (Donovan, 2022). This investment is necessary for plant-based beverage manufacturers to compete with the established dairy industry and to capture and maintain consumers' interest. To meet the evolving tastes, textures, nutritional requirements, and functional expectations of consumers, plant-based beverage producers must allocate resources to extensive research and development to create novel products. This involves continuously innovating and introducing new product lines, which can be a costly process.

3.4 Overcoming the barriers and future research

As we have discussed, several barriers prevent some consumers from choosing plant-based beverages. To overcome these barriers, food companies and businesses need to understand and address the concerns of consumers. Future research can focus on nutritional complementation, optimizing process parameters to become more efficient, with innovation in taste and texture, and focusing more on the sustainability of the process. The following sections will discuss recommendations to overcome barriers and suggestions for future research in this area.

3.4.1 Nutrient complementation

A major challenge with plant-based diets is the limited availability of certain essential amino acids in their protein sources, as discussed in Section 3.2 and often considered incomplete in nature. For example, cereals are found to be deficient in lysine and tryptophan, while legumes have a limited amount of cysteine and methionine (Lonnie et al., 2018). To overcome this challenge, different plant sources can be combined to create a balanced and complete nutrient profile, the process is known as nutrition complementation.

Table 3-3 presents some examples of protein complementation that can be explored and utilized to achieve a balanced protein profile.

Table 3-3: Limiting amino acids in plant proteins and their complementation (Staff, 2011)

Source of plant protein	Limited amino acid	Complementary
Grains (Oat, Brown Rice, Wheat)	Lysine, Threonine	Legumes (Soy, Pea, Lentils, Beans)

Nuts and seeds	Lysine	Legumes (Soy, Pea, Lentils, Beans)
Legumes	Methionine, cysteine	Brown Rice, Wheat

In addition to the quality and quantity of protein, the digestibility and bioavailability of protein and other nutrients should be an area of great focus. More research is needed to explore techniques and processes that can optimize conditions for reducing antinutrients and increasing the digestibility of plant-based beverages, while also retaining their nutritional quality. Some approaches that can be further explored are enzymatic reduction of antinutrients, novel processing technologies such as high-pressure processing, pulsed electric field, and ultrasound processing (Adeyemo & Onilude, 2013; Smith et al., 2009; Vanga et al., 2020; Zhao et al., 2014).

3.4.2 Adjustment of taste, colour, taste and texture based on the personal preference of the population.

A range of plant-based beverages present on the market use similar kinds of basic ingredients such as oats, almonds, rice, and soy but vary greatly in terms of their texture and taste, which can influence consumer choices. The main issues related to the development of plant-based beverages are the bitter, beany, metallic, or astringent taste associated with them. These off-notes associated with plant ingredients can be addressed by incorporating supplementary fresh dairy flavours and masking ingredients. Flavourings with modulating properties (FMPs), which are important components of flavour made up of various substances such as essential oils, extracts, natural aroma chemicals, flavouring preparations and reaction flavours, can be used to mask unpleasant tastes (Gomes et al., 2023; Guentert, 2018). Sugar and artificial sweeteners such as sucralose, stevia, and

sugar alcohol can be used as great options to add sweetness and mask flavours (Hilty-Vancura, 2017). The texture of the product is influenced by the main plant-based ingredient (oat, rice, soy, etc.), type of source (flour, paste, concentrate etc.) and processing technology used. The right combination of raw materials and a multitude of processing variables, order of ingredient addition, water temperature, hydration time, and homogenization pressure are critical elements that can impart textures, smoothness, and the mouthfeel preferred by consumers.

In the age of big data and artificial intelligence (AI), combinations and proportions of ingredients can be evaluated by using machine learning algorithms to identify patterns and novel combinations to design a particular flavour or nutrition profile. Machine learning algorithms can also be used to collect data on how people perceive flavours and offer predictions on how they might react to new products.

3.4.3 Expanding the health promotion credentials of plant-based beverages

Following the Covid-19 pandemic and the increasing availability of nutritional information on the internet, health has become a key priority for consumers all over the world, leading to an increase in the demand for functional food products. Consumers worldwide now want to choose food and beverages that positively boost nutrition and impart health benefits. This growing opportunity can be capitalized on using plant-based beverages that are already showing spectacular momentum in the market and provide a great platform for innovation with functional ingredients. Additional functional ingredients such as probiotics can also be added to diversify the functional portfolio of plant-based beverages. Another category whose market potential can be tapped is plant-based sports nutrition.

3.4.4 Addressing high prices and affordability

Although the plant-based beverage market is booming and innovations are driving this category of food products to new heights, these beverages are more expensive compared to their conventional counterparts. As discussed in Section 3.3.5, the high cost is due to the involved processes, packaging, marketing, and the need for constant innovation. Moreover, bovine milk is generally subsidized in many countries, which makes it a winner when prices are compared. However, as the plant-based industry continues its growth and market share gains, the high price associated with these beverages is likely to decrease due to economies of scale, and the increase in competition will further lead to improved production processes, efficient packaging, and better distribution strategies. In addition, food companies and businesses can optimize their supply chain to save the cost of raw materials and transportation by sourcing and working with local suppliers. Innovation in packaging and the availability of beverages in smaller packaging sizes can make plant-based beverages more accessible and affordable for customers.

3.5 Conclusion

In conclusion, the rise of plant-based beverages is a consumer-driven trend, based on changing preferences and concerns related to health, sustainability, and animal welfare. While there are challenges to overcome, such as the high price and sensory attributes, there are also opportunities for innovation and growth. The future of plant-based beverages looks promising, and it is an exciting time for consumers, companies, and researchers alike.

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Preface to Chapter 4

Successful formulation of any beverage heavily relies on the optimization of extraction parameters. The extraction process is a critical step that significantly influences the quantity and quality of the final product. To ensure the highest possible yield of beneficial components, it is essential to identify the optimal conditions that maximize extraction efficiency while minimizing undesirable compounds. Chapter 4 is aimed at optimizing the extraction parameters for the preparation of chickpea beverage. The significance of this research lies in understanding the impact of extraction parameters will not only optimize the yield of beneficial compounds but also contribute to the overall sensory and nutritional qualities of the final product.

Chapter 4: Optimization of extraction parameters for preparation of *Cicer arietinum*-based beverage using Response Surface Methodology

Abstract

Extraction parameters for the preparation of chickpea-based beverages were evaluated to maximize their protein content, carbohydrate content, and total solid content, and minimize specific LOX (lipoxygenase) activity. A Central Composite design was used to optimize three input factors namely water to chickpea ratio (6:1 to 12:1), extraction temperature (40 - 80°C), and extraction time (10-60 minutes). The predicted optimal conditions as determined by RSM (Response Surface Methodology) were water to chickpea ratio of 7.13:1, extraction temperature of 77.9°C, and extraction time of 51 minutes. At these optimized conditions, the protein content, total carbohydrate content, total solid content, and specific LOX activity were observed as 4.12 g/100mL extract, 5.78 g/100mL of extract, 10.57 g/100mL of extract, and 0.0084 units/mg of protein, respectively. The study provides the extraction parameters/conditions to be considered for the preparation of chickpea-based beverages for minimizing the beany flavor while retaining the highest nutritional properties.

Keywords: Chickpea, protein, carbohydrates, LOX (lipoxygenase), RSM (Response Surface Methodology)

4.1 Introduction

Milk is considered one of the most important commodities around the world as a rich source of macro and micronutrients. The advent of the internet and ample diverse online information sources focusing on health and well-being have led to a new era where consumers are increasingly aware

of their nutritional intake and nutritional needs. They are more well-informed than ever about the options available to them when they are unable to consume cow's milk due to allergy, lactose intolerance, hypercholesterolemia, and vegan preferences (Mäkinen et al., 2016). For a long time, animal milk has been the primary source of nutrition for humans, however, with the increasing commercial introduction of plant-based milk substitutes in the market, the scenario has changed drastically. Although soymilk and rice milk, have been available in the market for decades, it was not until 2012 that a huge surge has been recorded in the demand for plant-based milk amounting to a whopping 61% increase. Increasing demand and research in this field have opened numerous options for customers to choose from. Today a wide variety of cereals, seeds, nuts, and legumes are being investigated for their potential use as milk-like beverages. Legumes being inexpensive and nutritionally rich offer a great opportunity for the development of plant-based milk-like beverages and other novel food products.

Chickpea (*Cicer arietinum*) is considered one of the most important legume grains with multiple nutritional and health benefits. It is a good source of protein, healthy carbohydrates, folate, calcium, iron, and many other trace elements along with minerals like potassium, sodium, calcium, and magnesium also present in considerable amounts (El-Adawy, 2002). In addition, chickpea seeds also possess high protein digestibility, low glycemic index (GI), vitamin-rich, and relatively low anti-nutritional factors. The protein content in chickpeas ranges from 20-30% and is highly influenced by the genetic makeup, soil type, fertilization, irrigation conditions as well as the location of growth of the crop (Sali et al., 2016). The major storage protein found in chickpeas is globulin with relatively smaller quantities of glutelin, albumin, and prolamin. Among various legume seeds, the protein quality of chickpeas is considered to be the best, exhibiting higher NPU (Net Protein Utilization) than other legumes (Rachwa-Rosiak et al., 2015). In Chickpeas

carbohydrates range from 54-70% with the majority present as starch in most cultivars (Hirdyani, 2014). Chickpeas also have great nutraceutical value as they are found to be rich in numerous bioactive compounds that help in the management of cancer and cardiovascular ailments (Gupta et al., 2017). Chickpeas are classified under the low glycemic food category with a GI of 28 and are a recommended food choice for lowering the risk of diabetes (Aisa et al., 2019). Although chickpeas are found to be highly nutritious, the presence of certain compounds limits their consumption and acceptance among the general population. They contain antinutritional factors (ANFs) and many of these compounds have a deleterious effect on the human gut (Xu et al., 2016). Other than the presence of these factors, they also contain a group of enzymes known as lipoxygenases (LOX) that contribute to the typical beany flavor. Lipoxygenases cause deoxygenation of polyunsaturated fatty acids producing mono-hydroperoxides as primary products which, on further degradation produce carbonyl compounds such as hexanal, hexanol, and 2-pentylfuran, primarily responsible for off-flavors (Sanz et al., 1992). When legumes are utilized for the preparation of beverages, LOX converts unsaturated fatty acids to medium-chain aldehydes and alcohols producing an unpleasant beany flavor (Chong et al., 2019). Thermal treatment is the most common technology used for the inactivation of lipoxygenase and improvement of the beverage flavors. These legume-based beverages are mainly water extracts with varying water ratios, processed under different conditions of temperature and time. Therefore, these factors need to be optimized while processing, to create a beverage having high nutrition profile while eliminating or minimizing off-flavors and avoiding the deleterious effect of thermal treatment on protein and other bioactive compounds of the beverage. Previously, chickpea has been explored for the preparation of milk-like beverage to evaluate its properties as a substitute for soymilk, in sprouted and fermented form but studies regarding the optimized process of

processing, detailed nutritional characteristics, quality profiling and storage studies are still lacking (Wang et al., 2018).

In earlier studies on the preparation of soymilk, oat milk and peanut milk a varying degree of water ratio, extraction time and temperature has been used depending upon the source material used (Galvez, 1990). To the best of our knowledge, the optimization of these parameters has not been studied in the case of a chickpea beverage. In the present study, Kabuli-type chickpeas (*Cicer arietinum*) have been explored for the preparation of a plant-based beverage. For the preparation, the extraction parameters of the beverage have been optimized with the help of RSM (Response Surface Methodology). The parameters chosen for the optimization include water-to-chickpea ratio, extraction temperature, and time. The present study will provide optimum values of the parameters involved in the preparation of the chickpea beverage for maximizing its nutrient content (protein, total carbohydrate and total solid content) and minimizing off-flavor (lipoxygenase activity) thus improving its acceptability.

4.2. Materials and methods

4.2.1 Material and proximate analysis

Kabuli-type chickpeas (*Cicer arietinum*) Lot #15386, 9mm were obtained from AGT Clic Foods (Laval, Quebec). These were cleaned, sorted, and analyzed for their proximate composition namely moisture, total protein, total fat, total carbohydrates, and calorie content. Moisture content was calculated using the loss-by-drying method, keeping the milled samples in an oven at 105°C until a constant weight was achieved (Fruits, 2014). For protein estimation, a bicinchoninic acid assay (BCA) was carried out (Casal et al., 2000). For total carbohydrate content, the anthrone method was used (Widyastuti & Giarni, 2015). Fat content was estimated by the acid hydrolysis

method. The total caloric value (Kcal) of the sample was obtained using the Atwater factors for protein, carbohydrates, and fat (4, 9, and 4 respectively) (Achy et al., 2016).

4.2.2 The extraction process of the beverage

For the preparation of the chickpea-based beverage, the process followed by (Nelson et al., 1976) was used with some modifications (Figure 4-1). Chickpeas were soaked for 12 hours and rinsed well with water at the end of the soaking period. The soaked chickpeas were ground with different water-to-chickpea ratios for 1.5 minutes in the lab-scale food processor. After grinding, the chickpea-water slurry was heat-treated at different combinations of time and temperature as per the experimental design. The slurry was finally filtered through double-layer cheesecloth and the filtrate collected was homogenized for stability using a homogenizer (Fisherbrand 850 homogenizer). The obtained filtrate was cooled and stored at 4°C for further analysis. Beverage samples processed at different water-to-chickpea ratios, processing temperatures and times are shown in Figure 4-1.

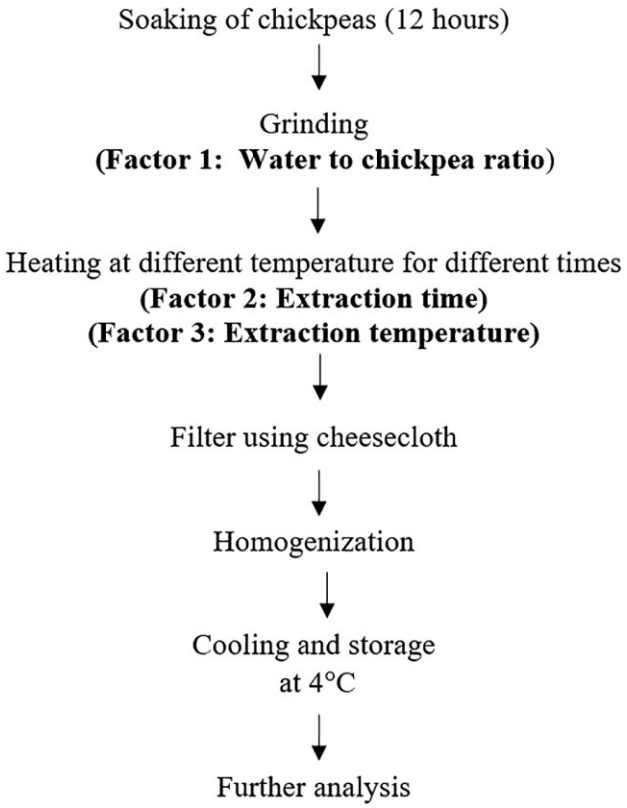

Figure 4-1: Processing of chickpea extract for preparation of the beverage

4.2.3 Experimental design and ranges for optimization

For optimization of the extraction process, Response Surface Methodology (RSM) with Central Composite Design (CCD) was used. Optimization was performed using OriginPro software by OriginLab. RSM is a set of statistical and mathematical techniques that are used to study the effects of independent variables on response variables. CCDs are factorial designs with center points augmented with a group of axial points widely used for fitting second surface response models. For optimization, three independent variables namely the water-to-chickpea ratio, extraction time, and the extraction temperature were chosen. The first factor, the water-to-chickpea ratio, was studied between 6:1 to 12:1, while the extraction time and extraction temperature were studied

between 10 minutes to 60 minutes and 40°C to 80°C respectively. The ranges of variables for optimization were decided based on preliminary studies and are presented in Table 4-1.

Table 4-1: Central composite design for process optimization with independent variables and their range of levels

Process parameters	Units	Range of levels		
		-1	0	1
Water to chickpea ratio	-	6:1	9:1	12:1
Extraction temperature	°C	40	60	80
Extraction time	Minutes	10	35	60

4.2.4 Responses and their estimation

The responses measured were total protein content, total sugar content, total solids, and specific lipoxygenase activity. For determining the protein content, the Bicinchoninic acid (BCA) method was used (Casal et al., 2000). BCA assay kit was obtained from Pierce Biotechnology, Rockford, IL, USA. The procedure mentioned in the user guide was followed. BCA kit contains two reagents labelled Reagent A (a solution of sodium bicinchoninate, sodium bicarbonate, sodium tartrate and sodium hydroxide in distilled water) and Reagent B (a solution of cupric sulphate pentahydrate in distilled water). The working reagent was prepared by mixing 50 parts of BCA Reagent A and 1 part of BCA Reagent B. Reaction mixture was prepared by mixing 0.1 mL of the sample and 2.0 mL of working reagent. The reaction mixture was incubated at 37°C for 30 minutes and thereafter absorbance was taken at 562 nm using a UV spectrophotometer. For the preparation of the standard

graph, Bovine serum albumin (BSA) was used. Total sugar content, both free and bound, was determined using the anthrone method (Widyastuti & Giarni, 2015). Anthrone reagent was freshly prepared by mixing 1g of Anthrone in 50 mL of 95% H_2SO_4. To the 1mL of sample solutions, 5mL of Anthrone reagent was added and mixed by vortexing. The obtained reaction mixture was incubated in a boiling water bath for 10 minutes and absorbance was taken at 620nm using a spectrophotometer after cooling down the reaction mixture to room temperature. Samples were diluted 100 times before adding the reaction mixture. Glucose was used to prepare the standard calibration graph. Total solid recovery or dry matter was estimated by the gravimetric method (mass basis) by drying the beverage at 105°C in the oven till constant weight is achieved (Achy et al., 2016). The fourth response variable, specific lipoxygenase activity, was estimated by the method developed by (de Barros et al., 2014; Navicha et al., 2017), and further described by (Chong et al., 2019). The prepared chickpea extract was centrifuged at 5000 rpm for 15 minutes for extracting the lipoxygenase enzyme. The supernatant obtained was pipetted out in an Eppendorf tube and stored in a freezer at -20 °C until further use. The stock solution for substrate (10 M sodium linoleate) was prepared by adding 156 µl of linoleic acid and 180 µl of Tween 20 to 20 mL of deionized water. To clear the solution 0.5 N NaOH was added dropwise, and repeated pipetting was done to avoid the bubbles. The stock solution was stored at -20°C in a falcon tube wrapped in aluminum foil.

For estimating specific lipoxygenase activity, 100 µl of enzymatic extract, 400 µl of sodium linoleate from the stock solution, and 5 mL of 50mM of phosphate buffer (pH 6.5) were added in a test tube. The mixture was rapidly shaken and transferred to a quartz cuvette for analysis. The reaction rate was determined with an increase in absorbance over 60 seconds at intervals of 20 seconds at 234 nm using a UV-Vis spectrophotometer. The increase in absorbance occurs due to

the formation of conjugated double-bond hydroperoxide moieties at 234 nm. For blank, the same amount of substrate and buffer was used without the enzymatic extract. In addition, the protein content of the enzymatic extract was estimated by the Bicinchoninic acid (BCA) method (Casal et al., 2000). The specific LOX activity was expressed as μmol/min mg of protein or simply as unit/mg of protein where a unit is defined as μmol/min, calculated using Equation [4-1]:

$$\text{Specific LOX activity} = (\Delta A \times DF \times Vc)/(Ve \times Lc \times \varepsilon \times Cp) \quad [4\text{-}1]$$

where ΔA is variation in absorbance at 234 nm (1/min), Vc is the volume of the enzymatic extract (L), DF is dilution factor, Ve is total volume in the cuvette (L), is the molar the absorption coefficient of linoleic acid hydroperoxide at 234 nm, i.e. 0.025 L/(μmol cm), Lc is the path length of the cuvette, i.e. 1 cm, and Cp is the protein concentration of enzymatic extract (mg/L).

4.2.5 Process Optimization

Optimization of the process parameters was performed using OriginPro software. Analysis of variance (ANOVA) was performed and regression coefficients of linear, quadratic, and interaction terms were obtained. Regression coefficients were then used for generating response surfaces and regression equations.

After obtaining polynomial regression equations, showing a relationship between responses and independent variables, optimization was done for input variables. For optimization, response variables namely protein content, total carbohydrate content, and total solid content were set to maximize while specific LOX activity was minimized. All input variables were kept within their range for optimization. Validation of the model was done by conducting experiments at predicted optimized conditions.

4.3. Results and Discussion

The obtained chickpeas were first analyzed for their proximate composition and the results obtained are shown in Table 4-2. For extraction of the beverage, experiments were performed in triplicates and in a completely randomized fashion with the center point repeated 6 times. The mean values of the response variables namely protein content, total carbohydrate content, total solid content, and specific LOX activity obtained from different experimental runs are presented in Table 4-3. The values were analyzed using ANOVA and results have been reported in Table 4-4 (for the fitted model) and Table 4-5 (individual terms) respectively.

Table 4-2: Proximate composition of chickpeas

Parameters	Composition (%) [§]
Moisture	9.56±.01
Protein	24.03±0.16
Total carbohydrates	65.24±0.18
Fat	6.17±0.07
Energy (kcal)	412.61

[§]Results are presented as Mean ± standard deviation, n=3

Table 4-3: Responses obtained for different process parameters combinations according to experimental design

Runs	Factor 1: water to chickpea ratio	Factor 2: Extraction temperature (°C)	Factor 3: Extraction time (minutes)	Response 1: Protein content (g/100mL	Response 2: Total carbohydrate content	Response 3: Total solid content (g/100mL	Response 4: Specific Lipoxygenase activity

				of the beverage)	(g/100mL of the beverage)	of beverage	(unit/mg of Protein)
1	9:1	40	35	3.23	3.89	7.97	0.0098
2	9:1	60	35	4.97	4.39	9.68	0.0079
3	12:1	80	10	0.98	3.83	4.89	0.0043
4	9:1	80	35	3.97	5.475	9.02	0.00628
5	12:1	40	10	0.73	3.235	4.10	0.00515
6	12:1	60	35	2.35	3.798	6.74	0.0053
7	9:1	60	35	4.85	4.353	9.99	0.0083
8	9:1	60	60	3.39	5.101	10.25	0.0071
9	6:1	40	10	3.56	4.781	9.98	0.0239
10	9:1	60	35	4.35	4.320	9.23	0.00815
11	9:1	60	10	2.98	3.958	9.12	0.0093
12	6:1	40	60	4.39	4.898	10.15	0.0211
13	9:1	60	35	4.59	4.397	9.93	0.00813
14	6:1	80	60	3.97	5.530	10.68	0.011
15	9:1	60	35	4.83	4.538	9.88	0.0071
16	6:1	80	10	3.73	5.670	10.65	0.0151
17	6:1	60	35	5.11	5.051	10.12	0.0189
18	12:1	40	60	2.11	3.590	7.78	0.0051
19	9:1	60	35	4.39	4.087	10.49	0.0082
20	12:1	80	60	1.26	4.760	7.88	0.00415

Table 4-4: ANOVA for the fitted model

	Source	Coefficient	SS	DF	MS	F	P	
	Model		33.31	9	3.70	22.88	<0.0001	Significant
	Residual		1.62	10	0.16			
	Lack of fit		1.28	5	0.25	3.83	0.08	not significant
Protein Content	Pure error		0.33	5	0.06			
	Total		34.93	19				
	R^2	0.95						
	Adj R^2	0.91						
	CV%	11.55						
	Model		7.46	3	2.49	34.18	<0.0001	significant
	Residual		1.16	16	0.07			
	Lack of fit		1.05	11	0.09	4.36	0.05	not significant
Total sugar content	Pure error		0.10	5	0.02			
	Total		8.63	19				
	R^2	0.86						
	Adj R^2	0.83						
	CV%	6.02						

	Model		63.46	9	7.05	31.40	<0.0001	significant
	Residual		2.25	10	0.23			
	Lack of fit		1.39	5	0.27	1.64	0.30	Not significant
Total solid recovery	Pure error		0.85	5	0.17			
	Total		65.70	19				
	R^2	0.96						
	Adj R^2	0.93						
	CV%	5.31						
	Model		0.0006	9	0.0001	232.52	<0.0001	significant
	Residual		2.87E-06	10	2.87E-07			
	Lack of fit		2.04E-06	5	4.08E-07	2.45	0.17	Not significant
Specific LOX activity	Pure error		8.32E-07	5	1.67E-07			
	Total		0.0006	19				
	R^2	0.995						
	Adj R^2	0.991						
	CV%	5.52						

Table 4-5: ANOVA for the effect of individual process parameters on response variables

Parameter	Sum of squares	df	Mean square	F-value	P-value
Protein content					
A	17.61	1	8.78	108.84	<0.0001*
B	0.0029	1	0.25	0.017	0.89
C	0.94	1	0.05	5.86	0.03*
AB	0.01	1	0.22	0.06	0.80
AC	0.05	1	0.55	0.32	0.58
BC	0.38	1	1.27	2.37	0.15
A^2	0.49	1	1.75	3.08	00.10
B^2	0.85	1	8.83	5.25	0.04*
C^2	2.59	1	0.11	16.02	0.0025*
Total sugar content					
A	4.51	1	4.51	61.99	<0.0001*
B	2.37	1	2.37	32.60	<0.0001*
C	0.58	1	0.58	7.95	0.01*
Total solid content					
A	40.76	1	40.76	181.55	<0.0001*
B	0.98	1	0.98	4.39	0.06
C	6.40	1	6.40	28.50	0.0003*
AB	0.012	1	0.012	0.053	0.821

AC	5.23	1	5.23	0.821	0.0007*
BC	0.086	1	0.086	0.549	0.54
A^2	2.39	1	2.39	0.008	0.008*
B^2	2.07	1	2.07	0.012	0.012*
C^2	0.287	1	0.287	0.284	0.28
Specific LOX activity					
A	0.0004	1	0.0004	1510.60	<0.0001*
B	0.0001	1	0.0004	205.50	<0.0001*
C	8.48E-06	1	8.48E-06	29.50	0.0003*
AB	0.00	1	0.00	125.77	<0.0001*
AC	5.76E-06	1	5.76E-06	20.04	0.0012*
BC	2.77-07	1	2.77-07	0.96	0.34
A^2	0.00	1	0.00	131.23	<0.0001*
B^2	3.47E-07	1	3.47E-07	1.21	0.29
C^2	1.05E-07	1	1.05E-07	0.36	0.55

4.3.1 Effect of process parameters on protein content

The protein content of the beverage is found to be a function of the linear effect of water to chickpea ratio while only quadratic effects are observed in the case of the extraction temperature. However, for extraction time, both linear and quadratic effects are observed as evident from the significant individual terms shown in Table 4-5. The protein content of the extracted beverage as influenced by the combined effect of chickpea to water ratio and the processing temperature is

shown in Figure 4-2a. As indicated in Figure 4-2a, the protein content of the beverage decreases as the water-to-chickpea ratio increases from 6:1 to 12:1, but an increase in temperature results in an increase in protein content up to 61°C after which point a decrease is observed. The initial increase in protein content can be attributed to mass transfer principles i.e., with an increase in temperature, solubility increases, and viscosity decreases, thus increasing the extraction rate of the solution (Zhang et al., 2009). But high temperatures could cause thermal denaturation of the protein leading to its aggregation and precipitation (Kumoro et al., 2010). Figure 4-2b shows the effect of the water-to-chickpea ratio and extraction time on the protein content. This result indicates an increase of the protein content up to 38-40 minutes, however, beyond that a decrease was observed. The observed results could be due to the solubility dynamism involved in the protein extraction process (Mang et al., 2016). Protein gets solubilized in the solution by establishing hydrogen bonds with the water till a saturation condition is achieved ((Nick Pace et al., 2004). In addition to this, the chickpea protein system (globular protein) contains hydrophobic groups inside and hydrophobic moieties outside. These hydrophilic amino acids present on the surface have hydrogen and oxygen atoms which form hydrogen bonds with water. As the temperature increases, thermal vibration in the medium results in exposing hydrophobic groups to the surface, thus increasing surface hydrophobicity and affecting solubility. The combined effect of extraction temperature and time is shown in Figure 4-2c. ANOVA results for the fitted model are shown in Table 4-4. The quadratic regression model is represented in the form of Equation [4-2]. The model is found to be significant with an F-value of 22.88. The R^2 and adjusted R^2 for protein content are 0.95 and 0.91, respectively. A higher R^2 value and lower p-value <0.0001 suggest appropriate fitting of the model is predicted. Actual and predicted values of the data points for protein content

of the beverage are plotted in Figure 4-2d. Lack of fit is non-significant with a p-value of 0.08 indicates adequacy of the model.

Protein content = 4.46 -1.33 A - 0.017 B+ 0.308 C – 0.036 AB + 0.081AC - 0.218BC -0.425 A^2- 0.56 B^2-0.97C^2 [4-2]

Where, A= water to chickpea ratio, B= processing temperature (°C), C= processing time (minutes)

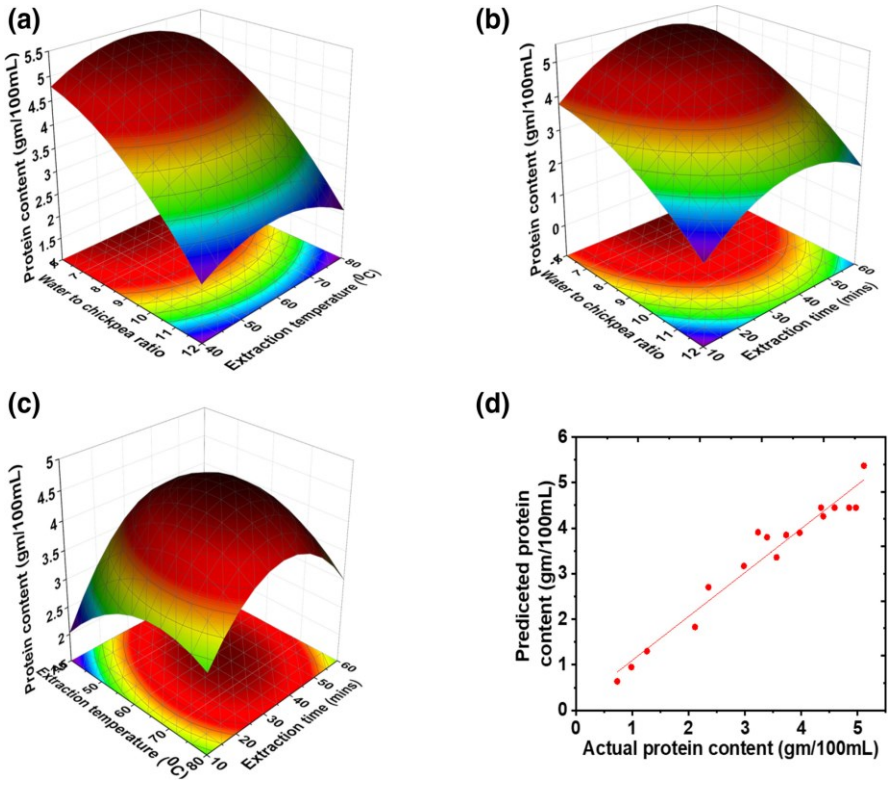

Figure 4-2: Response surfaces for correlating protein content with (a) water to chickpea ratio and extraction temperature at 35 minutes (b) water to chickpea ratio and extraction time at 60 °C (c) extraction temperature and extraction time at 9:1 water to chickpea

4.3.1 Effect of process parameters on the total carbohydrate content

The total carbohydrate content of the extracted beverage is affected by the chickpea processing parameters shown in Figure 4-3. As shown in Figure 4-3a, total carbohydrate content decreases with an increase in water to chickpea ratio from 6:1 to 12:1 and a decreasing trend is observed but

as extraction temperature is increased from 40 to 80°C, a linear increase is observed. Total carbohydrate content also increases with an increase in extraction time from 10 to 60 minutes as shown in Figure 4-3b-c. The increase in total carbohydrate content with an increase in both extraction time and temperature suggests a synergic effect of both factors however no significant interactions between them are observed. The decrease in sugar content with an increase in water to chickpea ratio may be attributed to the dilution principle i.e., with an increase in the water content there is dilution, and the concentration of the constituents decreases. Similar results for aqueous sugar extraction have been reported in other foodstuffs (Kuila et al., 2011). For all the factors, only linear terms were found statistically significant, and their values are presented in Table 4-5.

The ANOVA results for the fitted model for total carbohydrate content are shown in Table 4-4 and Equation [4-3] presents the predicted regression model. The model is significant at an F-value of 34.18. The R^2 and adjusted R^2 are 0.86 and 0.83 respectively indicating a good fit for the model. Lack of fit is also non-significant (p-value = 0.05) suggesting the adequacy of the model. Actual and predicted values of the data points are plotted in Figure 4-3d. The effects of individual factors on the total carbohydrate content of the beverage as analyzed by ANOVA are presented in Table 4-5.

$$\textbf{Total carbohydrate content} = 4.48 - 0.671\,A + 0.487B + 0.240C \qquad [4\text{-}3]$$

Where A= water to chickpea ratio, B= processing temperature (°C), C= processing time (minutes)

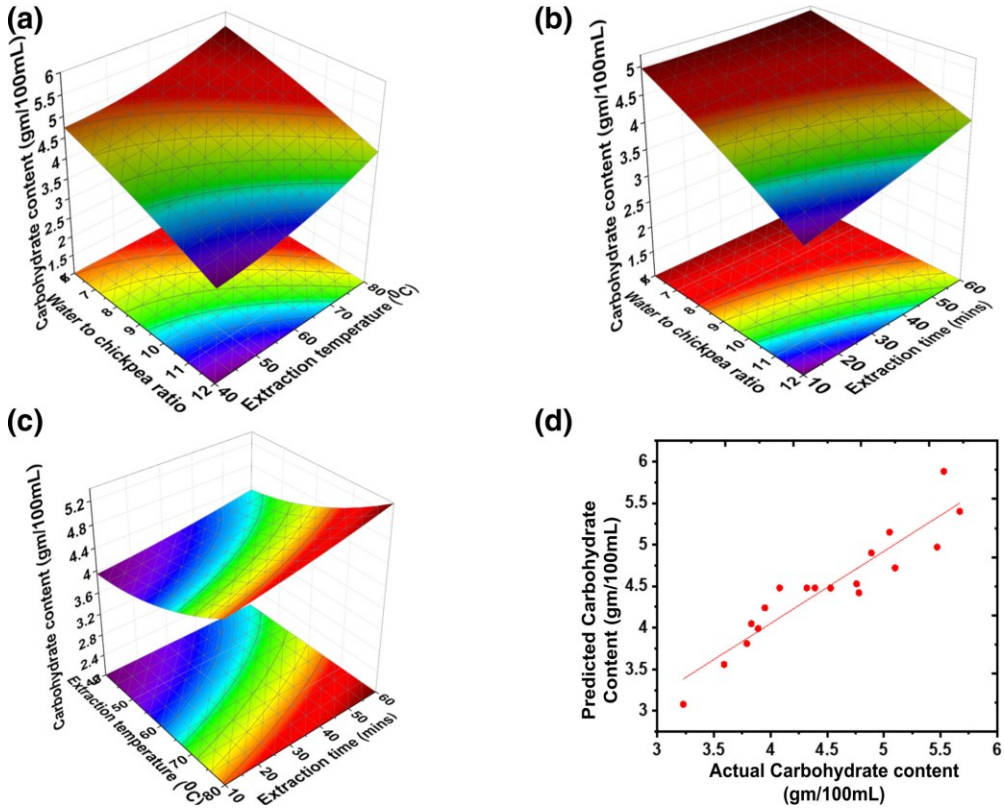

Figure 4-3: Response surfaces for correlating total carbohydrate content with (a) water to chickpea ratio and extraction temperature at 35 minutes (b) water to chickpea ratio and extraction time at 60 °C (c) extraction temperature and extraction time at 9:1 water

4.3.2 Effect of process parameters on total solid content

All components in the extract, other than water, represent the total solid content or dry matter of the solution. The response surfaces for total solid recovery of the beverage for water to chickpea ratio, extraction time, and temperature are shown in Figure 4-4a-c. Total solid contents decreased with an increase in water to chickpea ratio as observed in Figure 4-4a showing linear as well as quadratic effects. The total solid content increases up to 60°C and decreases on further increase as evidenced by the quadratic effects observed as shown in Figure 4-4b. In the case of extraction time, total solid content increases linearly with an increase in extraction time as shown in Figure

4-4c. The increase in solid content with time may be due to the release of more compounds from the solid part into the water as suggested by positive linear effects. Similar results on solid content have also been reported in other studies (Deswal et al., 2014b; Mang et al., 2016).

ANOVA results for the fitted model for total solids are shown in Table 4-4. The quadratic regression model is presented in the form of Equation [4-4]. The adjusted R^2 and predicted R^2 are 0.96 and 0.93 respectively. The lack of fit is non-significant with a p-value of 0.30 indicating the adequacy of the model. The actual and predicted values for total solids are plotted in Figure 4-4d. The effects of individual factors on the total solids content of the beverage as analyzed by ANOVA are presented in Table 4-5. In addition to linear and quadratic effects, significant interaction effects between the water-to-chickpea ratio and the extraction time are also observed.

Total solids content = $9.66 - 2.02\ A + 0.314\ B + 0.80\ C - 0.038\ AB + 0.808\ AC - 0.103BC - 0.93\ A^2 - 0.86\ B^2 + 0.32\ C^2$ [4-4]

Where A= water to chickpea ratio, B= extraction time (minutes), C= extraction temperature (°C)

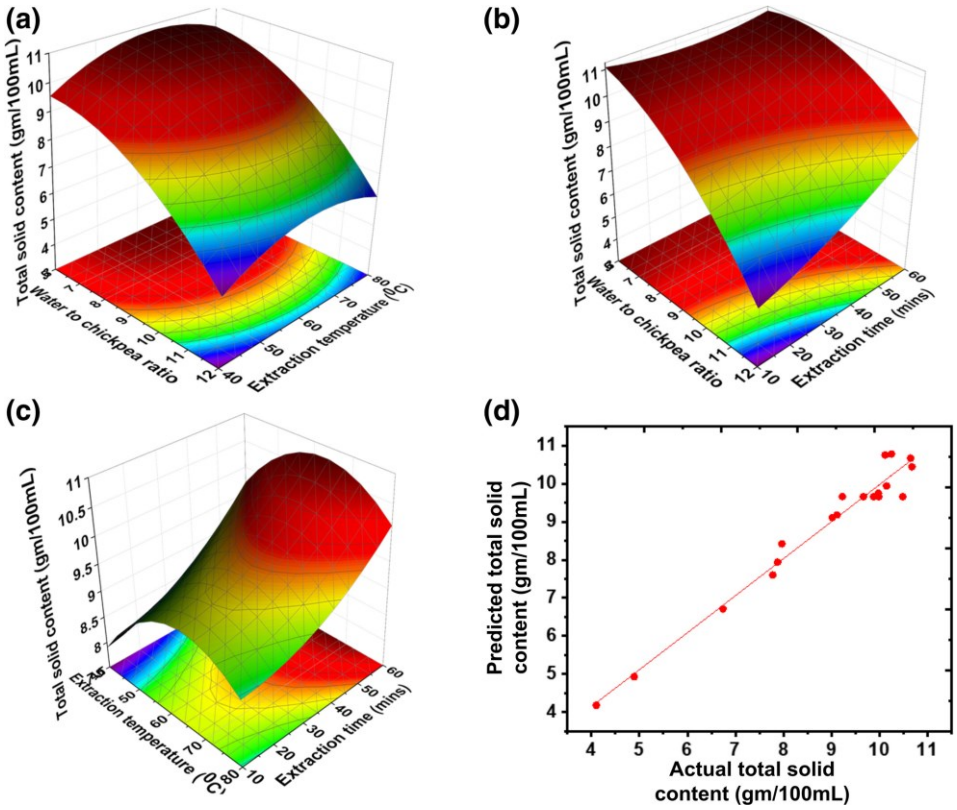

Figure 4-4: Response surfaces for correlating total solid content with (a) water to chickpea ratio and extraction temperature at 35 minutes (b) water to chickpea ratio and extraction time at 60 °C (c) extraction temperature and extraction time at 9:1 water to chic

4.3.3 Effect of process parameters on specific lipoxygenase activity

Response surfaces for specific lipoxygenase (LOX) activity as affected by the three input factors are shown in Figure 4-5a-c. As shown in Figure 4-5a, specific LOX activity decreases with an increase in water to chickpea ratio reflecting the simple rules of dilution i.e., a decrease in constituents with an increase in dilution. Also, with an increase in extraction time and temperature, specific LOX activity decreased as seen in Figure 4-5b-c. The lowest specific LOX activity was obtained when temperature and time were set at their highest values (80°C and 60 minutes). LOX is a group of enzymes responsible for imparting off-flavors in legumes and due to being heat-

sensitive could be inactivated at higher temperatures (Kumar et al., 2003). Similar results for LOX enzymes were obtained for soybean by (Chong et al., 2019). ANOVA results for the model fitting for specific LOX activity are shown in Table 4-4. The model is found to be significant with a p-value of <0.0001, with a non-significant lack of fit with a p-value of 0.17. The adjusted R^2 and predicted R^2 are 0.995 and 0.991 respectively suggesting a good fit for the model. The quadratic regression model is presented in the form of Equation [4-5]. Figure 4-5d presents the graph between actual and predicted values for specific LOX activity. The effects of individual terms on the specific LOX activity as shown in Table 4-5, suggests that all the linear effects are significant, but the quadratic effects are only significant in the case of water to chickpea ratio. In addition to this significant interaction, effects are observed between water to chickpea ratio with extraction temperature as well as extraction time.

Specific LOX activity = $0.008 - 0.0067\,A - 0.0024B - 0.0009C + 0.0021AB + 0.0008AC + 0.0002BC + 0.0037\,A^2 - 0.0004\,B^2 + 0.0002\,C^2$ [4-5]

Where, A= water to chickpea ratio, B= extraction time (minutes), C= extraction temperature (°C)

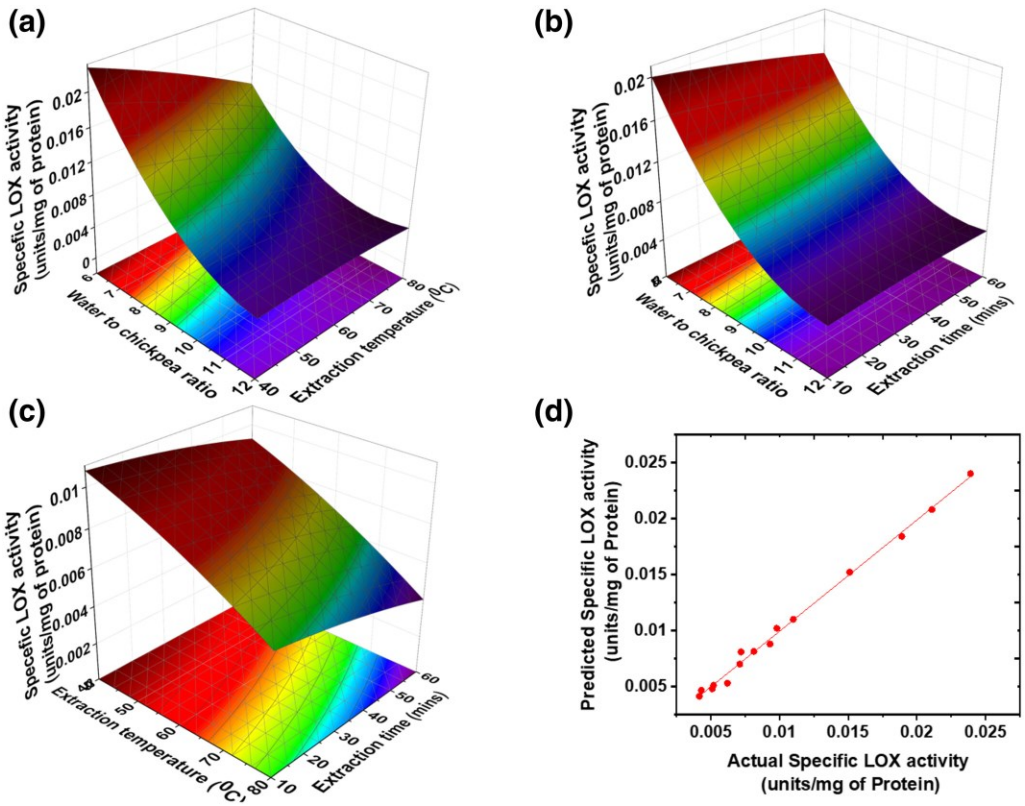

Figure 4-5: Response surfaces for correlating specific LOX activity with (a) water to chickpea ratio and extraction temperature at 35 minutes (b) water to chickpea ratio and extraction time at 60 °C (c) extraction temperature and extraction time at 9:1 water to chickpea ratio

4.3.4 Optimization of process parameters and validation of the model

After response surface analysis, numerical optimization was carried out using OriginPro software from OriginLab. Out of several combinations that can give desirable goals, the best one can be chosen based on the "desirability function". The desirability function consolidates multiple responses into one response with a choice of value varying from 0 (one or more product characteristics are unacceptable) to 1 (all product characteristics are on target). For optimization, all three factors were kept in the range i.e., water to chickpea ratio (6:1 to 12:1), extraction temperature (40 - 80°C), and extraction time (10-60 minutes). The desired goal for responses was

to maximize the protein content, total carbohydrate content, and solid content while specific LOX activity was minimized. From the obtained solutions, one with maximum 'desirability' i.e. one closest to value 1 was chosen as the optimized value. Predicted values of the factors at optimized conditions are presented in Table 4-6. The adequacy of the model was tested by conducting experiments at predicted optimum values of the selected factors. The obtained responses were compared with the predicted values to validate the model (Table 4-7). The closeness between the predicted values and experimental values suggests the suitability of the predicted model. The desirability of the predicted model is 0.89.

Table 4-6: Predicted optimum conditions for preparing chickpea-based beverage.

Factors	Low	High	Optimum
Water to chickpea ratio	6:1	12:1	7.13:1
Extraction temperature (°C)	40	80	77.9
Extraction time (minutes)	10	60	51

Table 4-7: Predicted and experimental responses for optimized process

Responses	Predicted value	Experimental value[§]	p-value (p< 0.05)
Protein content (g/100mL of beverage)	4.34	4.12 ± 0.20	0.19
Total sugar content (g/100mL of the beverage)	5.50	5.78± 0.18.	0.12
Total solid content	10.43	10.57±0.58	0.78

(g/100mL of the beverage)			
Specific LOX activity (unit/mg of Protein)	0.009	0.0084±0.0007.	0.27

§Results presented as Means±S.D., n=3

4.4 Conclusion

The optimization of process parameters was carried out using Response Surface Methodology. The predicted regression model suggested significant effects ($p < 0.05$) of all the input factors on the responses. The model was validated at the optimum conditions by experimentation and found to be a suitable fit. The present study provides insight on optimizing the extraction parameters for maximizing the nutritional content (protein, carbohydrate, and total solid content) while minimizing the LOX activity that is commonly responsible for the beany off-flavour in legume-based beverages. For the preparation of the chickpea-based beverage, further investigation of its nutritional profile, in-depth qualitative study of its components, sensory evaluation, shelf-life studies and fortification for taste enhancement is required.

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Preface to Chapter 5

The optimization of extraction parameters for the preparation of a *Cicer arietinum*-based beverage using Response Surface Methodology lays the groundwork for achieving an efficient and effective production process maximizing its nutritional value and minimizing its beany flavour, ultimately contributing to the development of a high-quality and nutritious beverage. Subsequently, in Chapter 5, the nutritional assessment, quality evaluation, and sensory studies of the optimized chickpea beverage were done to comprehensively understand its overall attributes and consumer acceptance.

Chapter 5: Quality evaluation and comparative physicochemical and sensory studies of optimized chickpea beverage

Abstract

A protein quality, nutritional assessment and sensory evaluation of a chickpea beverage were done, and results were compared to other commercially popular plant-based beverages. Results showed that the chickpea beverage demonstrated promising nutritional attributes, with notable protein content. Showing the highest protein content (5.59±0.17%) among other plant-based beverages (soy, oats, rice and almond milk). The PDCAAS (protein quality evaluation parameter) score of 0.89 indicated its potential as a good source of protein. The sensory evaluation of the chickpea beverage demonstrated its overall positive acceptability, with a respectable mean score of 6.09. The chickpea beverage received positive ratings for flavor, taste, and aftertaste, indicating consumer appeal. These results indicate the chickpea beverage's potential for further improvement and market success, making it an appealing option for health-conscious consumers seeking nutritious plant-based beverage alternatives.

Keywords: Protein quality, plant-based beverage, sensory analysis, protein content

5.1 Introduction

Over the past two decades, there has been a significant increase in the consumption of non-dairy plant-based beverages, commonly known as plant-based milk or beverages derived from sources such as legumes, cereals or nuts (Sethi et al., 2016). Non-dairy plant-based beverages are not only consumed as standalone "milk" but are also widely used as ingredients in various recipes, making them more popular than ever (Jaeger & Giacalone, 2021). Moreover, conventional milk has long

been recognized as a valuable source of essential nutrients, including proteins, calcium, and vitamins (Barłowska et al., 2011). However, the rise in lactose intolerance and the growing demand for plant-based alternatives have fueled the development of non-dairy beverages made from plant sources (Kundu et al., 2018).

The exploration of novel and nutritious plant sources has become a crucial area of research to meet the diverse needs of consumers worldwide. One such promising candidate is the chickpea (*Cicer arietinum*), an ancient legume crop with a rich nutritional profile and versatile culinary applications (Wood & Grusak, 2007). Traditionally, chickpeas have been widely consumed in the form of cooked whole grains or in flour form to prepare various savory dishes such as hummus, falafel, and curries (Jukanti et al., 2012). However, recent advancements in food processing and formulation techniques have opened new avenues for incorporating chickpeas into unconventional food and beverage products (Lopes et al., 2020).

In the preparation of plant-based beverages, optimization of extraction parameters is a crucial step to maximize the yield of desired compounds and enhance the overall quality of the beverage (Maghsoudlou et al., 2016; Plengsaengsri et al., 2019). Extraction parameters, such as extraction time, temperature, and solvent-to-material ratio play a significant role in determining the efficiency and effectiveness of the extraction process (Deswal et al., 2014b). Optimization ensures that the extraction process is efficient, minimizing losses and maximizing the extraction of beneficial components, ultimately leading to a high-quality plant-based beverage that meets consumers' expectations (Mensah-Brown et al., 2014). Optimizing process parameters is necessary for ensuring product consistency in the sensory profile and nutritional composition across different batches and production runs (Tzia & Liadakis, 2003). This improves product uniformity and enables manufacturers to meet consumer expectations consistently.

The present study was divided into three parts, wherein the first part, the protein quality evaluation of the optimized beverage was done by using protein digestibility-corrected amino acid score (PDCAAS). The Protein Digestibility-Corrected Amino Acid Score (PDCAAS) is the preferred method adopted by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) to evaluate the protein quality in human nutrition (Schaafsma, 2000). This method compares the concentration of the first limiting essential amino acid present in a test protein with the concentration of that amino acid in a reference (scoring) pattern. The scoring pattern is derived from the essential amino acid requirements of a preschool-age child, representing the amino acid needs of a population group with high protein requirements (Hughes et al., 2011). The second part of the study involved conducting a comparative analysis of the optimized chickpea beverage with other well-known plant-based beverages. The goal was to assess the relative nutritional value of the chickpea beverage in comparison with a few popular alternatives. Finally, a comparative sensory analysis was conducted on the optimized chickpea beverage to gather valuable feedback from consumers. This analysis aimed to evaluate the beverage's sensory attributes, in comparison to other popular existing plant-based beverages available in the market.

5.2 Material and methods

Kabuli-type chickpeas (*C. arietinum*) with lot number 15386 and a size of 9 mm were procured from AGT Clic Foods (Laval, Quebec) and were stored at room temperature after cleaning and sorting for further use.

5.2.1 Preparation of an optimized chickpea beverage

The beverage was prepared by using the optimized process established by (Sharma & Orsat, 2022). To begin with, the chickpeas were first rinsed thoroughly followed by soaking in water for a period of 12 hours. This soaking process allowed the chickpeas to soften and become more suitable for further processing. After the soaking, the chickpeas were ground to form a slurry using the RETSCH Knife Mill Grindomix GM200, (Thermo Fisher Scientific Inc., USA). The grinding process ensured that the soaked chickpeas were transformed into a smooth, paste-like consistency. Further, the slurry was heated using a water bath at the optimal conditions summarized in Figure 5-1. To complete the preparation of the beverage, the slurry obtained was subjected to a filtration process by passing it through a double-layer cheesecloth. The resulting liquid, known as the filtrate, was collected and further homogenized using a homogenizer (Fisherbrand 850) at 5000 rpm for 2 minutes to enhance the stability, consistency and uniform distribution of the components within the beverage. After homogenizing, the beverage was cooled and stored at 4°C till subsequent analysis and evaluation.

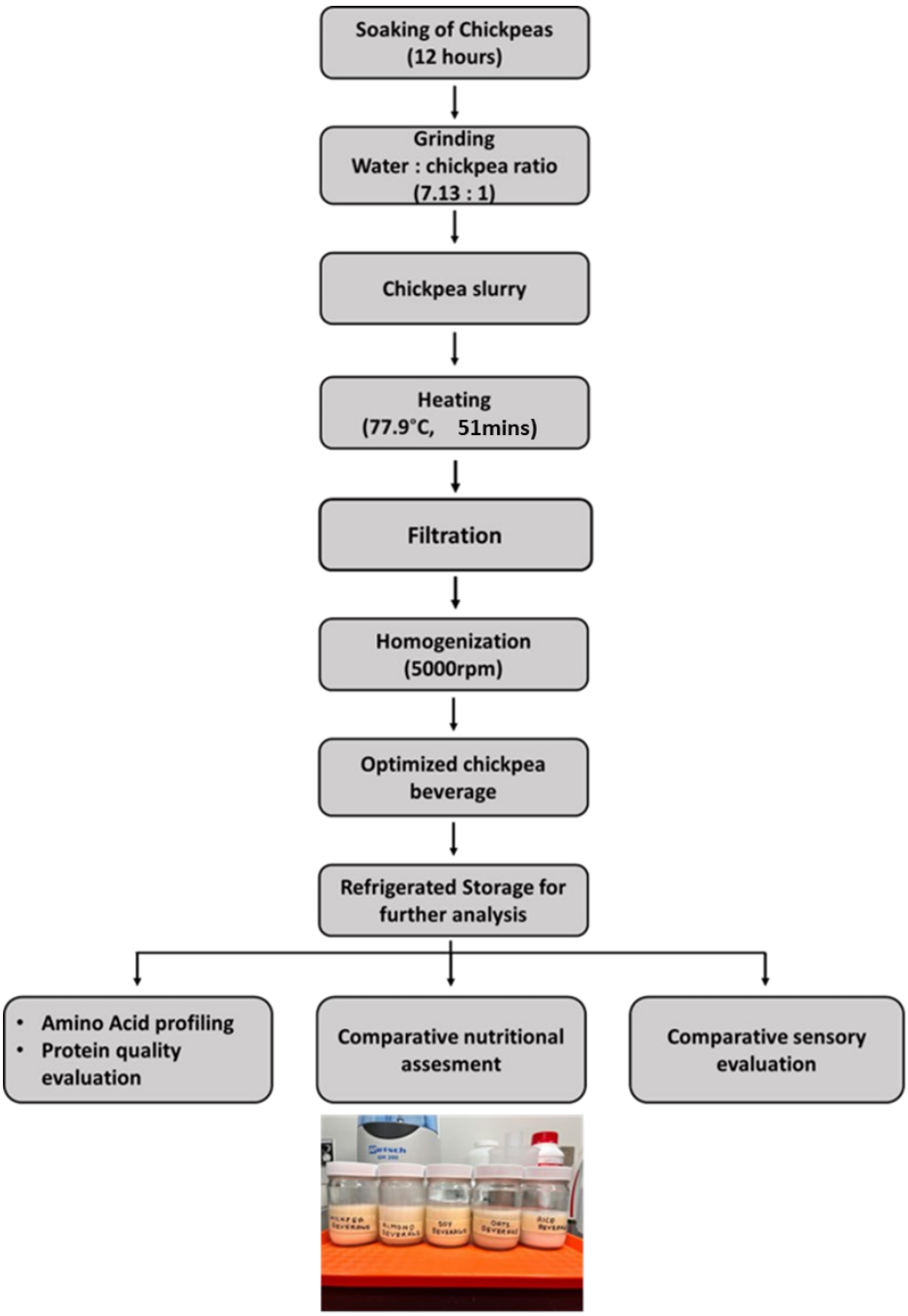

Figure 5-1: Schematic representation of optimized chickpea beverage preparation and study

5.2.2 Evaluation of optimized chickpea beverage

A) Protein quality evaluation: amino acid composition, amino acid score and calculation of Protein Digestibility-Corrected Amino Acid Score (PDCAAS)

The amino acid composition of the optimized beverage was evaluated using a Gas Chromatography system (HP-5, Agilent technologies. CA, USA) having a capillary column with 30mX 0.25mmX 0.25 µm dimensions as suggested by (Abadie et al., 2022; Savych et al., 2022) with suitable modifications. Freeze-dried beverage samples (0.1 g) were added to 10 mL of methanol in a flask and subjected to extraction in an ultrasonic bath at room temperature for a duration of 15 minutes. After extraction, the solution was centrifuged for 10 minutes at 5000 rpm and 25 °C to separate any solid residues. Next, 500 µL of the supernatant was collected and subjected to evaporation using nitrogen gas, resulting in the formation of a dry residue. This residue was dissolved in a solution comprising 100 µL of acetonitrile and 100 µL of MTBSTFA (N-methyl-N-tert-butyldimethylsilyl-trifluoroacetamide). The resulting solution was then heated at 100 °C for 2.5 hours in a glycerol bath to enable the derivatization of the analytes, making them suitable for analysis in the gas chromatograph. Finally, 1 µL of the prepared test solution was injected into the gas chromatograph, allowing for the identification and quantification of the compounds present in the sample. A volume of 100 µL of a standard mixture containing L-amino acids (Millipore Sigma, L-amino acid mix) was carefully measured and then subjected to drying under a gentle stream of nitrogen gas until a dry residue was obtained and the process of sample preparation mentioned above was repeated. The derivatized samples were then put to subsequent testing and characterization, providing valuable information on the amino acid composition.

The assessment of protein quality relies on two key factors: the amino acid composition and the inherent digestibility of the protein source (Boye et al., 2012). In this study, an alternative approach (in place of the traditional in vivo digestibility method using live organisms), the in vitro PDCASS was adopted. This in vitro method has been previously validated and shown to exhibit a strong correlation with in vivo data (Nosworthy & House, 2017). The in vitro PDCASS score was

calculated utilizing the method previously reported by (De Bhowmick & Hayes, 2022). Firstly, IVPD (In vitro protein digestibility) was calculated using the method suggested by (Chen et al., 2015) using a three-stage digestion method with the help of two enzymes: pepsin and pancreatin. Initially, 0.5 g of freeze-dried chickpea beverage samples were mixed with 20 mL of double-distilled water and incubated at room temperature for 30 minutes. After centrifugation at 5000×g for 10 minutes, the supernatant was collected as the initial protein extract solution. For the second stage of digestion, the pH of the first protein extract was adjusted to 1.5 using 1 M HCl to mimic the acidic conditions of the stomach. Then, a pepsin solution was added, and the mixture was incubated at 37 °C for 30 minutes. The digestion process was stopped by adding 1 M NaOH solution. Moving on to the third digestion stage, the pH of the mixture was adjusted to 7.8 using 1.0 M NaOH. Next, pancreatin solution was added, and the mixture was incubated at 40 °C for one hour. Finally, a 150 mM Na_2CO_3 solution was added to halt the overall digestion process. The total protein content during these three stages of digestion was measured using a BCA kit (Determination of total protein: complete process described in chapter 4). The in-vitro protein digestion percentage was then calculated using Equation [5-1], which compares the initial protein content (P0) with the final undigested protein content (P1).

$$\text{IVPD } (\%) = \frac{P_0 - P_1}{P_0}\ x\ 100 \qquad [5\text{-}1]$$

Further, the amino acid ratio was calculated using Equation [5-2] shown below:

Amino acid ratio (AA ratio) = (mg of limiting amino acid /g protein in sample)/(mg of same limiting amino acid/g protein in reference sample) [5-2]

Finally, in vitro, PDCASS was calculated using Equation [5-3] shown below:

$$PDCAAS = IVPD * AA\ Ratio \quad [5\text{-}3]$$

Where PDCASS= Protein digestibility corrected amino acid score

IVPD= In vitro protein digestibility

AA ratio= Amino acid ratio

B) Viscosity and particle morphology and dimension

The viscosity of chickpea beverage samples was measured using a cone/plate rheometer (Anton Paar rheometer) equipped with cone # CP-40. The measurements were conducted at a rotational speed of 12 rpm and room temperature (22°C). The gap between the cone and the plate was set to 12.7 mm. Before the measurements, the chickpea beverage samples were allowed to reach equilibrium with the room temperature. For comparison, the viscosity of the bovine milk (as it provides a familiar, consistent, and widely accepted benchmark for both producers and consumers) was also calculated as a reference sample. The morphology of suspended particles of chickpea beverage was investigated using an optical microscope (model TS100, Nikon Co., Tokyo, Japan). To prepare the samples for observation under the optical microscope, the freeze-dried beverage sample was appropriately diluted to a concentration of 1% (w/w). This dilution ensured that the particles were well-distributed and allowed for better visualization and analysis.

5.2.3 Comparison of the nutritional composition of optimized chickpea beverage with other plant-based beverages

The nutritional composition of the optimized chickpea beverage was compared to that of other popular plant-based beverages (soymilk, almond milk, oats milk and rice milk) to assess its relative nutritional value. This analysis aimed to provide insights into the unique attributes and potential

benefits of the chickpea beverage as a plant-based alternative. To conduct a fair comparison, other plant-based beverages were prepared using the same optimized parameters that were utilized for preparing the optimized chickpea beverage (Sharma & Orsat, 2022). This approach ensures consistency in the preparation methods and allows for a meaningful and accurate comparison of the nutritional composition. To determine the protein content, the Bicinchoninic acid (BCA) method was used as described by (Casal et al., 2000) with some modifications. The BCA assay kit used for this purpose was obtained from Pierce Biotechnology. Protein quantification was performed following the instructions provided in the user guide. To construct the standard graph, Bovine serum albumin (BSA) was used as the protein standard. For total carbohydrate content. Ash determination was conducted following the procedure outlined by (Czaja et al., 2020). To start with, 5 mL of the beverage sample was carefully measured and placed into ash dishes. Subsequently, the ash dishes were positioned inside a muffle furnace set at a temperature of 550 °C. The samples underwent incineration within the furnace until they transformed into a state of light gray ash and achieved a constant weight. The incineration process typically lasted for approximately 7 hours to ensure complete combustion and the removal of all organic matter, leaving behind only the inorganic mineral components known as ash. The total sugar content, which includes both free and bound sugars, was determined using the anthrone method as reported by (Widyastuti & Giarni, 2015) with some modifications (detailed methodology can be found in Chapter 4). To establish a standard calibration graph, glucose was utilized as the reference standard. To determine the fat content of the flour samples, the method described by Folch et al. (1957), with slight modifications, was employed. Initially, 0.2 g of freeze-dried samples were mixed with a 10 mL solution comprising chloroform and methanol in a 2:1 (volume/volume) ratio. The mixture was agitated for 20 minutes in a shaker. Subsequently, the solution was subjected to

vacuum filtration using a 125mm filter to separate solid particles from the liquid. To the filtered solution, 2 mL of a 0.3% sodium chloride (NaCl) aqueous solution was added, and the tube was vortexed for 1 minute to ensure thorough mixing. Next, the tube was centrifuged at 2000 rpm for 5 minutes, which led to the formation of two distinct layers: the upper layer containing methanol and NaCl, and the lower layer containing chloroform and lipids. After carefully removing the upper layer, the lower layer containing lipids was transferred to a pre-weighed aluminum dish.

To eliminate any remaining solvent, the aluminum dish was placed on a heating tray inside a fume hood, causing the chloroform to evaporate, leaving behind the lipid residue. The dish was then moved to an oven set at 100°C and left for 15 minutes to ensure complete removal of any traces of solvent. After cooling down, the dish was weighed again, considering the weight of the empty dish. The difference in weight between the empty dish and the dish with the lipid residue represented the amount of fat content present in the samples. Samples were analyzed in triplicates and reported as mean ± standard deviation.

5.2.4 Sensory evaluation

To assess the sensory characteristics of chickpea beverage and compare them with other popular beverages that are in high demand in the market, a sensory evaluation was conducted. The aim was to gather information about how chickpea beverage stacks up against beverages made from oats, soy, rice, and almonds. By including popular and highly demanded beverages made from oats, soy, rice, and almonds as reference points, a comprehensive comparison of the sensory attributes of chickpea beverage can be assessed. This information is valuable for market research and product development purposes, as it helps in understanding consumer preferences and identifying the unique selling points of chickpea beverage in relation to its competitors, allowing

a better understanding of its potential market appeal and positioning in the beverage industry. A sensory evaluation was conducted at the Macdonald campus of McGill University (Montreal, Canada) to compare the sensory properties of chickpea beverages with other plant-based beverages. The evaluation involved the participation of students and staff members as evaluators. Before the evaluation, the samples of chickpea beverages and other plant-based beverages were prepared a day in advance and stored at a temperature of 4°C. To enhance the palatability of the samples, a 3% sugar solution was added to the beverage samples. During the evaluation, the samples were served in plastic cups and each sample was assigned a unique three-digit code to ensure blind testing. To prevent any carryover effects, water was provided to the panel members to rinse their palate between samples.

A comprehensive test form was provided to the panel members, consisting of six sensory attributes: appearance, mouthfeel, flavor, taste, aftertaste, and overall acceptability. The panel members were asked to rate the acceptability of each attribute on a 9-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely) (Wichchukit & O'Mahony, 2015). The use of a hedonic scale allowed for capturing the evaluators' preferences on a continuum, classifying their responses into like and dislike categories (Fletcher et al., 1997; Heymann & Lawless, 2013). By employing this evaluation approach, the sensory properties of the chickpea beverages in comparison to other plant-based beverages were systematically assessed and quantified based on the panel members' hedonic ratings. This methodology provides valuable insights into the acceptability and sensory characteristics of the beverages, enabling a better understanding of consumer preferences and potential areas for improvement in formulation or production processes.

5.2.5 Statistical analysis

The results were compared by one-way analysis of variance (ANOVA). The Bonferroni test was used to identify differences between means using Origin Pro software.

5.3 Results and Discussion

5.3.1. Protein quality evaluation: amino acid composition, amino acid score and calculation of Protein Digestibility-Corrected Amino Acid Score (PDCAAS)

Table 5-1 presents the amino acid profile of optimized chickpea beverage with methionine and cysteine being present in the lowest concentration and referred to as the limiting amino acids present in chickpea beverage. Methionine and cysteine are sulphur-containing amino acids (essential and conditionally essential, respectively and highlighted in bold in Table 5-1) present in limiting amounts in legumes, especially chickpeas (Iqbal et al., 2006). The limiting amino acid is crucial for determining the overall quality of a protein source and ensuring that the body receives a balanced and complete set of essential amino acids (Yoshida et al., 1966).

PDCAAS is a scale that ranges from 0 to 1 where a value of 1 indicates a protein source that supplies all essential amino acids in the precise amounts required by the body (Han et al., 2015; Klamczynska & Mooney, 2017). Such proteins are considered of excellent quality and can fully support growth, tissue repair, and overall health. On the other hand, a PDCAAS score below 1 suggests a protein source that lacks one or more essential amino acids, making it incomplete in meeting the body's requirements (Bhagya et al., 2007). In the context of PDCAAS, if a protein source is completely lacking one or more essential amino acids, its score will indeed be 0 (Schaafsma, 2012). This means that the protein is unable to fulfill the body's minimum requirements for essential amino acids, resulting in a protein quality rating of "poor" or "inadequate." In vitro protein digestibility (IVPD) was found to be 82.15±0.57%. Further, the amino acid ratio was calculated by taking reference from the WHO standard recommendations for

amino acid daily intake (Table 5-2). As shown in Table 5-2 the value of PDCASS score for optimized chickpea beverage was calculated to be 0.89. A PDCAAS score of 0.89 means that the protein source can provide about 89% of the essential amino acids required by the reference population. As the PDCASS scale ranges from 0 to 1, a score of 0.89 (close to 1) can be considered a good source of protein. PDCAAS score of protein is valued for its simplicity and ability to directly correlate with human protein needs. However, the reference pattern used in this scoring system is based on the minimum amino acid requirements necessary for tissue growth and maintenance in healthy individuals (Schaafsma, 2012). This minimal approach may not represent the ideal or optimal intake of amino acids for everyone. Individual protein needs can vary based on factors like age, gender, physical activity level, and overall health status. The PDCAAS may not account for these variations adequately, potentially leading to discrepancies in evaluating protein quality for certain populations.

Table 5-1: Amino acid profile and PDCASS score of optimized chickpea beverage

Amino Acid	**Quantity: g amino acid/100 g of beverage sample**	**Quantity mg/g of total protein present in the beverage**	**Amino acid ratio for limiting amino acids = (mg of limiting amino acid /g protein in sample)/(mg of same limiting amino acid/g protein in reference sample).**	**Invitro PDCASS = PDCAAS = IVPD ∗ AA ratio**
Arginine	0.4	71.55		
Histidine	0.32	57.24		
Isoleucine	0.21	37.56		
Leucine	0.35	62.61		
Lysine	0.19	33.98		
Methionine	**0.08**	**14.31**		
Phenylalanine	0.53	94.81		
Theronine	0.3	53.66	**= (14.31+10.73)/23 =1.08**	**=0.82*1.08 =0.89**
Tryptophan	0.09	16.1		

Valine	0.44	78.71
Alanine	0.56	100.17
Aspartic acid	1.15	205.72
Cystine	**0.06**	**10.73**
Glutamic acid	1.1	196.77
Glycine	0.36	64.4
Proline	0.41	73.34
Serine	0.21	37.56
Tyrosine	0.14	25.04

Table 5-2: FAO/WHO/UNU amino acid scoring pattern mg/g requirement (FAO 2007), Age (yr) 3-10

Amino acid	**Requirement**
Histidine	16
Isoleucine	30
Leucine	61
Lysine	48
Methionine + Cysteine	23
Aromatic amino acids	41
Threonine	25
Tryptophan	6.6
Valine	402

**Source: From FAO/WHO/UNU Expert Consultation 2007

5.3.2. Viscosity and particle morphology and dimension

The viscosity of the optimized beverage sample was found to be 27.36±4.8 mPa.s (millipascals. sec) at a shear rate of 10 (1/sec) which was significantly higher ($p < 0.05$) than the viscosity of the

bovine milk 7.76±2.7 mPa.s. The beverage consists of a complex mixture of various components, including protein aggregates, oil droplets, and other insoluble solids. These elements form the distinct particle composition that contributes to the overall properties of the beverage. The morphology and particle distribution of optimized chickpea beverage is shown in Figure 5-2 (A, B). The optimized chickpea beverage showcases a well-controlled unimodal distribution curve, featuring particle sizes typically lying below 100 μm^2. The precise particle size distribution enhances the overall stability and mouthfeel of the beverage, making it more comparable to traditional dairy milk in terms of appearance and texture (Guinard & Mazzucchelli, 1996; Homer et al., 2021).

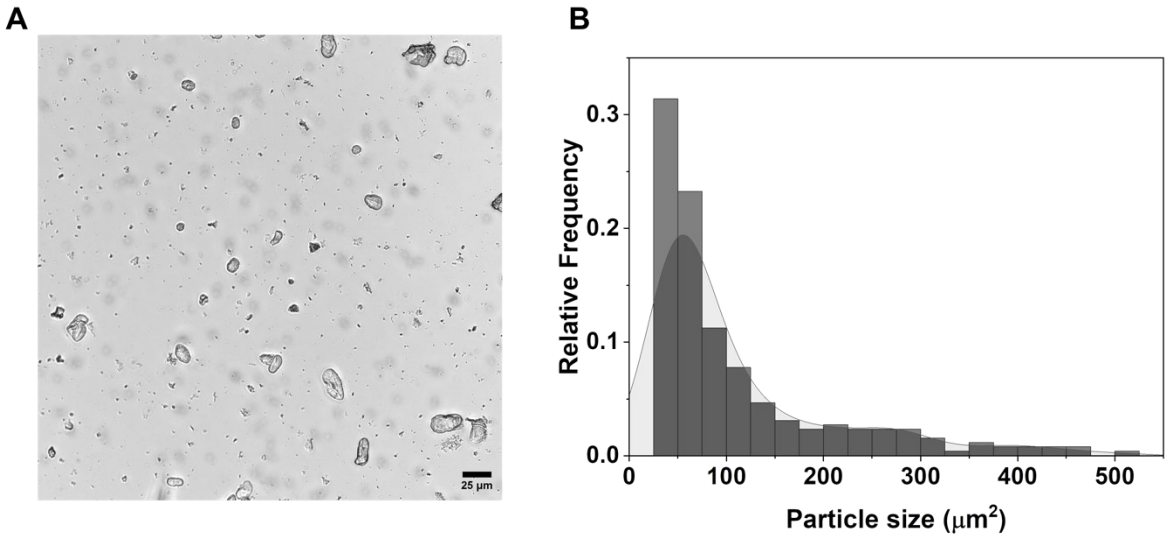

Figure 5-2: Particle study of optimized chickpea beverage (A) Particle morphology (B) Particle distribution

5.3.3 Comparison of the nutritional composition of optimized chickpea beverage with other plant-based beverages

The nutritional composition of various plant-based beverages prepared as the optimized chickpea beverage for comparison basis is shown in Table 5-3. As evidenced by Table 5-3, the protein content in different beverages varied significantly ($p<0.05$) among different beverages. The maximum protein content was reported in optimized chickpea beverage (4.86±0.60 %), followed by soy while the least amount of protein was present in rice beverage (0.15±0.02). The ability of a protein to dissolve and remain stably dispersed in the solvent is largely facilitated by favorable protein-protein and protein-solvent interactions, which actively promote solubility (Mcclements, 2007). Although various studies have reported that the protein content of soybeans is higher than chickpeas, the higher protein content of the chickpea beverage may be attributed to the higher solubility of chickpea protein in water i.e. a comparative study of chickpea and soy protein revealed solubility of chickpea protein to be 94% as compared to soy being only 50% (Johnston et al., 2015; Wang et al., 2018). In terms of carbohydrates also, there was a significant difference found among different beverages ($p <0.05$), with rice beverage having maximum carbohydrate (11.04±0.51%). There was no significant difference found in the ash content of the beverages, but lipid content was found maximum in almond beverage and differed significantly among different beverages. The findings of this study highlight the potential of chickpea-based beverages as a nutritious and protein-rich alternative to other plant-based beverages.

Table 5-3: Nutritional composition of plant-based beverages as compared to chickpea beverage

Parameters	**Chickpea beverage**	**Soy beverage**	**Almond beverage**	**Rice beverage**	**Oats beverage**
Protein (%)	4.86 ± 0.60^{a}	3.78 ± 0.55^{b}	1.33 ± 0.12^{d}	0.15 ± 0.02^{e}	2.24 ± 0.08^{c}

Carbohydrates (%)	5.07±0.19[d]	6.59±0.23[c]	2.15±0.21[e]	11.04±0.51[a]	8.85±0.55[b]
Ash (%)	0.34±0.09[a]	0.57±0.10[a]	0.51±0.13[a]	0.49±0.05[a]	0.63±0.20[a]
Total fats (%)	1.12±0.21[c]	2.42±0.25[b]	5.36±0.18[a]	0.41±0.31[d]	1.44±0.31[c]

5.3.4. Comparative sensory evaluation

Results of the sensory evaluation are presented in the form of a web chart are presented in Figure 5-3. The sensory evaluation revealed that the almond beverage received the highest overall acceptability rating among the beverages tested (mean score=6.18). Following closely behind was the chickpea beverage, which obtained a respectable level of acceptability (mean score=6.09). On the other hand, the soy beverage received the lowest level of overall acceptability among the evaluated beverages (mean score=4.09). Interestingly, when it came to appearance, the rice beverage received the highest score (mean score=6.9) among all the beverages evaluated, while the chickpea beverage obtained the lowest score (mean score=4.5) in this attribute. These results aligned with the sensory evaluation of the chickpea beverage in comparison to the soy beverage, as evaluated by (Wang et al., 2018). In terms of mouthfeel, the chickpea beverage received the highest score (mean score=6.54) among all the beverages evaluated, indicating that it excelled in this attribute. On the other hand, the oat beverage obtained the lowest score (mean score=5.27). These results align with the understanding that mouthfeel is a significant and appealing attribute to consumers (Isaskar et al., 2021). While appearance and taste are typically the initial factors that attract consumers, mouthfeel plays a crucial role in enhancing their overall sensory experience

(Coggins & Chamul, 2004). It encompasses the texture, thickness, smoothness, and other tactile sensations experienced while consuming a beverage. A pleasant and satisfying mouthfeel can greatly contribute to the overall enjoyment and perceived quality of a beverage (Civille, 1991). The fact that the chickpea beverage performed exceptionally well in terms of mouthfeel indicates that it provided a desirable texture and mouth-coating sensation to the evaluators. This may be attributed to the inherent properties of chickpeas or the formulation of the beverage itself. The sensory evaluation results revealed that the flavor and taste of the chickpea beverage received positive ratings, with the almond beverage scoring slightly higher, albeit by a small margin (mean score= 6.18, 5.63 respectively). Moreover, as reported in Table 5-3 fat content of chickpea beverage (1.12±0.21%) was far less than almond milk (5.36±0.18%), suggesting a clear preference for chickpea beverage over almond milk for individuals seeking a lower-fat alternative. This suggests that consumers generally appreciated the flavor and taste of the chickpea beverage, indicating its potential for further improvement and market success. Furthermore, the aftertaste of the chickpea beverage was also rated positively, although the almond beverage outperformed it in this attribute. This suggests that the chickpea beverage leaves a pleasant and satisfying lingering taste for consumers, contributing to their overall sensory experience.

Considering the positive feedback received for different sensory attributes, the chickpea beverage demonstrates consumer likeness and potential for further improvement. By focusing on enhancing these sensory attributes, such as exploring flavor variations or refining the taste profile, the chickpea beverage has the opportunity to strengthen its market appeal and attract a wider consumer base.

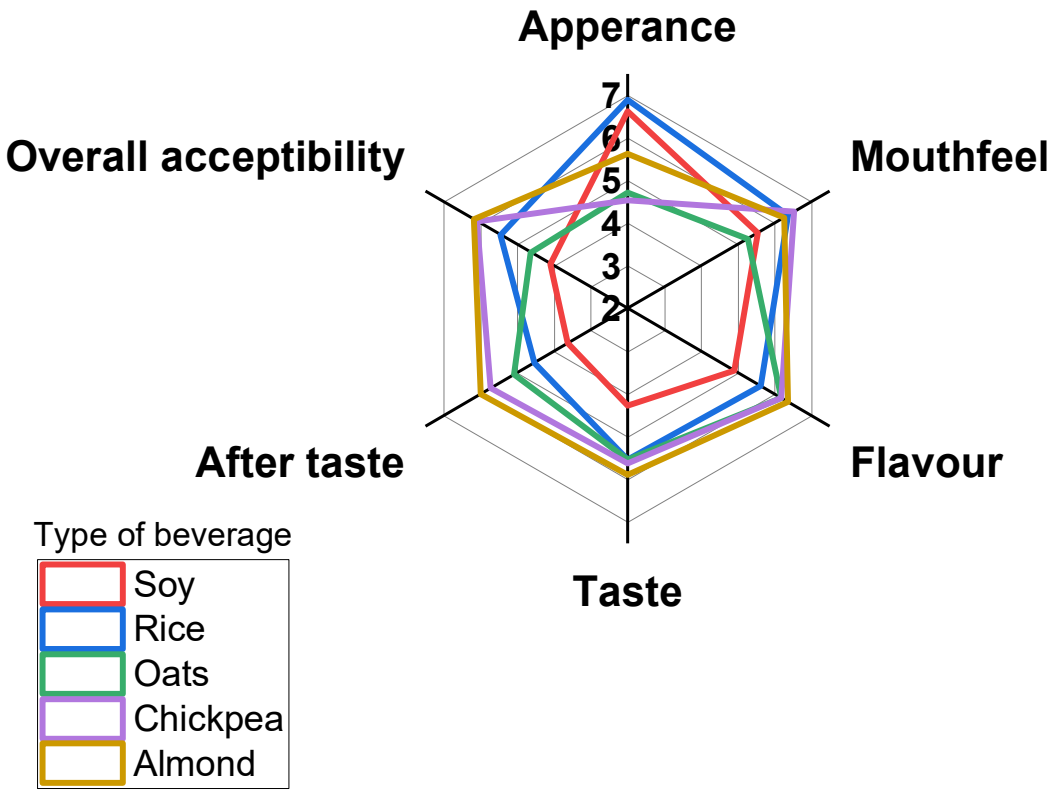

Figure 5-3: Sensory profile analysis of different plant-based beverages

5.4 Conclusion

In conclusion, the study highlights the potential of chickpea-based beverages as a nutritious and protein-rich alternative to other plant-based beverages. The optimized chickpea beverage showed promising nutritional and sensory attributes, making it a competitive option in the plant-based beverage market. By capitalizing on its high protein content and refining its sensory attributes further, such as exploring flavor variations, the chickpea beverage has the opportunity to strengthen its market appeal and attract a wider consumer base. Future research and development efforts can focus on tailoring the beverage to suit specific consumer preferences and dietary needs, further solidifying its position as a viable and appealing plant-based beverage choice.

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Preface to Chapter 6

After conducting a comprehensive quality evaluation of the optimized chickpea beverage and gaining valuable insights into its sensory profile and consumer acceptance, the investigation moved towards exploring the potential of alternative extraction techniques - ultrasound and microwave in chapter 6. These novel techniques were studied to understand their impact on the beverage's protein content and other crucial quality parameters. By investigating these innovative methods, the research aims to unlock potential benefits, such as increased protein yield and improved overall beverage quality. These findings have the potential to contribute to the development of more nutritious, functional, and appealing chickpea beverage, catering to the rising consumer demand for healthier and sustainable options.

Chapter 6: Effect of Microwave and Ultrasound extraction on protein and quality parameters of chickpea beverage

Abstract

Microwave and ultrasound were investigated for their ability to enhance protein content in chickpea beverage extraction, and the obtained results were compared to conventional beverage processing. Both microwave and ultrasound significantly increased ($p < 0.05$) the protein content of the chickpea beverage. Microwave enhanced protein content by 47.78%, while in the case of ultrasound, an increase of 21.22% was observed compared to conventional processing (traditional heating using water bath). Other beverage characteristics, like protein solubility, In vitro protein digestibility (IVPD) and Trypsin inhibitor (TI) activity, were also studied and compared at optimized conditions and a significant improvement ($p < 0.05$) was observed. The present study optimized microwave parameters (60- 100 °C, 2-10 minutes) and ultrasound parameters (duty cycle 50-100%, 5-15 minutes) using Response Surface Methodology (RSM). For microwave processing, optimal conditions for maximizing protein extraction were 65.8 °C and 6.7 minutes, and when ultrasound processing was used, protein content was maximized at 50% duty cycle and 13.5 minutes.

Keywords: Plant-based beverage, microwave, ultrasound, protein, protein solubility, IVPD (In vitro protein digestibility), TI (Trypsin inhibitor) activity

6.1 Introduction

Plant-based beverages (PBBs) are aqueous extracts of plant materials made by grinding them with water. These beverages are taken by individuals with dietary limitations due to allergies, lactose intolerance, or a special diet, and a growing number of vegetarians, vegans, and flexitarians are also opting for these. Plant-based beverages are winning the world today with their impressive nutritional profile and functional benefits and are presenting strong competition to the conventional dairy industry. With these beverages' increasing popularity and demand, new technologies are being tested and explored to enhance nutritional content, palatability, and overall acceptability. In addition, the food sector also aspires to create novel processing technologies to decrease energy consumption and optimize the value of raw materials to achieve economic and environmental sustainability.

One essential step in preparing plant-based beverages is heating, which enhances the extraction process and eliminates undesirable flavour, fragrance, and antinutrients while ensuring microbiological safety. However, the conventional heating process has disadvantages associated with the loss of certain (essential) nutrients due to degradation and chemical reactions, the formation of undesired compounds (acrylamide, esters, heterocyclic amines etc.) and the formation of sulphur compounds causing a loss in flavour perception and taste (Van Boekel et al., 2010). Legume-based beverages such as soymilk, chickpea, peanut, and lupin beverages are rich in protein and denatured protein from the heat processing used in preparing them (Nawaz et al., 2020). Due to heat damage to the natural protein structure, insoluble denatured protein fractions are generated. Removing these fractions during filtering reduces the protein level of filtered beverages (Varghese & Pare, 2019). To address the above-mentioned caveats, innovative heat treatments like ultra-high-temperature processing, high-temperature short-time processing, ohmic

heating, and microwave heating have been explored and employed for improving the nutritional quality of plant-based beverages (Kwok et al., 2002; Varghese & Pare, 2019; Zhang et al., 2003). Ultrasound (non-thermal) and microwave (thermal) are two of the most critically emerging technologies in the food industry owing to their economic and processing advantages and energy efficiency. Power ultrasound waves, characterized by high intensity (10 to 1000 W/cm^2) and low frequency (20 to 100 kHz), induce substantial physical and chemical effects in food processing during extraction procedures and have been successfully utilized in effective protein extraction from rice bran, soymilk, edible insect and other such food systems (Ly et al., 2018; Mishyna et al., 2019; Varghese & Pare, 2019). The primary phenomenon of ultrasonication that determines the effectiveness of protein extraction is the cavitation effects of ultrasound, which might lead to the bursting of tiny bubbles due to pressure fluctuations. This process results in tissue disintegration and particle size reduction, enhancing mass transfer (Petcharat et al., 2021). Similarly, the microwave has been studied widely to extract essential plant components. Microwave-assisted extraction (MAE) induces cell wall breakdown by forced superheating of entrapped water molecules and constant collisions inside the matrix. This cellular rupture induces abrupt exudation of components inside cells into the surrounding solvent (Barać & Stanojević, 2005; Kute et al., 2015).

Our previous study has optimized the conventional extraction process for chickpea beverages to maximize their nutritional composition and quality (Sharma & Orsat, 2022). In this study, microwave and ultrasound processes were tested and optimized to extract an aqueous beverage from a chickpea matrix by subjecting the chickpea slurry to various input factors. Further, the optimized beverage obtained by these two processes has been compared to the conventional optimized beverage process in terms of protein, in vitro protein digestibility, protein solubility and

trypsin inhibitor activity. To the best of our knowledge, microwave and ultrasound optimization for chickpea beverage preparation has never been studied.

6.2 Material and Methods

6.2.1 Sample preparation and experimental scheme

Kabuli-type chickpeas (*C. arietinum*) with lot #15386, were acquired from AGT Clic Foods (Laval, Quebec). These were sorted, cleaned and stored in a clean and dry place for further use. For the beverage preparation, chickpeas were soaked for 12 hours after being thoroughly washed with water. The soaked chickpeas were crushed in a RETSCH Knife Mill Grindomix GM200 from Thermo Fisher Scientific Inc., USA, with a 7.13:1 water-to-chickpea ratio optimized by (Sharma & Orsat, 2022). According to the experimental scheme, the chickpea-water slurry was processed using Microwave and Ultrasound at different input conditions, as shown in Figure 6-1 and described in subsequent sections. Finally, the slurry was filtered through two layers of cheesecloth, and the recovered filtrate was homogenized for stability using a homogenizer (Fisherbrand 850 homogenizer). The resulting filtrate was refrigerated and kept at 4 degrees Celsius for subsequent examination.

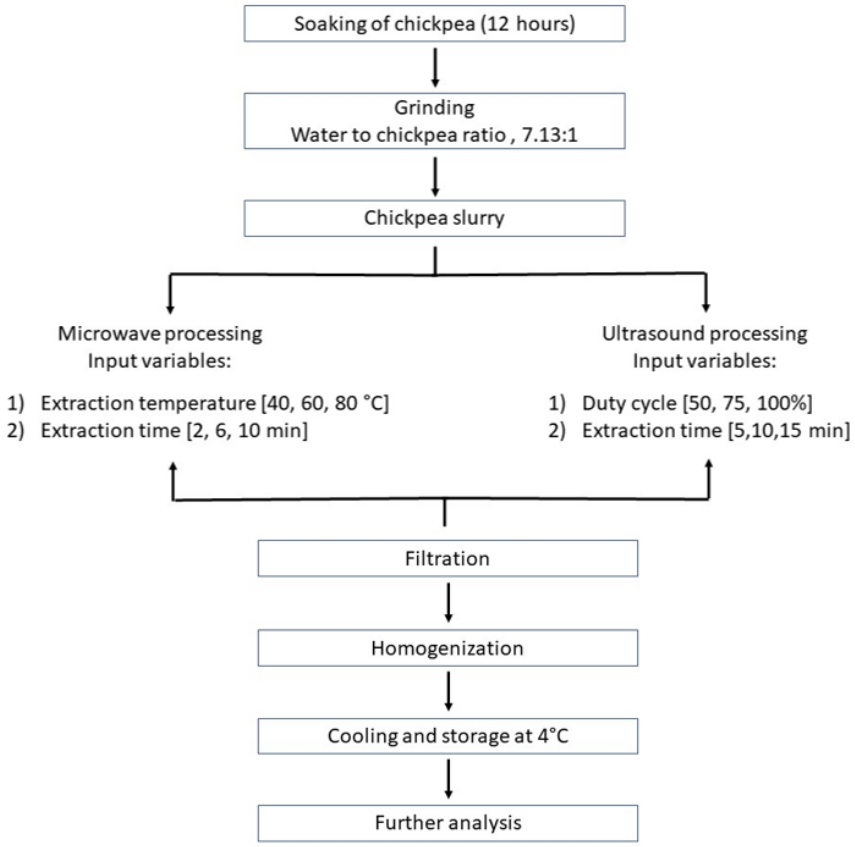

Figure 6-1: Process scheme for preparation and processing of chickpea beverage

6.2.2 Microwave-assisted extraction (MAE)

The Mini Wave digestion system (SCP Science, QC Canada) was used for microwave processing at a frequency of 2450 MHz at 1000 watts. The loading chamber was loaded with 50 mL of chickpea slurry in six identical quartz tubes. Inside the chamber, infrared sensors monitored the temperature of the samples in the tubes. The processing temperature was set to 60, 80 and 100 °C and samples were treated for 2, 6 and 10 minutes; each treatment slurry was filtered through double-layered cheesecloth and stored at 4 °C for further analysis. All treatments and measurements were carried out in three replicates.

6.2.3 Ultrasound-assisted extraction (UAE)

For UAE, a probe sonicator (Branson Sonifier 450, Branson Ultrasonics Corporation, Danbury, CT) was used for processing the samples. The factors studied were duty cycle (on time/off time) and processing time. The duty cycle was set at three levels (50%, 75% and 100%) while ultrasound application time was kept at 5, 10 and 15 minutes, as shown in the process scheme in Figure 6-1. For each run, 100 mL of sample was weighed and taken in a glass beaker and sonicated with the help of a probe for different combinations of input factors as mentioned above. An ice bath was used throughout the ultrasonic processing to keep the samples cool. Following treatment, the samples were filtered through a double-layered cheesecloth. The samples were then stored at 4 °C for analysis. All treatments and measurements were carried out in three replicates.

6.2.4 Control samples (Optimized conventional extraction)

In this study, three factors were considered, namely water to chickpea ratio, extraction time and temperature (using conventional heat), and were optimized using response surface methodology. For comparison purposes, conventional heat treatment optimized by (Sharma & Orsat, 2022) for preparing chickpea-based milk beverages was used. The optimum extraction time and temperature obtained were 51 minutes and 77.9 °C, respectively, for maximizing the nutritional content of the beverage. These optimized parameters were used to prepare a conventional chickpea-based beverage to compare with MW-assisted and US-assisted beverage extraction. Samples were prepared fresh and stored under refrigeration until further analysis.

6.2.5 Determination of the total protein

The Bicinchoninic acid (BCA) assay kit from Pierce Biotechnology was used to determine the protein content (Casal et al., 2000), and the method outlined in the user manual was followed. Reagent A (a solution of sodium bicinchoninate, sodium bicarbonate, sodium tartrate, and sodium hydroxide in distilled water) and Reagent B are included in the BCA kit (a solution of cupric sulphate pentahydrate in distilled water). Fifty parts of BCA Reagent A were combined with 1 part of BCA Reagent B to create the working reagent. 0.1 mL of the sample and 2.0 mL of the working reagent were combined to create the reaction mixture. After incubating the reaction mixture at 37°C for 30 minutes, the absorbance at 562 nm was measured using an ultraviolet spectrophotometer. Bovine serum albumin (BSA) was used to produce the standard graph.

6.2.6 Protein Solubility

The protein solubility of the prepared chickpea beverage was tested according to the technique reported by (Morr et al., 1985) with modifications. The beverage samples (25mL) were maintained at a pH of 4.5 by adding 0.1N HCl. The samples were centrifuged for 10 minutes at 12,000 RPM, and the supernatant was collected after filtration. The protein contents of both the supernatant and the beverage were measured. Protein solubility was determined using Equation [6-1].

$$PS\ (\%) = \frac{P_s}{P_{beverage}}\ x\ 100 \qquad [6\text{-}1]$$

Where P_s is the crude protein content of the supernatant and $P_{beverage}$ is the protein content of the beverage.

6.2.7 IVPD

In-vitro protein digestibility was determined by applying two enzymes (pepsin and pancreatin) in a three-stage digestion method as described by (Chen et al., 2015; J. Wang et al., 2020) with some

modifications. 0.5 g of freeze-dried chickpea beverage samples were mixed with 20 mL of double-distilled water and incubated at room temperature for 30 min, and then the mixture was centrifuged at 5000×g for 10 min. The supernatant obtained was considered as an initial protein extract solution. For the second stage of digestion, the pH of the first protein extract (10 mL) was adjusted to 1.5 using 1 M HCl to simulate in-vivo gastric conditions. The mixture was incubated at 37 °C for 30 minutes after adding 100 µL of a pepsin solution (10 mg pepsin/mL in 0.01 M HCl). Following incubation, 100 µL of 1 M NaOH solution was added to the samples to terminate the second-stage digestion. During the third digestion stage, the mixture's pH was adjusted to 7.8 with 1.0 M NaOH, and 300 µL of pancreatin solution (10 mg/mL in sodium phosphate buffer, pH 7.0) was added. After incubating the mixture at 40 °C for one hour, 100 µL of a 150 mM Na_2CO_3 solution was added to halt the overall digesting process. Using a BCA kit, the total amount of protein in the samples during these three stages of digestion was measured (Determination of total protein, section 3.5).

The in-vitro protein digestion percentage was calculated using the following Equation [6-2].

$$IVPD\ (\%) = \frac{P_0 - P_1}{P_0}\ x\ 100 \qquad [6\text{-}2]$$

Where P_0 is the initial protein content, and P_1 is the final undigested protein content.

6.2.8 Trypsin Inhibitor Assay

Trypsin inhibitors are one of the prominent antinutrients found in legumes and are responsible for impeding the functioning of trypsin and chymotrypsin by creating stable complexes, hence reducing protein digestibility (Vanga et al., 2020). Trypsin inhibitor activity was calculated based

on the method used by (Jiang et al., 2013) with slight modifications. Freeze-dried beverage (0.5 g) was extracted with 50 mL of 0.01 N NaOH at room temperature for 3 hours while continuously shaking and left to stand for another 2 hours at 4 °C. The supernatant was collected and diluted such that 2 mL of the extract could inhibit trypsin by 40% to 60% (trypsin, type 1 from bovine pancreas, Sigma Chemical Company, USA). Standard and standard blanks were prepared by adding 2mL of distilled water to two test tubes, while sample and sample blanks were prepared by adding 2mL of sample extract to the test tubes, respectively. Then, 2 mL of trypsin standard solution (20 mg in 0.001 M HCl) was added to the sample and standard tubes, and the tubes were vortexed and incubated at 37 °C for 10 minutes. After preheating the tubes at 37 °C for 10 minutes, 5 mL of pre-warmed benzyl-DL-arginine-para-nitroanilide (BAPNA) was added to each vortexed tube. After incubating this mixture at 37 °C for 10 minutes, 1 mL of 30% acetic acid was added to end the process. The obtained mixture was centrifuged for 10 minutes at 3000 g, and the absorbance of the clear supernatant was measured at 410 nm. The absorbances of the standard and samples were compared to their respective blanks. Trypsin inhibitor activity (TIA) was reported as trypsin inhibitor units/mg protein (TIU/mg of protein). One unit of TIU was defined as reducing A410 nm of 0.01 relative to trypsin control reactions.

6.2.9 Optimization of the process conditions

OriginPro software was used to optimize the process parameters. We ran an analysis of variance (ANOVA) and got regression coefficients for linear, quadratic, and interaction factors. Regression coefficients were then utilized for building the response surfaces and regression equations. After obtaining polynomial regression equations demonstrating a connection between responses and independent factors, input variables were optimized. The response variable, i.e. protein content,

was maximized and all input variables for Microwave and Ultrasound processing were maintained within their respective ranges. The model was validated by performing tests under projected optimal circumstances.

6.3. Results and Discussion

6.3.1 Effect of microwave-assisted extraction on the protein content of the chickpea beverage

Protein extraction yields (dependent variable) obtained from 14 experimental combinations of microwave temperature and time are shown in Table 6-1. The relationship between independent variables and response value in the 3-D surface plot is shown in Figure 6-2. It was observed that the microwave temperature and time had a quadratic effect on the protein yield, as evidenced by Figure 6-2. When microwave temperature increased (40 °C to 80°C), total protein yield increased up to 66°C, after which it decreased. Similarly, protein content increased when microwave time was increased from 2 to 10 minutes, reaching a maximum of 8.34g/100mL around 6 minutes and declining beyond that. An increase in protein content can be attributed to the disruption of hydrogen bonds caused by the microwave-induced dipole rotation of molecules and the migration of ions that enhance solvent penetration into the matrix. As a result, the cell wall is disrupted, causing the release of an intracellular product (Phongthai et al., 2016). However, a drop in protein yield at extended periods of microwave time and temperature could be a result of irreversible protein aggregation and covalent complex formation, resulting in reduced protein solubility and extractability as heat treatment causes denaturation and unfolding of proteins, resulting in the

surface exposure of hydrophobic and sulfhydryl groups present inside the molecule (Amponsah & Nayak, 2016; Renkema et al., 2000).

The quadratic regression model equation for protein content obtained after the application of RSM is shown in Equation [6-3].

$$\text{Protein content} = 7.76 + 1.23A + 0.734B - 0.71AB - 1.89A^2 - 1.34B^2 \quad [6\text{-}3]$$

Where, A= Microwave extraction temperature (°C), B= Microwave extraction time (min)

The model terms' significance and individual coefficients were statistically analyzed using F-test and P-value. The generated model was found statistically significant ($P< 0.05$) in explaining the connection between the response and the independent variables with an F value of 24.63. ANOVA results for the fitted model are presented in Table 6-2. The higher R^2 and adjusted R^2 values (0.93 and 0.90, respectively), lower $p <0.0001$ and a non-significant lack of fit (0.44) at $p>0.05$ suggested an appropriate model fitting. The protein content of the chickpea beverage was found to follow a linear function as well as a quadratic effect for microwave extraction time, temperature and their interaction, as shown in the ANOVA for the individual term presented in Table 6-3.

Table 6-1: Responses obtained for different process parameters combinations according to experimental design

	Microwave Assisted Extraction		
Runs	**Factor 1: A Extraction temperature (°C)**	**Factor 2: B Extraction time(mins)**	**Response: Protein Yield (g/100mL)**

1	60	6	7.99
2	80	6	6.21
3	60	6	6.79
4	80	10	5.93
5	60	6	7.90
6	40	6	4.13
7	40	2	2.12
8	60	10	6.77
9	40	10	4.97
10	80	2	5.92
11	60	6	8.13
12	60	6	8.23
13	60	2	5.23
14	60	6	8.34

Ultrasound-Assisted Extraction

Runs	**Factor 1: A Duty Cycle (%)**	**Factor 2: B Extraction time(mins)**	**Response: Protein Yield (g/100mL)**
1	75	5	1.47
2	75	10	3.89
3	100	15	0.88
4	75	10	4.9
5	100	5	0.22

6	75	10	3.66
7	75	10	4.43
8	75	10	4.02
9	100	10	1.54
10	75	10	3.46
11	50	10	4.35
12	50	15	5.13
13	75	15	3.47
14	50	5	1.51

Table 6-2: ANOVA for fitted model

Microwave Assisted Extraction

Source	**Coefficient**	**SS**	**DF**	**MS**	**F-value**	**p-value**	**Comments**
Model		40.04	5	8.01	24.63	0.0001	Significant
Residual		2.60	8	0.32			
Lack of Fit		1.01	3	0.34	1.05	0.44	Not significant
Pure Error		1.60	5	0.31			
Total		42.64	13				
R^2	0.93						
Adjusted R^2	0.90						
CV%	8.95						

Ultrasound-Assisted Extraction						
Model	31.93	5	6.39	31.20	<0.0001	Significant
Residual	1.64	8	0.20			
Lack of Fit	0.24	3	0.08	0.29	0.83	Not significant
Pure Error	1.39	5	0.28			
Total	33.57	13				
R^2	0.95					
Adjusted R^2	0.92					
CV%	14.75					

Table 6-3: ANOVA for the effect of individual process parameters on response variable

Microwave Assisted Extraction					
Parameters	**Sum of Square**	**DF**	**Mean Square**	**F-value**	**p-value**
A	9.15	1	9.15	28.15	0.0007*
B	3.23	1	3.23	9.92	0.0136*
AB	2.02	1	2.02	6.20	0.0375*
A^2	10.10	1	10.10	31.06	0.0005*
B^2	5.11	1	5.11	15.72	0.0042*
Ultrasound-Assisted Extraction					
A	11.62	1	11.62	56.77	<0.0001*

B	6.57	1	6.57	32.11	0.0005*
AB	2.19	1	2.19	10.70	0.0113*
A^2	2.01	1	2.01	9.81	0.0140*
B^2	4.91	1	4.91	24.01	0.0012*

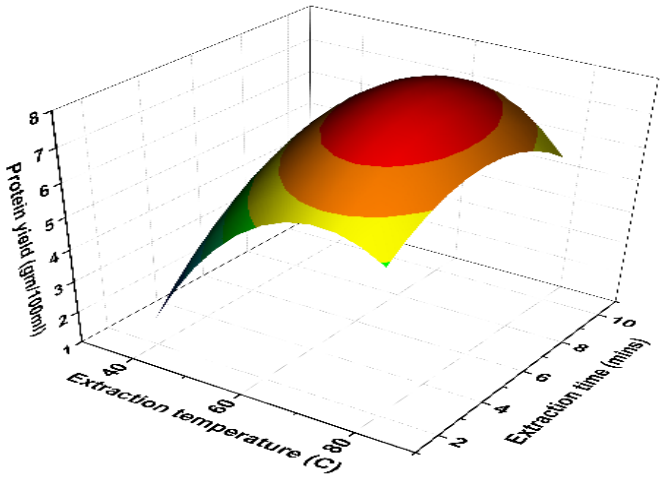

Figure 6-2: Response surface for correlating microwave input variables with output variable (protein yield)

6.3.2 Effect of ultrasound-assisted extraction on the protein content of the chickpea beverage

Table 6-1 displays the protein extraction yields obtained from 14 experimental ultrasound-assisted extraction combinations with input time and duty cycle input factors. The combined effect of the duty cycle and extraction time of ultrasound is shown in Figure 6-3. As illustrated in Figure 6-3, as the duty cycle is increased from 50% to 75%, a slight increase in protein yield is observed, but when the duty cycle was 100% (continuous mode), the yield decreased. The result shows that the

use of ultrasonic in pulse mode with the appropriate pulse intervals may be more effective than continuous sonication (duty cycle 100%) which also minimizes the electrical energy consumption, which is consistent with the work of (Kobus, 2008), that suggested the effectiveness of pulsed ultrasound on dry matter extraction of valerian roots (*Valeriana officinalis L*.). Similarly, (Xu & Pan, 2013) found that compared to continuous sonication, 50% duty cycle sonication had a positive effect on the extraction process and enhanced the extraction yield of lycopene from red grapefruit *(Citrus paradise Macf.).* The observed effect could be due to the generation of degassing bubbles forming clusters under continuous mode ultrasound, which may limit the effective zone of the sonochemical reaction due to the dramatic expansion and contraction of the spatial area. On the other hand, under pulse mode operation, the inactive time of the ultrasound pulse might facilitate the clarity of the cavitation zone and the acceleration of sonochemical processes (Mitome & Hatanaka, 2002; Yolmeh et al., 2014). In addition, the presence of residual cavitation nuclei during the inactive time of pulsed ultrasound facilitates the formation of cavitation bubbles during the subsequent active ultrasonic period, thus accelerating/promoting the yield (Tuziuti et al., 2008). For ultrasound extraction time, protein yield increased up to 13 minutes of operation and started decreasing beyond that, as shown in Figure 6-3, which is attributed to the acoustic cavitation, which damaged the cell walls, broke molecular bonds, boosted mass transfer, and expedited the washing out of the proteins and as the time progressed an increasing number of cells were broken and proteins were progressively liberated (F. Wang et al., 2020). However, there was no apparent improvement in extraction yield when the ultrasound duration increased further, which may be due to the shifting of the diffusion front deeper into the tissues, decreasing diffusion area and increasing diffusion distance, ultimately decreasing diffusion rate proportionally (Hemwimol et al., 2006;

Zhao et al., 2007). The quadratic regression model equation obtained using RSM is represented as Equation [6-4].

$$\text{Protein content} = 3.99 - 1.39A + 1.05B - 0.74AB - 0.84A^2 - 1.32B^2 \quad [6\text{-}4]$$

Where, A= Ultrasound duty cycle (%), B= Ultrasound extraction time (min)

The statistical significance of the model terms and coefficients was assessed using the F-test and P-value. With an F value of 31.20, the developed model was determined to be statistically significant ($P<0.05$) in describing the relationship between the response and the independent variables. The model-specific ANOVA results are provided in Table 6-2. The higher R^2 and adjusted R^2 values (0.95 and 0.92, respectively), lower $p< 0.0001$, and a not statistically significant (0.82 at $p>0.05$) lack of fit indicate a good model fit. ANOVA for the individual terms is presented in Table 6-3, showing linear, quadratic and interaction effects for the predicted model.

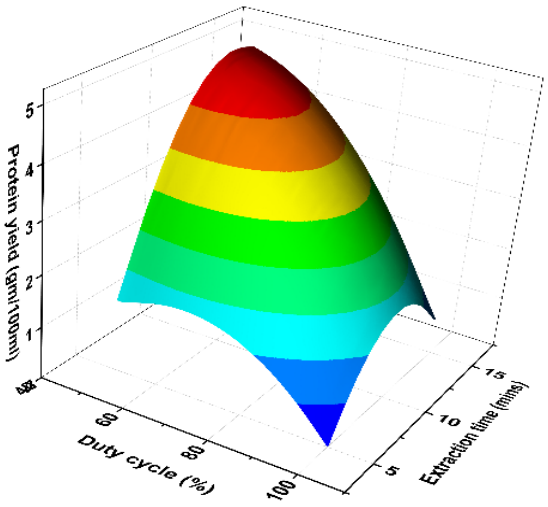

Figure 6-3: Response surface for correlating ultrasound input variables with output variable (protein yield)

6.3.3 Verification and Optimization

Using the desirability function, the protein content of the chickpea beverage extracted with the help of microwave-assisted extraction and ultrasound-assisted extraction was optimized. The desirability function combines numerous responses into a single value ranging from 0 (one or more product attributes are unsatisfactory) to 1 (all product attributes are desirable). The chickpea beverage was prepared using optimized MAE and UAE conditions for model validation, and the protein concentration was measured. The experimental and projected values were compared to ascertain the model's validity and are shown in Table 6-4. The best conditions to maximize protein content using MAE were 65.8 °C temperature for 6.7 minutes. The optimum protein content obtained using these conditions was 8g/100mL of beverage with desirability of 0.94.

Similarly, for UAE, optimized conditions obtained were an extraction time of 13.5 minutes operated at 50% duty cycle. The predicted optimum value of protein content using obtained UAE conditions was 5.09 g/100mL of chickpea beverage with desirability 1. As shown in Table 6-4, the

experimental values obtained were reasonably close to the predicted one confirming the validity and adequacy of the predicted model.

Table 6-4: Optimized process parameters and responses (predicted and experimental) using MAE and UAE

Microwave Assisted Extraction	
Factors	**Parameters**
Extraction temperature (°C)	65.8 °C
Extraction time(mins)	6.7 minutes
Predicted Protein content (g/100mL of beverage)	8.00
Experimental Protein content (g/100mL of beverage)	7.89
Ultrasound-Assisted Extraction	
Duty Cycle (%)	50
Extraction time(mins)	13.5
Predicted Protein content (g/100mL of beverage)	5.09
Experimental Protein content (g/100mL of beverage)	5.37

6.3.4 Effect of conventional, MAE and UAE processing on the quality parameters of the beverage

The optimized conditions obtained after MAE and UAE processing were used to prepare chickpea beverages for quality assessment. The optimized beverages obtained from MAE and UAE were compared for some crucial quality parameters with the beverage prepared by conventional method optimized by (Sharma & Orsat, 2022) treated as a control sample.

6.3.4.1. Effect on protein content and protein solubility

The protein content of chickpea beverages is a crucial parameter that significantly impacts their nutritional value. A significant increase ($p < 0.05$) in protein content was noticed when the beverage was produced using microwave and ultrasound processing as compared to the conventional optimized extraction method used by (Sharma & Orsat, 2022). The extracted chickpea beverage by microwave processing contains 7.89±0.11g of protein per 100mL beverage. In the case of ultrasound processing, 5.23 ±0.23 g of protein per 100 mL was observed at optimized conditions. Therefore, an increase of 47.78% in protein content was observed when microwave processing was applied, while for ultrasound processing, an increase of 21.22% was noticed as compared to the traditional method (4.12± 0.20 g of protein per 100 mL) employed by (Sharma & Orsat, 2022). Solubility is the most realistic metric of protein denaturation and aggregation and is thus an excellent indicator of protein functioning. At optimized conditions for conventional processing, the protein solubility of the beverage was found to be 13.58%±0.35, while for microwave processing and ultrasound processing, it was found to be 18.54±0.23 and 15.28%±0.19

respectively. Both microwave and ultrasound processing enhanced protein solubility significantly ($p < 0.05$). The microwave process enhanced the protein solubility of the beverage by 36.52% as compared to conventional processing. Microwave heating is a faster way of heating that decreases protein structure's exposure to high temperatures for longer durations, resulting in less protein denaturation and more protein solubility (Hemwimol et al., 2006).

By ultrasound treatment, an alteration in protein conformation and structure occurs and hydrophilic amino acid side chains become exposed to water (Morel et al., 2000). For ultrasound processing, protein solubility increased by 12.81% compared to conventional processing. This increase in solubility might be attributed to alterations in the three-dimensional structures of globular proteins, which led to an increase in the amount of charged groups (NH_4^+, COO^-) with higher electrical conductivity, increasing protein–water interactions, thus enhancing solubility (Jambrak et al., 2008). Comparison results for the beverage processed by three different methods are shown in Table 6-5.

6.3.4.2 Effect on protein digestibility and trypsin inhibitor activity of the beverage

Both microwave and ultrasound processing resulted in a significant increase ($p < 0.05$) in the protein digestibility of the chickpea beverage. The protein digestibility of traditional beverages was determined to be 80.59±0.19%, whereas when microwave was used for processing, it was observed to be 90.79±0.64 %, indicating an increase of 12.65%. The results are in accordance with previously reported studies conducted by (Sun et al., 2020) and (Hafez et al., 1985). Microwave treatment may lower the activity of anti-nutritional factors and accelerate the hydrolysis of protein macromolecules into small molecules, exposing more cleavage sites for digesting proteases. Thus

the protein interacts readily with pepsin/trypsin to enhance the IVPD of the beverage (S. Wang et al., 2022). In addition, microwave processing also modifies the protein's secondary structure, increasing its flexibility and expansion and making it more susceptible to enzymes for digestion (Hassan et al., 2019). Studies have also suggested that anti-nutritional factors such as trypsin inhibitors are decreased or digested by protease, enhancing the IVPD (Sharma et al., 2019). In the case of ultrasound, IVPD was found to be 87.56±0.43 indicating an increase of 8.64% compared to the conventionally processed beverage. The cavitation effect of sonication alters the molecular structure of the protein by breaking hydrogen bonds, converting them to smaller aggregates being extremely vulnerable to proteolysis (del Rio et al., 2020). Intense cavitational pressures may also be responsible for the partial unfolding of proteins and reduced intermolecular interactions making the action of digestive enzymes easier and more effective (Xiong et al., 2018).

Trypsin inhibitor activity is considered an essential factor adversely affecting protein digestibility in legumes and is often studied with protein digestibility. Initial TI activity was found to be 24.73±0.21 TIU/mg of protein. When the beverage was processed with the help of a microwave at optimized conditions (65.8 °C and 6.7 minutes), TI activity was 15.26±0.15 TIU/mg of protein, indicating a reduction of 62.15% in comparison to conventional processing. Similar results regarding the microwave processing of soymilk were obtained by (Vagadia et al., 2018), which resulted in a significant reduction in trypsin inhibitor activity. Similarly, an estimated reduction of 84% in the TI activity of soymilk was achieved when soymilk was processed by microwave for 10 minutes at 100°C (Vanga et al., 2020). Several other studies have also reported that a significant reduction in trypsin inhibitor activity occurred by microwave cooking in broad bean, soybean seeds and edible cereal brans (Barać & Stanojević, 2005; Kaur et al., 2012; Pysz et al., 2012). An oscillating electric field during microwave treatment might have destroyed the trypsin-binding site

on the trypsin inhibitor and diminished its inhibitory efficacy; also, the higher temperature might produce a structural change in the protein, leading to the loss of trypsin inhibitors (Wallace et al., 1971).

Both microwave and ultrasound treatments resulted in a significant decrease ($p < 0.05$) in the TI activity of the chickpea beverage. When the beverage was processed using optimized ultrasound conditions (50% duty cycle and 13.5 minutes), TI activity obtained was 18.55±0.14 TIU/mg of protein, showing a 21.59 % reduction in activity as compared to conventional processing optimized by (Sharma & Orsat, 2022). A previous study by (Huang et al., 2008) suggested a 55% reduction in TI activity following 20 minutes of ultrasound processing in soybeans. Similarly (Vanga et al., 2020), achieved the highest reduction of 52% in TI activity when soymilk was ultrasonically treated for 16 minutes. Reduction in TI activity by non-thermal ultrasound treatment is attributed to a change in secondary structure due to disruption of disulphide bonds leading to reduced activity. However, when microwave and ultrasound treatments are compared for the reduction in TI activity, microwave being a thermal technique, is found to be more effective. However, despite its non-thermal nature, ultrasonication processing appeared to be successful in significantly lowering the trypsin inhibitor activity considerably.

Table 6-5: Effect of conventional, MAE and UAE processing on the quality parameters of the beverage

Parameters	Conventional Processing	Microwave processing	Ultrasound Processing
Protein (g/100g of beverage)	4.12±0.20	7.89±0.11	5.23±0.24

Protein solubility (%)	13.58±0.35	18.54±0.23	15.28±0.19
IVPD (%)	80.59±0.19	90.79±0.64	87.56±0.43
TIA (TIU/mg of protein)	24.73±0.21	15.26±0.15	18.55±0.14

6.4. Conclusion

The objective of this study was to optimize the microwave and ultrasound extraction of chickpea beverages to maximize their protein content and compare the chickpea beverages with conventional beverage processing as optimized earlier (Sharma & Orsat, 2022). In addition, a comparison of protein solubility, IVPD and TI activity at optimized processing conditions by all three processes (conventional, microwave and ultrasound) was also done. Overall, protein yields obtained from microwave and ultrasound extraction were found to be higher as compared to conventional processing. Also, these higher yields are achieved by microwave and ultrasound in relatively shorter processing time as compared to conventional processing proving to be more economical. Comparison studies at optimized conditions also suggested the effectiveness of microwave and ultrasound processing compared to conventional ones. Microwave and ultrasound processing both enhanced protein solubility and IVPD significantly. TI activity was also effectively reduced by microwave and ultrasound processing, which positively affected protein digestibility. However, compared to microwave processing, ultrasound was found to be moderately effective (to a certain extent only) and milder in enhancing the nutritional properties of the

beverage. In conclusion, it can be safely said that both microwave and ultrasound are way better techniques than conventional processing and can be successfully utilized for enhancing the nutritional profile of plant-based beverages, but further research is still required for scale-up and industrial utilization of these techniques for the commercial processing of chickpea-based beverages.

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Preface to Chapter 7

Previous study suggested that both microwave and ultrasound processing proved to be superior techniques to conventional processing for improving the nutritional profile of chickpea-based beverages. These findings highlight the potential of microwave and ultrasound techniques for enhancing plant-based beverage nutritional properties and the potential benefits and limitations of these novel techniques in comparison to traditional methods. Building upon the insights gained from the initial research, the study then progressed to conduct comparative storage studies of the chickpea beverage in Chapter 7. The focus during this phase was to examine how conventional, ultrasound and microwave processing methods influenced the physicochemical and functional properties of the beverage over its storage period. Additionally, microbial analysis was conducted to ensure the safety and stability of the beverage under different processing conditions during its shelf life.

Chapter 7: Storage studies of chickpea beverage: Effects on the physicochemical and functional properties and microbial analysis

Abstract

Storage studies play a crucial role in assessing the stability and quality of foods and beverages over time. An optimized chickpea beverage was prepared using three processing techniques i.e., conventional, ultrasound and microwave processing for storage studies for 21 days to examine changes in physicochemical, microbial, and functional properties. The study demonstrated that both ultrasound and microwave processing techniques significantly outperformed conventional processing in terms of the yield of bioactive compounds. Ultrasound processing increased total phenolic compounds by 27.11% and total flavonoid content by 29.16%, while microwave processing led to a remarkable increase of 47.32% in total phenolic compounds and 58.34% in total flavonoid content. The storage study conducted with the chickpea beverage revealed a decline in bioactive compounds and antioxidant activities over the storage duration (21 days). However, it was observed that the application of ultrasound and microwave processing techniques resulted in retaining the TPC more effectively than the other techniques during the storage period up to 18.71% and 17.79% respectively. The microbial study revealed less total bacterial and fungal counts in microwave and ultrasonically processed beverages over the storage period. Moreover, the study also revealed that the processing treatments applied did not result in significant alterations in the pH and acidity values of the chickpea beverage.

Keywords: chickpea beverage, microwave, ultrasound, bioactive compounds, storage.

7.1 Introduction

In recent decades, plant based beverages have become more popular than ever owing to their healthy and functional properties along with their sustainable and cruelty-free claims (McClements et al., 2019). Plant-based drinks are made from water-based extracts of cereals, pseudo-cereals, nuts and legumes (Sethi et al., 2016). Plant-based beverages, derived from sources such as soy, almond, oat, and rice, have gained popularity as alternatives to traditional dairy products (Penha et al., 2021). However, their storage stability is crucial to monitor for maintaining product quality and consumer acceptance. The storage conditions to which food products are exposed play a significant role in determining their shelf life, overall quality and consumer acceptance (Beacom et al., 2021). Commercial production of these beverages is mainly done through conventional methods followed by pasteurization before aseptic packaging. The shelf-life of plant-based beverages can vary depending on a range of factors, including the ingredients used, the processing methods, the packaging, and the storage conditions (Khodke et al., 2014). In general, plant-based beverages have a shorter shelf-life than dairy-based products with their high moisture content inherent processing conditions and low acidity levels (Helsing, 2019).

Shelf-stable plant-based beverages often undergo specific processing techniques, such as ultra-high temperature (UHT) processing, High-Pressure processing (HPP) or similar technologies for effectively killing microorganisms and extending shelf life (Dhankhar & Kundu, 2021). In addition, aseptic packaging is often considered to ensure the beverage is packaged in a sterile environment, further preventing microbial contamination. While in the case of refrigerated plant-based beverages milder processing techniques like pasteurization are used to ensure product safety. The combination of processing techniques and packaging methods used in shelf-stable beverages helps to inhibit microbial growth and preserve product quality, allowing for a shelf life of up to 12

months, depending on the specific product and packaging, while refrigerated beverages typically have a shorter shelf life, ranging from a few weeks to a couple of months (Blonz, 2017).

Storage conditions can influence the nutrient content of plant-based beverages. Studies have shown that packaging materials' storage temperature, light exposure, and oxygen permeability can affect the stability of vitamins, minerals, and bioactive compounds (Achouri et al., 2007). Therefore, although shelf-stable plant-based beverages have longer shelf life than refrigerated beverages, once open, they should be stored properly under refrigerated conditions to retain their nutritional quality.

As discussed, storage conditions can significantly influence the quality and shelf life of food products, and chickpea beverages are no exception. Various physicochemical parameters, such as pH, color parameters and total dissolved solids may undergo alterations during storage, impacting their sensory attributes and overall consumer acceptance. Microbial safety is another critical aspect that needs to be assessed during storage. The presence of microorganisms can lead to spoilage and pose potential health risks to consumers (Poliseli-Scopel et al., 2013). Furthermore, various bioactive compounds are present in plant-based beverages with antioxidant properties, such as phenolic compounds and flavonoids (Ma & Huang, 2014). Antioxidants play a vital role in protecting against oxidative stress and contribute to the potential health benefits of plant-based diets (Bernhoft et al., 2010). Monitoring the changes in antioxidant activity during storage provides insights into the beverage's stability and its ability to retain its beneficial properties over time.

In our previous study, we optimized the processing parameters for preparing a chickpea beverage (Sharma & Orsat, 2022). The present study aims to investigate the changes in physicochemical parameters, microbial profiles, and antioxidant parameters of chickpea-based beverages during storage prepared by three processing methods i.e., conventional, microwave and ultrasound. By

comprehensively assessing these aspects, we can gain a better understanding of the beverage's quality and stability, enabling manufacturers to optimize storage conditions and extend product shelf life while preserving sensory attributes and health benefits.

7.2 Material and Methods

7.2.1 Beverage preparation and treatments

Kabuli-type chickpeas (*C. arietinum*) with lot number 15386 and a size of 9 mm were procured from AGT Clic Foods (Laval, Quebec). These were sorted, cleaned and kept in a clean and dry area for later use. For the beverage preparation, chickpeas were soaked for 12 hours after being well-rinsed with water. A slurry was obtained by grinding soaked chickpeas in a RETSCH Knife Mill Grindomix GM200 from Thermo Fisher Scientific Inc., USA, keeping the water-to-chickpea ratio at 7.13:1 as optimized by (Sharma & Orsat, 2022). Further, the beverage was prepared using three processing methods i.e., conventional, microwave and ultrasound. For the selected processing parameters, only optimal conditions studied and optimized earlier were used for conventional processing as shown in Figure 7-1 (Sharma & Orsat, 2022). The optimum microwave and ultrasonic settings for this comparative storage study were selected based on our prior findings, indicating the best outcomes showing maximum protein content in the beverages (Chapter 6). These optimum settings are summarized in Figure 7-1. Before storage, beverages obtained by the three processing methods were pasteurized at 72°C for 15 seconds followed by rapid cooling by transferring the samples to ice water to prevent any further physical or chemical changes (Kwok & Niranjan, 1995).

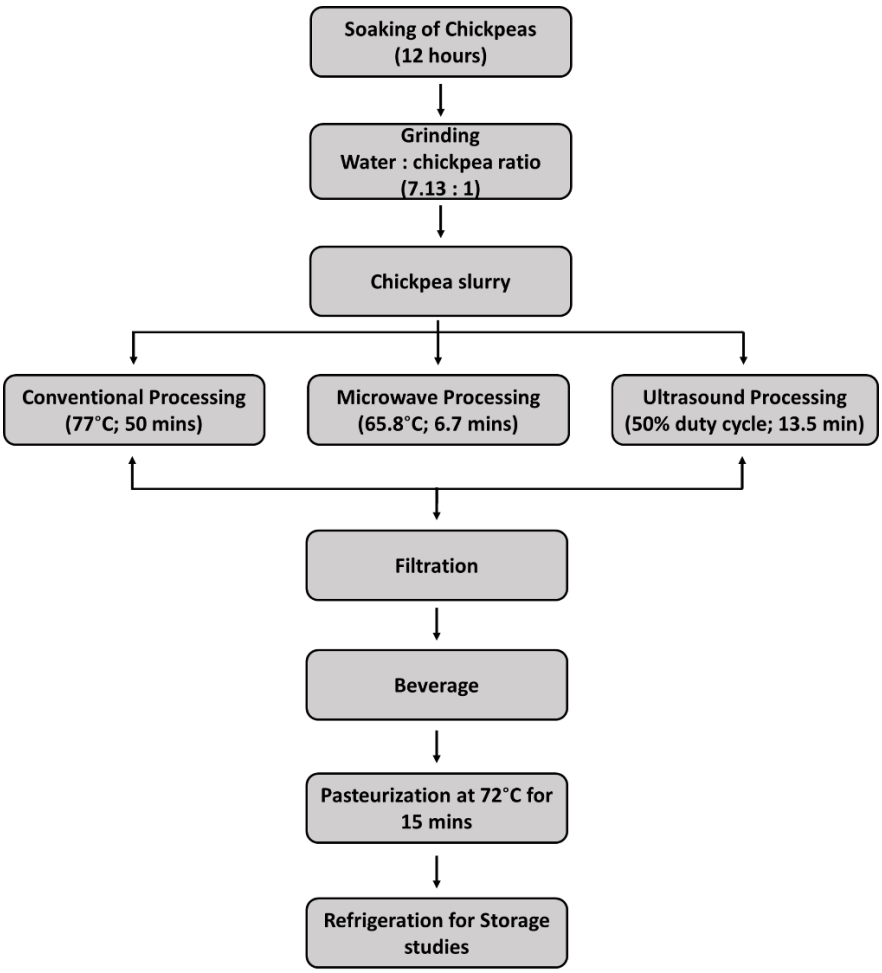

Figure 7-1: Schematic process for the processing of chickpea beverage prepared for storage studies.

7.2.2 Storage conditions

After the preparation of the beverage and treatment, samples were filled into sterilized containers and falcon tubes and kept in the refrigerator at 4°C for conducting the storage study for 21 days. Its chemical, microbiological, and functional properties were assessed immediately after the processing and weekly at 7, 14 and 21 days as the estimated shelf life of refrigerated plant-based beverages is 2-3 weeks approximately (Macedo et al., 1999). For estimating the bioactive/functional properties the samples were freeze-dried first before analysis.

7.2.3 Determination of physicochemical properties

The pH of prepared beverage samples was determined by a digital pH meter (Fisher Scientific, USA). The total soluble solids (TSS) of beverage samples were measured using a portable handheld refractometer (Cole-Parmer, Quebec, Canada), and the findings were represented as °Brix at room temperature. Color characteristics of the beverage were determined by collecting lightness and chromaticity coordinates in the L, a, b color space (CIELAB) using a portable colorimeter (CR-300 Chroma, Minolta, Japan) to evaluate color changes in the samples during the tested storage period. The color index (ΔE) was determined using Equation [7-1] following the method used by (Romano et al., 2008)

$$\Delta E = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{1/2} \quad [7\text{-}1]$$

where ΔL, Δa, and Δb represent the differences in tristimulus coordinates between the sample being analyzed and a reference sample of bovine milk used as the control. These tristimulus coordinates are part of the CIELAB color space, which quantifies color using three dimensions: L (lightness), a (red-green axis), and b (yellow-blue axis). The titrable acidity of the samples was estimated by the method used by (Kim et al., 2012) with slight modifications. To begin with, samples were subjected to a titration process using sodium hydroxide as the titrant, with phenolphthalein serving as an indicator. To the 10mL sample, 10 mL of distilled water and a few drops of phenolphthalein indicator were added. The resulting solution was then subjected to titration using a 0.1 N NaOH solution until the color of the solution turned pink. The titrable acidity, (TA%) of the samples was calculated using Equation [7-2] shown below.

$$TA\ (\%) = \frac{V_1\ X\ N\ X\ E}{V_2\ \ X\ 1000}\ X100 \qquad [7\text{-}2]$$

Where,

V_1 = volume of the NaOH used

N = Normality of NaOH

E = Equivalent weight of acid

V_2 = Equivalent volume of acid

7.2.4 Microbial analysis

A microbial study of the beverage samples was conducted by following the method cited by (Khodke et al., 2014). Tryptone agar medium was used for determining the total plate count. The total count was reported as colony-forming units per mL of beverage samples (CFU/mL). All plates were incubated for 24 h at 37°C. After 24 hours colonies were counted with the help of open-access image processing software ImageJ.

7.2.5 Determination of functional properties

7.2.5.1 Extraction of functional compounds from beverage samples

Bioactive compounds were extracted by the process previously used by (Chandra et al., 2014; Yu et al., 2021) with suitable modifications. Freeze-dried beverage samples (0.5g) were mixed with 10 mL of acetone/water/acetic acid (0.7:29.5:0.5, v/v/v) solvent and extraction was carried out on an orbital shaker at 300 rpm for 3 hours at room temperature. The obtained extracts were left in the dark overnight for another 12 hours followed by centrifugation at 3000 rpm for 10 minutes. Extraction was done twice following the process mentioned above. The two-time extracts were combined and stored at 4 °C till needed for further analysis.

7.2.5.2 Determination of total phenolic content (TPC) of the beverage samples

Total phenolic content was determined using FC (Folin–Ciocalteu) reagent according to a laboratory procedure described by (Ma & Huang, 2014; Xu & Chang, 2007) with suitable modifications. The method works on the principle that when FC reagent, which is a mixture of phosphomolybdate and phosphotungstate, reacts with phenolics present in the medium it results in the formation of a blue colour complex which can be detected at 765nm by spectrophotometry. Briefly, 2.95mL distilled water was added to 100 µl of sample extract. Further, 250 µl of FC reagent solution and 750 µl of Na_2CO_3 (7%) was added along with 950 µl of distilled water. The mixture was allowed to stand in the dark for 2 hours and absorbance was taken at 765 nm using a UV visible spectrophotometer. The calibration curve was prepared using gallic acid as the standard. The experiments were carried out in triplicates and results were reported as mg of gallic acid equivalent (GAE) /g of freeze-dried samples.

7.2.5.3 Determination of total flavonoid content (TFC) of the beverage samples

The total flavonoid content of the sample was determined using the aluminum chloride colorimetric technique earlier reported by (Bag et al., 2015; Chandra et al., 2014) with some modifications. This method works by forming stable complexes between the aluminum chloride and certain functional groups existing on flavonoid compounds, such as keto groups and hydroxyl groups (Bag et al., 2015) which can be detected by colorimetry at 420 nm. An amount of 1.0 mL of extracted samples was mixed with 1.0 mL of 2% aluminum chloride. Following mixing, the solution was incubated at room temperature for 60 minutes and the absorbance of the reaction mixtures was measured at a wavelength of 420 nm. The calibration curve was prepared using

quercetin as the standard. Using the calibration plot, the concentration of total flavonoid content in the test samples was determined and reported as mg of quercetin equivalent (QE) /g of freeze-dried samples. Experiments were performed in triplicates and results are reported as mean±standard deviation.

7.2.6 Determination of antioxidant properties

7.2.6.1 DPPH free radical scavenging assay of the beverage samples

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a common method used to measure the antioxidant activity of compounds. DPPH is a free radical, which is stable at room temperature and deep purple in colour in solution. But, in the presence of an antioxidant, the colour of the solution changes from violet to yellow, indicating the reduction of DPPH to its non-radical form (Garcia et al., 2012). The experiment was performed according to the protocol followed by (Payum et al., 2013; Xiao et al., 2020) with some modifications. The DPPH working solution (0.1 mM) was prepared by dissolving 4mg of DPPH in 100 mL of methanol. Briefly, 0.1 mL of sample extracts or standards were mixed with 2.9 mL of DPPH and the reaction mixture was stored in the dark for 30 minutes. Further, the absorbance of the reaction mixture was taken at 517 nm using a UV-visible spectrophotometer. Trolox was used for preparing a standard calibration curve and results were reported as µM of Trolox per g of freeze-dried samples.

7.2.6.2 Ferric ion reducing antioxidant power assay of the beverage samples

For further evaluation of the antioxidant activity of the samples, FRAP/ Ferric ion-reducing antioxidant power assay was used. The standard protocol for FRAP assay, previously used by (Amarowicz & Pegg, 2019; Benzie & Strain, 1996; Sudan et al., 2014), was used with some

modifications. This assay works on the principle of ferric reducing ability i.e. Fe^{3+}-TPTZ, (2, 4, 6-tripyridyls-triazine) complex is reduced to Fe^{2+} in the presence of antioxidants, imparting a change in colour in the solution which can be detected by spectrophotometer at 593nm (Amarowicz & Pegg, 2019). Experiments are conducted by preparing FRAP working reagent by mixing acetate buffer (300mM, pH 3.6), TPTZ (10mM in 40mM HCl) and $FeCl_3$. $6H_2O$ (20 mM) in a ratio of 10:1:1 v/v/v. Further, 0.1 mL of test samples were mixed with 2.9 mL of FRAP reagent and absorbance was measured at 593 nm after incubation in the dark for 30 minutes. $FeSO_4 \cdot 7H_2O$ was used for the preparation of a standard calibration curve of known Fe^{2+} concentration. Experiments were performed in triplicates and results are reported as mean±standard deviation. Results are reported as the mM equivalent of Fe^{2+} per 100 g of freeze-dried samples.

7.3 Results and Discussion

7.3.1 Effect of storage on the physicochemical properties of the beverages

The physicochemical parameters of a beverage play a crucial role in ensuring its overall quality. Therefore, it is essential to thoroughly investigate the optimal conditions under which these parameters can be maintained to enhance the sensory attributes of the final product. A comparative change in pH during the storage period in the chickpea beverage is shown in Figure 7-2.

The pH of the chickpea beverages showed significant variations ($p \leq 0.05$) during different storage time intervals, as indicated in Figure 7-2 (A). But a non-significant difference was found between the pH of the conventional and microwave-processed samples. The findings from the storage analysis revealed a slight decrease in pH over time compared to the pH of the fresh beverages as evident from Figure 7-2 (A). Similarly, no significant difference was found among different

processes used in the case of titrable acidity (TA%) as shown in Figure 7-2 (B). During the storage period, an increase in TA% was observed in all three types of processed beverages. However, in practical terms, the observed variations in titrable acidity (TA%) of the beverage can be considered to be negligible, as these slight variations in TA (0.10 – 0.22%) levels are not expected to have significant implications in real-world applications. The range observed in the acidity levels suggests that the beverage samples analyzed exhibit relatively consistent acidity. Similarly, the pH values fall within a relatively narrow range (6.99-6.34), indicating that the samples maintain a reasonably stable pH.

The values for total soluble solids of conventionally processed beverage samples were found to be significantly different ($p \leq 0.05$) from microwave and ultrasound processing as shown in Figure 7-2 (B). Over the observed storage period, TSS values decreased significantly ($p \leq 0.05$) in all treatments, but the maximum reduction was found in conventionally processed beverage samples. The better performance of ultrasound and microwave processing in retaining TSS content over the storage period can be attributed to the efficiency of these novel processing techniques in improving the mixing and homogenization of the beverages during production, leading to a more uniform distribution of solids within the sample. This enhanced uniformity plays a crucial role in preventing the settling or separation of solids during storage, thereby contributing to the stabilization of the total soluble solids (TSS) content (Bocker & Silva, 2022; Rojas et al., 2022). The color indices (ΔE) were calculated based on the L, a, b tristimulus values and found to be significantly different ($p \leq 0.05$) among beverage samples. Samples with lower ΔE values are perceived as having a whiter color due to their reduced divergence from the standard (Achouri et al., 2008). The lowest value of ΔE (15.52) was found with the microwave samples followed by ultrasound (16.26) and conventionally processed (16.78) beverage samples. The obtained results indicate that microwave

and ultrasound processing facilitates the improvement in whiteness with reduced browning reactions (Maillard reaction or enzymatic browning) as compared to conventional processing (Al Faruq et al., 2019). However, at the end of the storage period, an increase in ΔE (statistically non-significant) values was noticed as evident in Figure 7-2(D), among all treatments studied, indicating a more noticeable and pronounced difference between sample colors and reference sample (bovine milk).

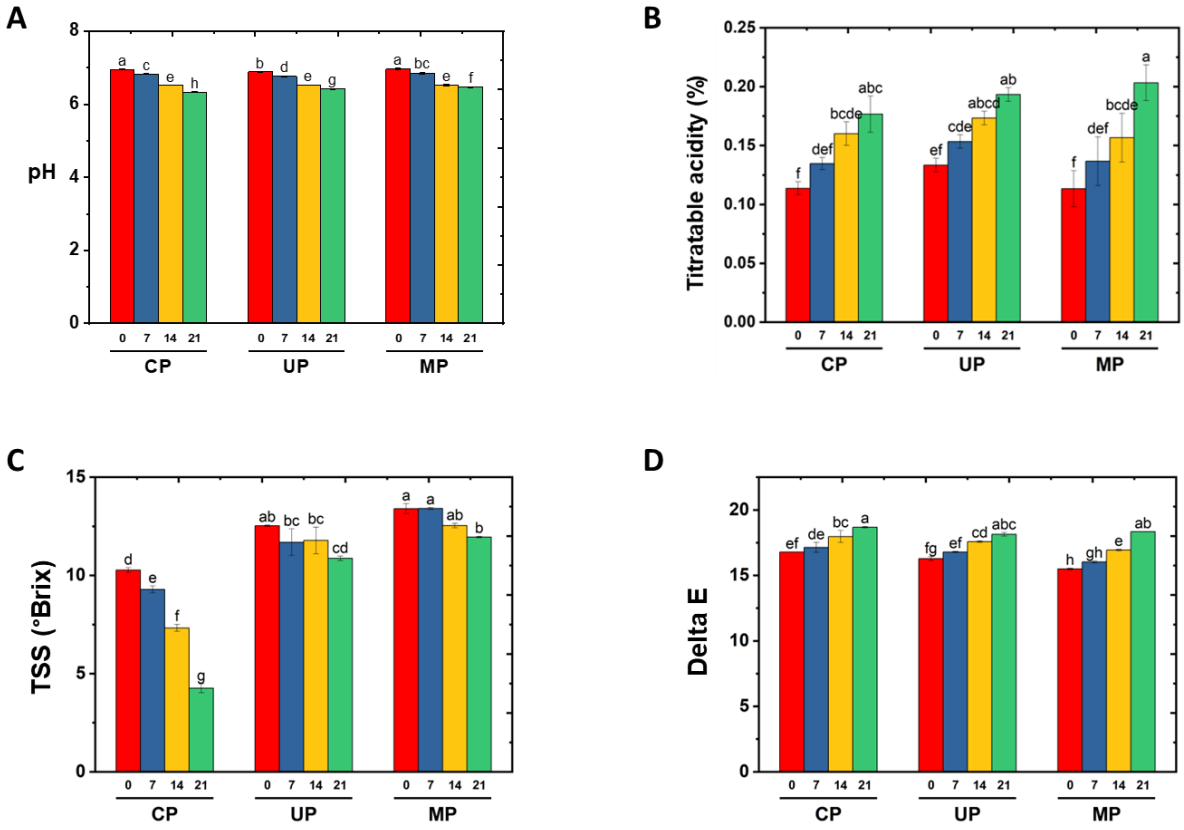

Figure 7-2: Effect of storage on the physicochemical properties of beverage samples (A) changes in pH, (B) changes in titrable acidity, (C) changes in total soluble solids (TSS), (D) changes in color indices (ΔE). CP= conventional processing, UP= ultrasound processing, MP= microwave processing

7.3.2 Microbial analysis

A total plate count for bacteria and fungi was done on the day of beverage preparation and each week during the storage period and results are presented in Figure 7-3. As evidenced by Figure 7-3 (A) the total plate count was found to be minimal in the microwave-processed beverage followed by ultrasound and conventionally processed beverage samples. Similar results were obtained in the case of total fungal counts as shown in Figure 7-3 (B). The bacterial counts consistently exhibited higher values compared to the fungal counts suggesting that bacterial populations were more abundant and prevalent in the studied environment than fungal populations. In addition, during the entire storage period, the total bacterial counts remained well under the safe acceptable limits (20,000/mL) as described by FDA Pasteurized Milk Ordinance (Food & Administration, 2017).

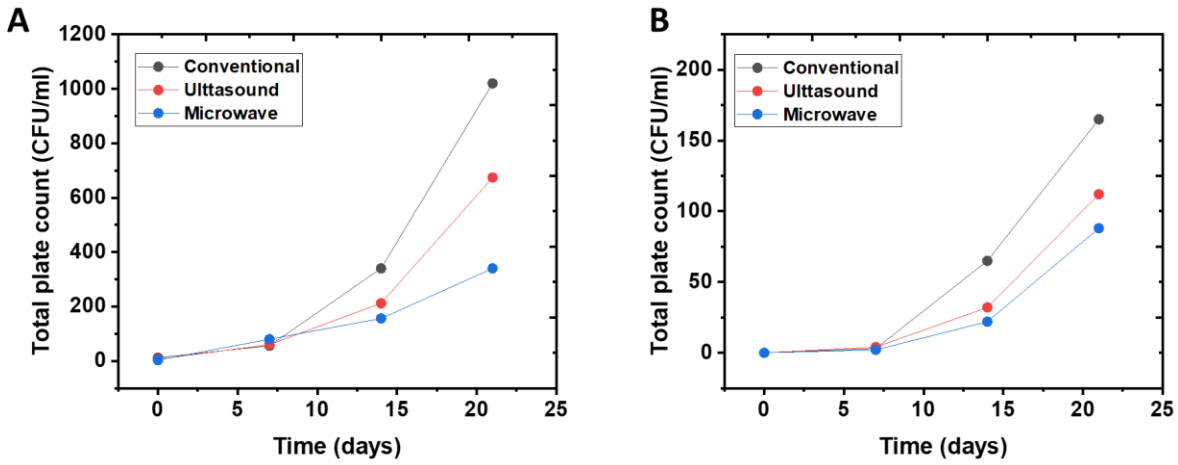

Figure 7-3: Total plate count for (A) bacteria (B) fungal over the storage periods

7.3.2 Effect of storage on the functional properties of the beverages

7.3.2.1 Effect on total phenolic contents (TPC) and total flavonoids contents (TFC)

Bioactive compounds are natural components found in foods that have potential health benefits beyond their basic nutritional value (Afshar et al., 2022). Figure 7-4 presents the bioactive compounds of interest, namely Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) in different beverage samples prepared by different processing techniques. Both TPC and TFC of the chickpea beverages exhibited substantial and statistically significant variations ($p \leq 0.05$) with different processing techniques and storage time intervals, as shown in Figure 7-4 (A, B). Among the three processing techniques, microwave processing resulted in the highest yield (TPC:47.32%, TFC:58.34%), followed by ultrasound (TPC:27.11%, TFC:29.16%) in comparison to conventional processing. Similar results regarding TPC and TFC extraction from *Cassia alata* and peach beverage respectively using microwave and ultrasound were reported by (Ling et al., 2019; Sattar et al., 2020). The obtained results can be attributed to the basic principle that microwaves in the frequency range of 300 MHz to 300 GHz can heat substances by utilizing the combined effects of ionic conduction and dipole rotation (Bagade & Patil, 2021; Woodhouse, 2005). The interaction between microwaves and polar solvents leads to heating through frictional forces and the attempted alignment of molecules with the alternating electric field (Emilie et al., 2013). In the case of plant material, when moisture within the deepest plant cells is exposed to microwaves, it undergoes heating, which leads to moisture diffusion and evaporation (Bagade & Patil, 2021). This evaporation and mass transfer process creates significant pressure on the cell wall, exerting force from within (Mandal et al., 2007). As a result, the cell wall weakens and eventually ruptures. This rupture allows for the release of the constituents contained within the cell (Azmir et al., 2013; Kingston, 1998). This phenomenon contributes to the increased extraction yield of phytoconstituents from the plant material. Similarly, an increase in yield of bioactive compounds utilizing ultrasound as compared to conventional processing, can be linked to the combined

influence of cavitation effects, thermal dissipation, and mechanical effects (Rostagno et al., 2003; Toma et al., 2001). These effects play a significant role in cell wall destruction, particle size reduction, and enhancing the rate of reaction through improved mass transfer, without causing changes to the structure and function of the extracted compounds (Wen et al., 2018). The significant increase in TPC and TFC observed in this study holds considerable value in terms of using extracts for their potential as antioxidant ingredients and for the overall health benefits they offer to consumers (Skrovankova et al., 2015).

Further, during the studied storage period, a significant reduction in TPC and TFC was observed over the 21 days but the reduction in microwave (TPC:18.71%, TFC:23.68%) and ultrasound (TPC:17.79%, TFC:22.58%) processed beverages was less as compared to conventional processing (TPC:25.20%, TFC:45.83%). The decrease in TPC and TFC over the storage period can be related to the autooxidation of bioactive compounds by oxidase enzymes during storage (Bakowska-Barczak & Kolodziejczyk, 2008; Šamec & Piljac-Žegarac, 2015). Similar results regarding the storage study of almond milk were reported by (Manzoor et al., 2020). Other studies, regarding the relatively little decrease in bioactive compounds in microwave and ultrasonically processed juices and beverages, have been reported by several authors (Agbenorhevi & Marshall, 2012; del Socorro Cruz-Cansino et al., 2015; Dima et al., 2015; Igual et al., 2010).

Numerous studies have been conducted to assess the impact of storage on the levels of different phenolic compounds in various food products. However, the observed changes in these studies exhibit significant variations, which can be primarily attributed to several factors, including the composition of the food matrix, the specific treatment and storage conditions employed, and particular phenolic compounds being investigated (Klimczak et al., 2007; Morales-De La Peña et al., 2011; Piljac-Žegarac et al., 2009).

7.3.2.2 Effect on antioxidant properties

To evaluate the antioxidant activity of the processed chickpea beverages during different storage time intervals, two distinct antioxidant analyses were conducted namely DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) assay. The antioxidant activity was found highest in microwave-processed samples (DPPH:1.46 µmol Trolox equivalent/g, FRAP: 2.01 mmol equivalent of Fe/100 g), followed by ultrasound (DPPH:1.16 µmol Trolox equivalent/g, FRAP:1.24 mmol equivalent of Fe/100 g), and conventional (DPPH:0.68µmol Trolox equivalent/g, FRAP: 0.92 mmol equivalent of Fe/100 g) treatments. Previous studies have also reported higher antioxidant activity in microwave and ultrasonically processed beverages and fruit juices (Saikia et al., 2016; Sattar et al., 2020). As shown in Figure 7-4 (C, D), beverage samples exhibited significant variations ($p \leq 0.05$) in DPPH and FRAP values, when subjected to different processing techniques and storage time intervals. Further, a substantial decrease in the antioxidant activity of the beverage samples was observed during the storage period. However, the reduction was observed to be least in the microwave (DPPH:16.40%, FRAP:20.31%), followed by ultrasound (DPPH:26.70%, FRAP:28.22%). The beverage processed using conventional methods exhibited a considerable decrease in antioxidant activity, retaining only approximately half of its original potency (DPPH: 52.90%, FRAP: 50.01%). Similar reduction trends in antioxidant activity were reported in storage studies of *Adansonia digitata* fruit pulp (Tembo et al., 2017) and roselle-mango juice blends (Mgaya-Kilima et al., 2015). However, some studies have also reported a slight increase in antioxidant activity at the end of tested storage periods which may occur due to polymerization reactions of polyphenol compounds (Sattar et al., 2020).

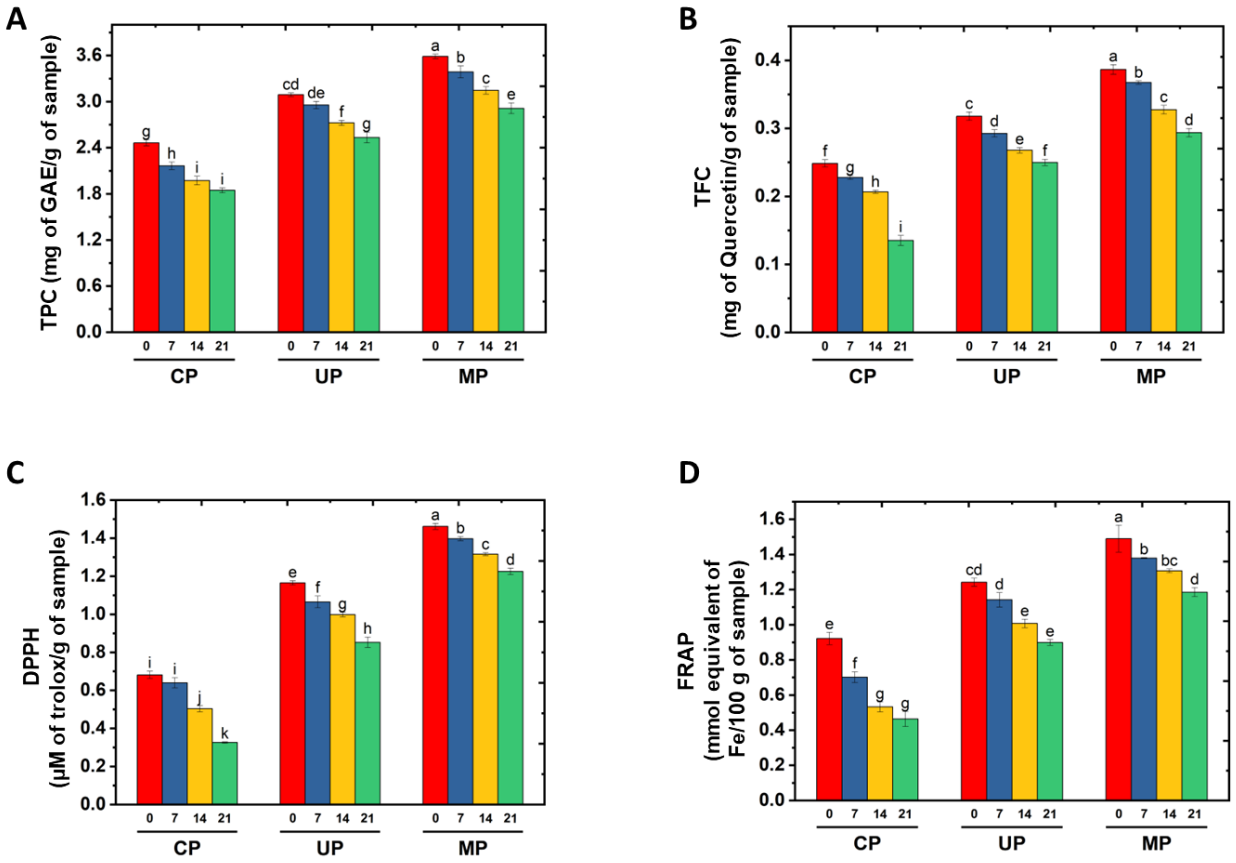

Figure 7-4: Effect of storage on functional and antioxidant properties of beverage samples (A) changes in TPC, (B) changes TFC, (C) changes in antioxidant properties (DPPH assay), (D) changes antioxidant properties (FRAP assay), CP= conventional processing, UP= ultrasound processing, MP= microwave processing

7.3.3 Correlations between TPC, TFC and antioxidant activities

Phenolics and flavonoid compounds are recognized for their ability to scavenge free radicals in various samples counteracting the harmful effects of reactive oxygen species by accepting electrons or hydrogen radicals resulting in their transformation into stable diamagnetic molecules (Prakash et al., 2007). In the present work, correlations between TPC, TFC and antioxidants were analyzed, and significant correlations were found between DPPH, FRAP and TPC (r = 0.97-0.98)

as shown in Figure 7-5. The antioxidant activities exhibited by plant materials are closely linked to their composition and concentration of bioactive compounds such as phenols, vitamins, flavonoids, and carotenoids (Ma & Huang, 2014).

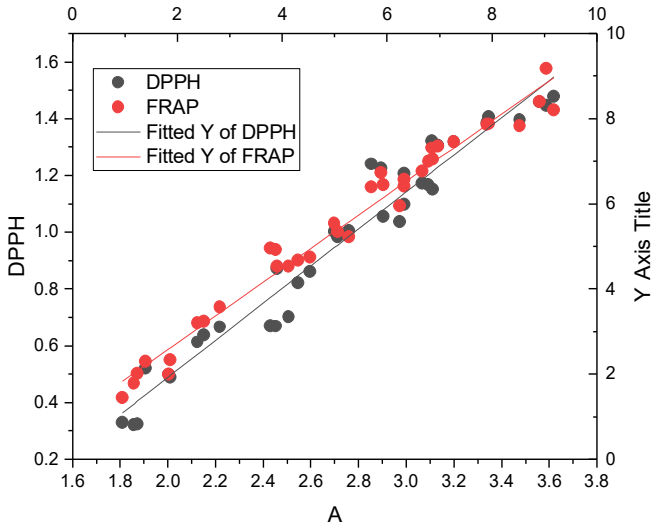

Figure 7-5: Correlation between Total Phenolic compounds and antioxidant activities of the beverage samples.

7.4 Conclusion

In today's consumer-driven market, there is a growing demand for products that not only taste fresh but also retain their nutritional properties for extended periods. Consumers are increasingly conscious of the importance of consuming foods that offer both great taste and optimal nutrition. To meet these expectations, it is essential to emphasize the development of processing techniques that can effectively preserve the freshness and nutritional quality of products over time. The findings of our study indicate that ultrasound and microwave processing techniques exhibit distinct advantages over conventional processing in terms of not only enhancing the yield but also retaining the bioactive and functional compounds during storage. This retention of valuable compounds is

crucial as it ensures the maintenance of the desired properties and benefits of the processed products over an extended period. The results highlight the significant potential of ultrasound and microwave processing as promising alternatives to conventional processing methods in various industrial applications opening up new opportunities for developing high-quality products with improved nutritional and functional attributes.

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Preface to Chapter 8

After completing the optimization, sensory evaluation, and nutritional analysis of the chickpea beverage, the research delved further into exploring the potential of its byproduct, chickpea okara. This subsequent study in Chapter 8 centred around a comparative analysis of chickpea okara flour obtained using three processing methods: microwave, ultrasound, and conventional processing. These findings offer an opportunity for sustainable food waste management and the development of healthier and more innovative food products.

Chapter 8: Physicochemical, microscopical and functional properties of chickpea okara flour extracted by different processing methods

Abstract

This study investigated the physicochemical, microscopical, and functional properties of chickpea (*Cicer arietinum*) okara flours. The flours were prepared from chickpea okara obtained as a by-product following the preparation of chickpea beverages using conventional, microwave and ultrasound processing. The assessment of the okara flours focused on evaluating the influence of the processing methods on their physicochemical, functional, and microstructural characteristics. Through comprehensive analyses, the study examined how the different processing techniques affected the composition and properties of the resulting okara flours. Furthermore, the study included a comparative mass balance analysis to assess the extraction efficiency of the three processing methods. The findings revealed significant variations in the composition and properties of the okara flours among the different processing methods. Each method exhibited unique effects on the physicochemical, functional, and microstructural attributes of the resulting flours. Consequently, it was not possible to identify a single "best" processing method for obtaining optimal okara flour characteristics since all flours had interesting and unique properties. Overall, this study provides valuable insights into the effects of different processing methods on chickpea okara flour. The findings highlight the importance of selecting an appropriate processing technique based on the desired properties and applications of the flour. The results can contribute to the development of tailored processing approaches for enhancing the utilization of chickpea okara flour in various food formulations, thereby promoting sustainability and reducing waste in the food industry.

Keywords: chickpea beverage, chickpea okara, conventional processing, ultrasound processing, microwave processing.

8.1 Introduction

The proper management of food waste is crucial for the sustainable growth of food systems, both economically and nutritionally and the health of our planet. The reduction of food waste has become a major focus of research and businesses as it has significant effects on food security, quality, safety, and the environment. Food processing waste often contains a range of valuable ingredients that can be used to create healthy products (Mckee & Latner, 2000). For example, dietary fiber, which is a vital component of a balanced diet, can be extracted from food processing waste and used as an ingredient in healthy foods like whole grain bread and cereals (Lu et al., 2013). Additionally, pigments like β-carotene and anthocyanins, which have strong antioxidant properties, can be extracted from food processing waste and used to create vibrant and nutritious foods (Kantifedaki et al., 2018). Organic acids, such as citric acid, have been recovered from apple pomace, pineapple peel, carrot waste and other similar food processing waste and have been used to add flavour and nutrition to foods (Nayak & Bhushan, 2019; Varshney, 2016). Finally, biodegradable and antimicrobial packaging for food materials has been produced and tested from food processing waste materials like grape pomace and lime peel (Zhang & Sablani, 2021).

By utilizing food processing waste in this way, the food industry can create healthier, more sustainable products while reducing waste.

When plant-based beverages are processed, a substantial amount of waste (also known as okara in the case of soymilk) is also produced after the filtration of the beverage and is often considered as

waste. However, it is a rich source of protein, fiber, and other valuable nutrients that can be recovered and repurposed through nutrition upcycling (O'Toole, 1999). A huge amount of okara is produced annually worldwide with an estimation of 8.5 metric tons in China only from tofu processing industries making its disposal significantly challenging (Ahn et al., 2010; Lu et al., 2013). According to an estimate, approximately 250 kg of okara can result from the production of 1000 L of soy beverage, and based on soy beverage consumption, it is estimated that approximately 14 million tonnes of okara are produced annually around the world, which is associated with serious environmental issues (putrefaction) and massive loss of valuable nutrients (Nguyen et al., 2013). Due to its high levels of water (80%), protein, dietary fiber, and lipids, okara decomposes naturally when left at room temperature (D'Toole, 1999). Okara can be converted to a dried flour form by drying and grinding with reduced storage volume and enhanced storage stability (Kamble & Rani, 2020). Being nutrient-dense, dried okara can not only be utilized to enrich foods but also to improve their quality and usability (Campderrós, 2017). In recent years, researchers have investigated the potential for enhancing the nutritional and functional properties of various food products, including bread, cookies, cakes, noodles, and yogurt, through the incorporation of okara (Kang et al., 2018; Mbaeyi-Nwaoha & Uchendu, 2016; Park et al., 2015). Earlier studies have shown that the structural features and digestibility of food items can be altered and enhanced by integrating them with okara (Kang et al., 2018; Schved & Hassidov, 2010). The incorporation of okara in different food products leads to an increase in the in vitro digestibility of protein and carbohydrates, which are important nutrients for the human body (Kamble & Rani, 2020). The fibre content of gluten-free cookies has been reported to increase substantially by the incorporation of the soymilk by-product okara (Ostermann-Porcel et al., 2017), which upon consumption can improve and regulate intestinal functions (Li & Komarek, 2017). Similarly addition of okara to

vegetable paste resulted in a notable enhancement of its protein (from 3.0 to 4.7 g/100 g) and lipid (from 5.6 to 6.6 g/100 g) contents (Guimarães et al., 2018). The observed improvements in protein and lipid contents highlight the potential of okara as a valuable ingredient for enhancing the nutritional composition of vegetable paste which can be utilized to enhance the nutritional content of other foodstuffs as well. Similarly, by-products/wastes obtained after processing of other plant-based averages (almond, oats, rice milk) can be utilized to prepare value-added food products with superior nutrition quality and sensory attributes (Lorente et al., 2023).

A lot of research has been done and reported regarding the physicochemical and functional properties of soymilk by-product okara and its utilization in various food products (Katayama & Wilson, 2008; Li et al., 2012; Wickramarathna & Arampath, 2003) but there is not much data and studies related to the by-products produced by processing of other types of plant-based beverages. In our previous research, we have optimized various parameters during the preparation of chickpea-based plant beverage (Sharma & Orsat, 2022). Further, in addition to conventional processing, the effects of microwave and ultrasound processing were also studied on chickpea beverage quality. The present study has focussed on a comparative study of the physicochemical parameters, and functional and microstructural properties of chickpea okara flours, by-product obtained from three different methods (conventional, microwave and ultrasound processing) of processing a chickpea beverage.

8.2 Material and Methods

The production and characterization of chickpea okara was carried out according to the schematic diagram shown in Figure 8-1. This diagram provides a concise overview of the prevailing

approaches in adopting production methods that are capable of minimizing waste generation and upcycling it to better use.

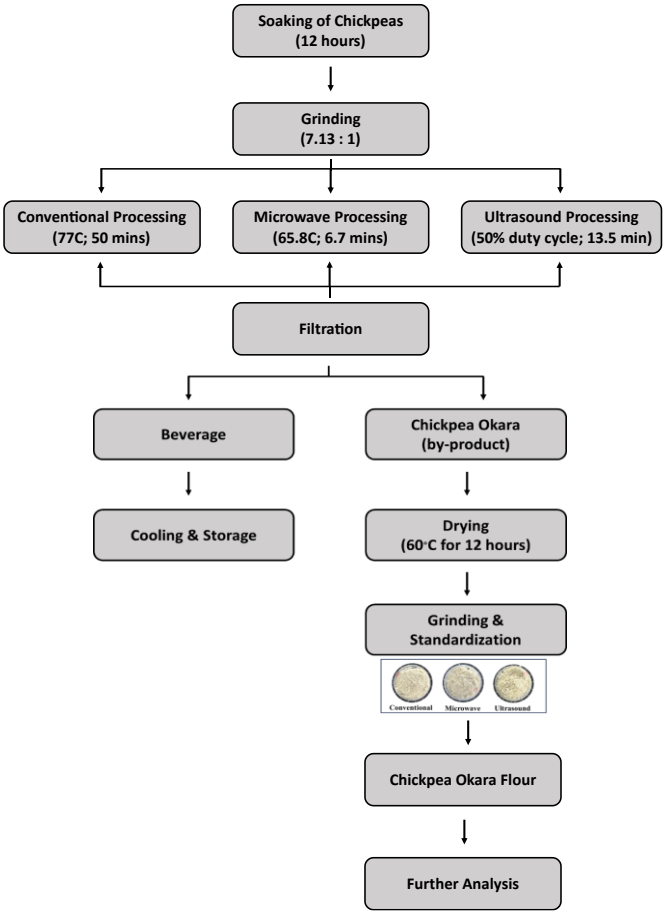

Figure 8-1: Schematic representation of the process followed to obtain chickpea okara flours.

8.2.1 Preparation of beverages and obtaining wet chickpea pulp

Chickpea okara (residue left after the filtration process) obtained after the preparation of a chickpea beverage by three optimized processes was dried for preparing the flour. For conventional processing, the parameters were a processing temperature of 77°C and 50 minutes duration

(Sharma & Orsat, 2022). For microwave processing, the process parameters were 65.8°C temperature for 6.7 minutes, while ultrasound processing used a 50% duty cycle for 13.5 minutes while maintaining a low temperature using an ice bath. The optimized processing conditions were obtained based on preliminary experiments and findings, indicating the best outcomes showing maximum protein content in the beverage (Chapter 6).

8.2.2 Drying chickpea okara to prepare flour samples

Further, chickpea flours were obtained using the procedure described by (Kamble & Rani, 2020; Lian et al., 2020). Chickpea beverages were filtered using a double-layered cheesecloth to separate the beverages from the chickpea okara. Wet okara samples were further dried in a conventional oven at 60°C for 12 hours. Dried chickpea pulp was further ground and standardized by passing through sieves to obtain uniform fine-grained flours. The flour samples were packed in airtight containers till further analysis.

8.2.3 Mass balance, extraction yield and comparison of the three processes

Extraction yield and protein content comparison between the three processing procedures used for the preparation of the beverages was compared using a mass balance approach i.e. amount of beverage produced per kg of chickpeas (raw material) used. The comparison was done between the beverage, wet okara and dried okara flour.

8.2.4 Proximate composition and physicochemical properties of flour samples

Moisture content was tested according to the methods mentioned in (AOAC, 1990). To begin with, a 3g sample was taken and subjected to a drying process at 105°C in a conventional oven until a

constant weight was achieved. The moisture content was then calculated as the percentage of water present in the sample relative to its total weight. Ash determination was done using the method described by (Czaja et al., 2020). Flour samples were measured out (5g) in ash dishes and placed in a muffle furnace, at a temperature of 550 °C. The samples underwent incineration in the furnace until they reached a state of light gray ash and achieved a constant weight. The incineration process typically lasted for a period of 7 hours. For determining the protein content, the Bicinchoninic acid (BCA) method was used as described by (Casal et al., 2000). For this method, a BCA assay kit obtained from Pierce Biotechnology was used and the procedure outlined in the user guide provided with the BCA assay kit was followed. To create a standard graph for protein quantification, Bovine serum albumin (BSA) was used as a reference standard. The total sugar content, including both free and bound sugars, was measured using the anthrone method as described by (Widyastuti & Giarni, 2015) with some modifications. To generate a standard calibration graph, glucose was used as the reference standard. Fat content was calculated using the method outlined by (Folch et al., 1957) with some modifications. A 10 mL mixture of chloroform and methanol in a ratio of 2:1 (volume/volume) was added to 0.2 g of flour and the tubes were then placed in a shaker and agitated for 20 minutes. Afterward, the solution was vacuum filtered using a 125mm filter to separate the solid particles from the liquid. 2 mL of a 0.3% sodium chloride (NaCl) aqueous solution was added to the filtered solution. The tube was then vortexed for 1 minute to ensure thorough mixing of the solution. Following the vortexing step, the tube was centrifuged at a speed of 2000 rpm for 5 minutes. This centrifugation step allowed the separation of two layers: the upper layer containing methanol and NaCl, and the lower layer containing chloroform and lipids. After decanting the upper layer, the lower layer was transferred to a pre-weighed aluminum dish. To remove the remaining solvent, the dish was placed on a heating tray

located inside a fume hood. The heat caused the chloroform to evaporate, leaving behind the lipid residue in the dish.

After evaporating the solvent, the aluminum dish was transferred to an oven set at 100°C and left for 15 minutes to ensure complete removal of any remaining traces of solvent. Once the dish had cooled down, it was weighed again, considering the weight of the empty dish. The difference in weight between the empty dish and the dish with the residue represented the amount of fat content in the original sample. Total dietary fibre content was determined by the enzymatic-gravimetric method described by (Krishnan et al., 2012) using Megazyme, KTFDR-200 A kit. For pH determination, samples were prepared by mixing chickpea pulp flours with distilled water in a ratio of 1:10. After mixing thoroughly reading was taken with a digital pH meter (AB15, Thermo Fisher Scientific, USA) after proper calibration (Kamizake et al., 2014). The color of the dried pulp samples was assessed using CIE -Lab values using a colorimeter (Konica Minolta, Osaka, Japan). The measurements provided values for L* (representing lightness), where 0 corresponds to black and 100 corresponds to white, a* (representing the red-green component) and b* (representing the yellow-blue component) (Yoshida & Prudencio, 2020).

8.2.5 Functional properties of the flour samples

A) The water-holding capacity (WHC) and oil-holding capacity (OHC)

The water-holding capacity (WHC) and oil-holding capacity (OHC) were determined according to the methods followed by (Kaur & Singh, 2005; Lian et al., 2020). To prepare the samples, distilled water (for WHC) and canola oil (for OHC) were added to dried chickpea pulp flour in an 8:1 ratio in a 50mL centrifuge tube. Samples were mixed by vortexing (10 sec), followed by

centrifugation at 1260g for 10 minutes using a centrifuge (Sorvall Legend X1R Centrifuge, Thermo Fisher Scientific, GmbH, Germany). After centrifugation, the supernatant was removed, and the remaining pellet was weighed. Further, WHC/OHC was measured using the following Equation [8-1].

$$WHC\ or\ OHC = \frac{weight\ of\ the\ pellet\ (gm)}{weight\ of\ chickpea\ okara\ (gm)} \quad [8\text{-}1]$$

B) Emulsifying activity index (EAI), Emulsifying stability index (ESI) and Emulsifying activity (EA)

For calculating the EAI, the method used by (Lian et al., 2020; Pearce & Kinsella, 1978) was used with certain modifications. A mixture consisting of 1.25 g of samples, 10 mL of canola oil, and 10 mL of distilled water was homogenized in a 50-mL centrifuge tube using a homogenizer (Fisher brand homogenizer 850) at a speed of 5000 rpm for 60 seconds. The resulting pre-emulsions were then vortexed, and their absorbance (A_o) was measured at a wavelength of 500 nm using a spectrophotometer (Thermo Fisher Scientific, USA). EAI was calculated using tabletion [8-2] as described by (Jiang et al., 2009) and shown below.

$$EAI\ (m^2/g) = \frac{2\ X\ T\ X\ A_0\ X\ N}{C\ X\ U\ X\ 100} \quad [8\text{-}2]$$

Where T is constant with a value of 2.303, A is the absorbance of the sample solution, N is the dilution factor, C is protein concentration before emulsion (g/mL), and U is the oil fraction volume at 0.25.

Further, for the Emulsifying Stability Index (ESI), the absorbance of the emulsion mixture was taken again after 10 minutes (A_{10}) and calculation was done using Equation [8-3] shown below.

$$ESI\ (min) = \frac{A_0}{A_0 - A_{10}}\ X\ 100 \qquad [8\text{-}3]$$

For calculating the emulsifying activity index (EA), the procedure described above for emulsifying activity (EAI) was used for the preparation of the emulsions. Further, the emulsion was separated from the rest of the solution using a centrifuge at 1000g for 5 minutes (ThermoFisher Scientific, Sorvall Legend X1R centrifuge). After centrifugation, the emulsified layer was distinct from the rest of the solution. The height of the emulsified layer was then measured, and EA was calculated using Equation [8-4] mentioned below.

$$EA\ (\%) = \frac{height\ of\ emulsified\ layer}{total\ height\ of\ mixture}\ X\ 100 \qquad [8\text{-}4]$$

C) Swelling capacity

Swelling capacity is an important property of flour which refers to its ability to absorb water and expand when hydrated. Swelling capacity plays an important role not only in the texture, structure, and volume of baked goods but helps in imparting thick and smooth texture when flours are used as thickeners in sauces, gravies, soups, and other dishes (Awuchi et al., 2019; Huang et al., 2019). The swelling capacity is an important parameter as it provides insights into the water absorption and expansion characteristics of the sample. By understanding how much water the sample can absorb and the subsequent increase in volume, it becomes possible to evaluate its suitability for various applications. Swelling capacity was determined using the method described by (Mateos-Aparicio et al., 2010) with certain modifications. In a graduated cylinder, 250 mg of the sample flours were mixed with 5 mL of distilled water, mixed well for 30 minutes to remove air bubbles

and left overnight undisturbed. The next step involved measuring the volume occupied by the sample in the graduated cylinder, expressed in millilitres (mL), indicating the expansion of the sample due to its ability to absorb water. The swelling capacity of the samples, expressed as mL/g, is calculated using Equation [8-5] shown below.

$$Swelling\ capacity\ (ml/g)\ = \frac{measured\ volume\ of\ the\ sample}{weight\ of\ initial\ sample} \quad [8\text{-}5]$$

D) Protein solubility in water

Protein solubility refers to the proportion or fraction of proteins that successfully dissolve in each solution, relative to the total protein content present. Studying protein solubility is critical in understanding flour properties and behaviour during baking, new product development, quality control and optimization. The protein solubility was estimated by the method described by (Kamizake et al., 2014; Raikos et al., 2014) with suitable modifications.

A mixture of the sample and distilled water in a ratio of 1:50 (mass/volume) was subjected to vigorous stirring using a homogenizer (Fisher brand homogenizer 850) for a duration of 2 minutes. Further, the mixture was centrifuged at 3000g for 25 minutes using a centrifuge (ThermoFisher Scientific, Sorvall Legend X1R centrifuge). Protein quantification was done using a BCA protein estimation kit. For estimating protein solubility in water, the protein content in the supernatant, obtained after centrifugation, was compared to the total protein content in the sample. The results were expressed using Equation [8-6] as a percentage in grams of soluble proteins/ total proteins present in the samples.

$$PS\ (\%) = \frac{protein\ content\ in\ supernatant}{total\ protein\ content\ in\ the\ sample} X\ 100 \quad [8\text{-}6]$$

8.2.6 Microstructure of the flour samples

The morphological characteristics of the flour samples were examined using a scanning electron microscope (SEM, TM3000, Hitachi, Japan). The samples were mounted on aluminum stubs using double-sided carbon adhesive tabs to ensure secure attachment. The SEM analysis was conducted at magnifications of 500X and 1500X, allowing for detailed observation of the morphological details. To prevent any heat-related modifications to the sample, an accelerating voltage of 5 kV was applied instead of higher voltages. The SEM micrographs were captured using the auto-contrast mode at a working distance of 9.5–9.8 mm.

8.2.7 Statistical analysis

All experiments were performed in triplicate, and the data were expressed as means with standard deviation. The one-way ANOVA (P-value < 0.05) and Tukey's test were performed and analyzed in Origin Pro by Origin Labs, USA.

8.3 Results and Discussion

8.3.1 Extraction yield and mass balance comparison of the three processes

A significant increase in the extraction yield was observed when microwave and ultrasound extraction was used in comparison to conventional extraction during the chickpea beverage production process. A comparative mass balance equation showing the total extraction yield and protein yield obtained by the three extraction processes is shown in Table 8-1. The beverage yield increased from 5.79±0.14 litres /kg of chickpeas used to 6.18±0.12 litres/kg when microwave extraction was used in place of conventional extraction while ultrasound extraction further increased the yield to 6.93±0.14 litres of beverage/kg of chickpeas. Therefore, the microwave

method led to a 6.73% increase in extraction yield, while the ultrasound method resulted in a more substantial improvement of 19.68%. The protein content obtained from all three extraction processes showed a similar trend, with notable increases observed in the case of microwave and ultrasound extraction methods. As indicated in Table 8-1, the protein content of the obtained beverage increased by 45.86% when using the microwave method, while the ultrasound method resulted in a slightly lower increase of 23.16%, as compared with the conventional extraction. The protein content of the chickpea okara also decreased accordingly i.e. as more protein leached out in solution, less protein was left out in the remaining okara. As indicated in Table 8-1, the weight of chickpea okara flour was lower when using microwave and ultrasound extraction which can be attributed to the increased leaching out of soluble components from wet-milled chickpeas into the aqueous phase facilitated by cell wall solubilization by the heating effect of microwaves and cavitation effect of ultrasound respectively (Preece et al., 2017; Varghese & Pare, 2019).

Table 8-1: Mass balance of chickpea beverage and leftover chickpea okara

Method of extraction	**Total Extraction yield**		**Protein content**	
	Beverage L/kg of chickpea	**Chickpea okara flour g/kg of chickpea**	**Beverage (%)**	**Chickpea okara flour (%)**
Conventional Extraction	5.84 ± 0.15^{b}	195.6±0.13^{c}	4.23±0.07^{c}	17.64±0.44^{a}
Microwave Extraction	6.18 ± 0.29^{b}	231.33±0.73^{b}	6.17±0.25^{a}	14.08±0.27^{c}

Ultrasound Extraction	6.93 ± 0.17[a]	250.20±0.91[a]	5.21±0.166[b]	15.77±0.36[b]

** Different letters refer to significant differences within each column (P<0.05)

8.3.2 Proximate composition and physicochemical properties of flour samples

The proximate composition of chickpea okara flour differed significantly among different processing methods (Table 8-2). Moisture content was found maximum (5.919±0.02 %) in chickpea okara flours obtained by microwave processing in comparison to conventional (5.378±0.06 %) and ultrasound processing (5.815±0.04%). The moisture content of food samples plays a crucial role in determining their microbiological stability and shelf life. The presence of low moisture content in food samples has been identified as a critical factor in inhibiting the growth and proliferation of microorganisms (Kavitha & Parimalavalli, 2014). Maintaining a low moisture content is highly desirable as it reduces microbial activity, extends the storage period of food products, and helps prevent food spoilage (Temple et al., 1996; Udedi et al., 2013). In addition to this, it is important to mention that the moisture content of okara can vary depending on the specific drying processes employed, this has been studied in soy okara, a by-product of soymilk and tofu processing (Guimaraes et al., 2020). A maximum amount of carbohydrate was observed in conventionally obtained chickpea okara (37.76±0.64) followed by ultrasound and microwave processing. Ultrasound and microwave processing are known to affect the extraction of carbohydrates and result in an increase in their yield in solvent as compared to conventional extraction techniques (Garcia-Vaquero et al., 2020; Mena-García et al., 2019). In terms of plant beverages, a large proportion of soluble carbohydrates get leached out in the water and the leftover okara is found to be rich in soluble carbohydrates and fibres (Lian et al., 2020; Santos et al., 2019). Soluble carbohydrates play a crucial role in the nutritional composition and sensory attributes of

plant-based beverages. They contribute to the overall sweetness, mouthfeel, and flavour profile of the final product. Earlier study have shown the presence of a relatively higher starch content in chickpea okara as compared to soy okara, suggesting it can act as a better thickening and binding agent (Lian et al., 2020). There was no significant difference found in fat, ash content and pH of the okara flours obtained by the three tested processes as shown in Table 8-2. Okara, despite being a by-product, serves as an excellent source of dietary fiber, making it a valuable ingredient for boosting the fiber content of food products (Vong & Liu, 2016). In the present study, there was no significant difference found in the total dietary fibre (TDF) of the okara flours obtained from conventional and microwave processing while the okara flour obtained after ultrasound processing was significantly lower. All color parameters for flours obtained by conventional, microwave and ultrasound processing are shown in Table 8-2. The study revealed that all color parameters of okara flours were affected by ultrasound, microwave, and conventional processing and were found to be significantly different. L* values representing brightness were found to be lower in okara flours obtained by ultrasound processing (81.46±0.04) as compared to those of microwave and conventionally processed flours. There was an increase in all color parameters (minimum in ultrasound processing) when conventional and microwave processing was used indicating intensification of luminosity, red and yellow tonalities. The colour changes may be attributed to the thermal effect of conventional and microwave processing in comparison to the nonthermal ultrasound process.

Table 8-2: Proximate composition and physicochemical properties of the chickpea okara flour samples.

Components	**Chickpea okara flour (CP)**	**Chickpea okara flour (MWP)**	**Chickpea okara flour (USP)**

Moisture (%)	5.378±0.06[b]	5.919±0.02[a]	5.815±0.04[a]
Carbohydrates (%)	37.76±0.64[a]	29.98±0.29[c]	32.567±0.42[b]
Fat (%)	2.57±0.51[a]	1.65±0.52[a]	2.19±0.43[a]
Ash (%)	1.39±0.29[a]	1.26±0.11[a]	1.312±0.13[a]
Fibre (TDF) (%)	20.70±0.63[a]	20.549±.35[a]	17.065±.46[b]
pH	6.26±0.11[a]	6.20±0.02[a]	6.37±0.03[a]
Color parameters			
L*	84.14±0.03[a]	82.88±0.1[b]	81.46±0.04[c]
a*	3.91±0.05[a]	3.48±0.09[b]	2.67±0.08[c]
b*	21.39±0.02[b]	21.68±0.01[a]	21.05±0.03[c]

** Different letters refer to significant differences within each row (P<0.05)

CP: Conventional processing

MWP: Microwave processing

USP: Ultrasound processing

8.3.3 Functional properties of the flour samples

Leguminous fibres i.e. dietary fibres from soy, chickpea, cowpea and other similar legumes are found to be rich in pectin and possess notable hydration properties, such as high Water Holding Capacity (WHC) (Huang et al., 2021). This can be attributed to the hydrophilic nature of pectin, a complex polysaccharide found in plant cell walls (Boulos et al., 2000). This property of legume

fibers, makes them suitable for applications as thickeners in various food products (Tosh & Yada, 2010).

As shown in Table 8-3, the WHC was found to be significantly different ($p < 0.05$) among different processing methods used. Maximum WHC was found in okara flours obtained after ultrasound processing i.e. 12.34±0.16. The observed result may be attributed to the change in the hydrophilic groups and the okara fiber structure by ultrasonic cavitation microstreaming effects (Wang et al., 2019). Studies have also suggested that improvement in WHC can also be due to the reduction in particle size, increased surface area, and thinner structure of the fibre particles imparted by ultrasonic processing, all of which can contribute to enhanced water absorption and retention capabilities (Chen et al., 2023; Ullah et al., 2018). A similar trend was observed in the case of swelling capacity which was found to be significantly higher in ultrasonically processed chickpea okara flour due to the same reasons mentioned above for WHC. In addition to this, microwave irradiation generates heat within the fibre matrix through molecular friction, leading to localized heating and potential degradation in fiber structure leading to a reduction in water holding capacity and swelling capacity (Gan et al., 2020). The oil holding capacity (OHC) plays a crucial role in various food applications and can have a significant impact on the quality and sensory attributes of the final product in terms of moistness, tenderness, and overall mouthfeel (Schneeman, 1999). There was no significant difference between the OHC of the conventionally and ultrasonically treated chickpea okara flour but was significantly higher than for the microwave-treated chickpea okara flours as listed in Table 8-3.

Okara fibres may possess inherent properties that promote strong bonding or adsorption of lipids, regardless of the physical processing method applied. These interactions could be attributed to the presence of specific functional groups or surface characteristics of the fibers that facilitate lipid

absorption and retention (Nguyen et al., 2015). The results suggested that interactions between the okara fibres and lipids have not been affected by the physical processing i.e. ultrasonic or conventional heat treatment at the given processing conditions. But the localized heating effect may have destroyed the complex porous structure of fibres resulting in a reduction in OHC (Gan et al., 2020). In addition to okara fibres, proteins also play a significant role in the absorption of oil and the binding capacity of lipids is enhanced by the presence of hydrophobic proteins (Du et al., 2014).

The emulsifying activity index (EAI) is a measure of a protein's ability to create a stable emulsion, which is like a well-mixed blend of two liquids that don't naturally 2mix, such as oil and water. The EAI helps in understanding how effectively the protein can hold the two liquids together at their boundary and is measured in terms of interfacial area per unit weight of the protein (Karaca et al., 2011). Also, emulsifying activity (EA) refers to the capability and capacity of proteins to form emulsions. It is a measure of the protein's ability to interact with both water and oil at the interface of an emulsion. The emulsifying activity of proteins is closely linked to their capacity to absorb and stabilize the interfacial area between water and oil in an emulsion (Du et al., 2014), and the duration or degree of emulsion stability is known as the emulsion stability index. The highest Emulsion activity index (EAI) was obtained for conventionally treated chickpea okara flour (8.29±0.11) m^2/g, followed by ultrasound and microwave treatments and a similar trend was observed for Emulsion activity EA as well, however Emulsion stability index (ESI) of microwave processed okara flour was found to be highest (1.67±0.08) min. The emulsifying properties of legume products, such as okara, is not solely attributed to the protein content only. Other components, such as starch, fat, and sterols, have also been suggested to contribute to the emulsifying properties and overall emulsion stability (Du et al., 2014; Lian et al., 2020).

Plant proteins can exhibit varying degrees of solubility in water, depending on several factors including the specific plant protein, structure, and the conditions under which solubility is being evaluated. The presence of hydrophilic amino acid residues that are capable of forming hydrogen bonds with water, leads to their dissolution in aqueous solutions (Qing et al., 2022). The highest protein solubility was reported in ultrasonically processed chickpea okara flour (53.57±0.63) %, followed by microwave, being significantly higher than conventionally processed samples. The results can be attributed to the cavitation effect generating localized high temperatures and pressures, resulting in protein denaturation and enhanced solubility. Additionally, the mechanical forces exerted by ultrasound can disrupt protein aggregates and improve the dispersion of proteins in water, further enhancing the solubility (Alavi et al., 2021; Jambrak et al., 2008). On the other hand, the microwave treatment results in exposing the protein-water mixture to microwave energy, which generates heat through molecular friction. This localized heating can lead to protein denaturation and unfolding, resulting in increased protein solubility (Varghese & Pare, 2019). The heat generated by microwave treatment can disrupt protein-protein interactions and expose hydrophobic regions, allowing for better interaction with water molecules and improved solubility (Hafez et al., 1985).

Table 8-3: Functional properties of the chickpea okara flour samples

Functional Properties	**Chickpea pulp flour (CP)**	**Chickpea pulp flour (MWP)**	**Chickpea pulp flour (USP)**
Water Holding Capacity (g/g sample)	11.52±0.22^{b}	10.49±0.40^{c}	12.34±0.16^{a}

Oil Holding Capacity (g/g sample)	4.61±0.29[a]	3.11±.15[b]	4.08±0.07[a]
Emulsifying activity index (m^2/g)	8.29±0.11[a]	5.56±0.33[c]	6.57±0.18[b]
Emulsifying stability index (min)	1.17±0.10[b]	1.67±0.08[a]	1.48±0.12[ab]
Emulsifying activity (%)	59.35±0.12[a]	53.77±0.15[c]	55.63±0.24[b]
Swelling capacity (mL/g sample)	3.86±0.10[b]	2.32±0.19[c]	4.37±0.13[a]
Protein solubility in water (%)	43.83±0.37[c]	49.61±0.46[b]	53.57±0.63[a]

** Different letters refer to significant differences within each row (P<0.05)

8.3.4 Microstructural Analysis

The microstructural analysis of particles of chickpea okara flour samples was carried out by scanning electron microscopy as shown in Figure 8-2. The micrograph of the flour samples displayed a heterogeneous composition with both loose and agglomerated components. It is evident from Figure 8-2(B), that the surface of the okara samples following microwave processing was found greatly destroyed suggesting microwave irradiation played an important role in breaking up cell walls. The interaction between microwaves and polar molecules of water results in molecular movement and rotation. This continuous reorientation of polar molecules generates molecular friction and rapid heating and a rise in pressure aiding in the disruption of the cell walls and the subsequent extraction of target compounds (Chan et al., 2011; Mandal et al., 2007). In

comparison to microwave processing, the impact on cell surfaces by ultrasonic processing was relatively milder as shown in Figure 8-2(C), due to the acoustic cavitation effect. The disruption of cellular structures, by microwave and ultrasound processing, results in better leaching of cellular components and enhanced yield of target compounds.

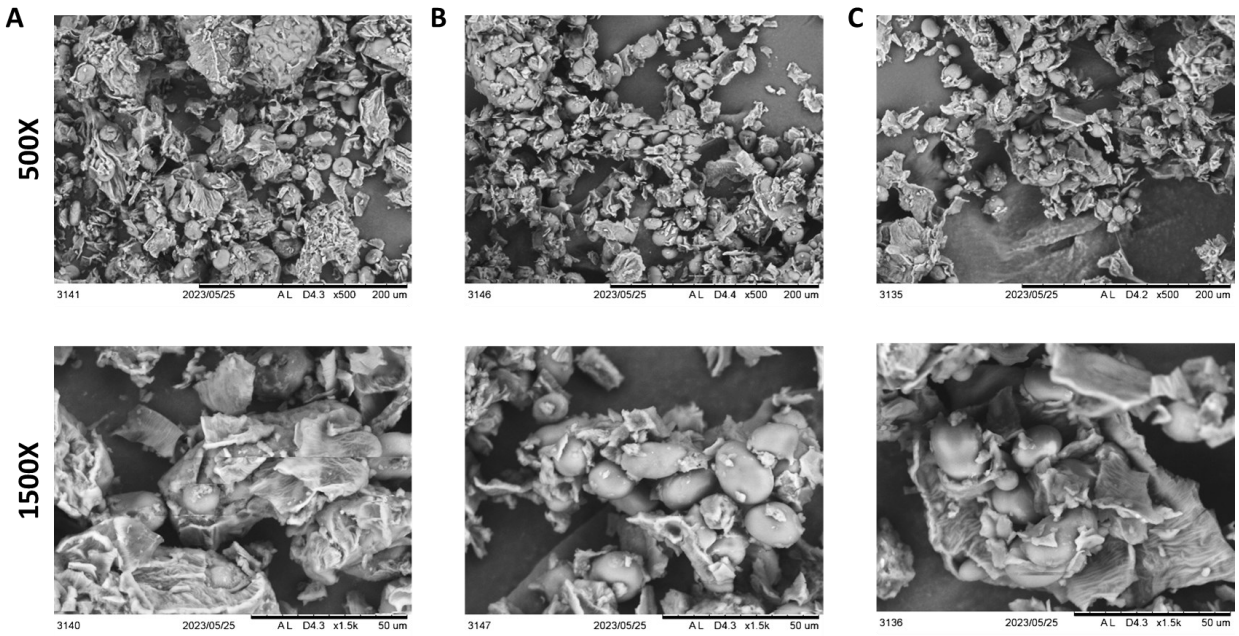

Figure 8-2: Scanning electron microscopy (SEM) images of chickpea okara flour samples obtained by (A) Conventional processing, (B), Microwave processing, (C) Ultrasound Process

8.4 Conclusion

In conclusion, the different processing methods employed in the preparation of chickpea beverages significantly influenced the physicochemical, microscopical, and functional properties of the okara flours prepared from the by-product of the beverage production. Each method produced okara flour with distinct physicochemical, microscopical, and functional properties, offering the potential to customize the flour for specific applications. By understanding the effects of different processing

methods, food manufacturers can tailor the properties of okara flour to meet specific product requirements. For example, although the protein content of the conventionally processed okara flour was significantly higher than the other two processes, the water holding capacity (WHC) of the ultrasonically processed flour was found to be highest. The findings of this study contribute to expanding the knowledge base regarding the effects of processing methods on okara flour properties. This understanding enables food scientists and manufacturers to harness the potential of chickpea okara flour as a valuable ingredient in various food formulations. Incorporating okara flour into food products can not only enhance their nutritional profile but also contribute to waste reduction by utilizing a by-product that would otherwise go to waste. Further research could delve into exploring the sensory attributes and nutritional composition of products incorporating okara flour.

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Chapter 9: Discussion

In recent years, the world has witnessed a significant shift towards healthier and more sustainable lifestyles. The rise in popularity of plant-based beverages is indicative of a broader shift in consumer behavior. Today's consumers are increasingly conscious of the environmental impact of their food choices and are seeking sustainable and eco-friendly alternatives. They are also more health-conscious, opting for products that align with their dietary requirements, such as lactose-free, gluten-free, or allergen-free options. Furthermore, the availability of information through social media, online platforms, and scientific research has empowered consumers to make informed choices about their diets. As a result, there is a growing demand for transparency and clean-label products, leading to a rise in plant-based beverages made with simple, recognizable, and natural ingredients. One of the most prominent developments in this space is the rise of new-edge plant-based beverages. These innovative drinks, derived from a variety of plant sources, have gained immense popularity due to their numerous health benefits, environmental sustainability, and ethical considerations. Protein-rich plant-based beverages are in high demand among consumers seeking alternatives to animal-derived proteins. However, the main caveat that has spurred new research in plant-based beverages is the need to overcome the limitations and challenges associated with existing products. Consumers are increasingly seeking plant-based alternatives, but there are concerns regarding nutritional balance, taste, texture, allergenic potential, sustainability, functional benefits, and alignment with consumer preferences. To address these concerns, researchers are actively pursuing innovative solutions. Moreover, researchers are exploring novel approaches to improve the taste and texture of plant-based beverages, aiming to offer sensory experiences comparable to traditional dairy products. Additionally, researchers are

focusing on sustainable sourcing, manufacturing processes, and eco-friendly packaging to reduce the environmental impact. The inclusion of functional ingredients to provide additional health benefits and understanding consumer preferences through market studies are also driving new research efforts.

Addressing allergenic potential by investigating alternative plant sources is another critical aspect of the ongoing plant-based research focus. **The present study focuses on a similar aspect by exploring chickpeas (*Cicer arietinum*) as a potential source of plant-based beverages.** Chickpeas being nutrient-dense, are a rich source of protein, dietary fiber, vitamins, and minerals. This nutritional profile aligns with the growing consumer demand for wholesome and nutritious plant-based options. Chickpea-based beverages can provide a well-rounded and balanced alternative that caters to the nutritional needs of health-conscious individuals.

While popular plant-based beverages like almond milk and soy milk have gained popularity, they also have certain caveats that chickpea-based beverages can address. For example, almond milk has raised concerns regarding its environmental impact due to the water-intensive cultivation needs of almonds. Chickpeas, on the other hand, require less water and have a lower environmental and carbon footprints, making them a more sustainable choice. Additionally, soy milk, while widely consumed, can trigger allergies in some individuals due to soy allergens. Soy is recognized as one of the prominent allergens and is often ranked among the top 10 common food allergens globally. On the contrary, chickpea-based beverages can offer an allergen-friendly alternative, appealing to those with soy allergies or sensitivities. Furthermore, chickpeas offer a versatile and neutral flavor profile that can be easily customized and adapted to various taste preferences, setting them apart from the sometimes-distinct flavors of other plant-based beverages.

Taking the above points into consideration, the present study was conducted for preparing plant-based beverages from chickpeas and further nutritional evaluation was carried out. The initial step in the preparation of chickpea-based beverages involves process optimization to determine the impact of various process parameters. This optimization aims to identify the ideal conditions and parameters that can maximize the quality and nutritional composition of the beverage. Therefore, **objective 1** of this thesis titled **"Optimization of extraction Parameters for Preparation of *Cicer arietinum*-based Beverage using Response Surface Methodology"** was aimed to optimize the extraction parameters for the preparation of chickpea-based beverages to maximize their nutritional properties and minimize their beany flavor. The factors investigated were the water-to-chickpea ratio, extraction temperature, and extraction time. The water-to-chickpea ratio varied between 6:1 and 12:1, while the extraction temperature ranged from 40°C to 80°C, and the extraction time varied from 10 minutes to 60 minutes.

The responses measured in the study included the total protein content, total sugar content, total solids, and specific lipoxygenase (LOX) activity. For optimization, a Central Composite design, coupled with Response Surface Methodology (RSM), was used to determine the optimal extraction conditions. Based on the predictions, the optimal conditions were found to be a water-to-chickpea ratio of 7.13:1, an extraction temperature of 77.9°C, and an extraction time of 51 minutes. When the extraction was performed under these optimized conditions, the resulting chickpea-based extract exhibited the following properties: a protein content of 4.12 g/100 mL, a total carbohydrate content of 5.78 g/100 mL, a total solid content of 10.57 g/100 mL, and a specific LOX activity of 0.0084 units/mg of protein.

Optimizing the nutritional content, specifically protein, in preparing chickpea beverages not only provides consumers with a convenient and accessible protein source but also helps in achieving a

balanced nutrient profile. Protein-rich plant-based beverages can contribute to satiety, aid in weight management, and support overall health and wellness. Moreover, the minimized specific LOX activity helps to mitigate the beany flavor commonly associated with chickpeas. This is particularly important as it enhances the overall taste profile of the beverage, making it more appealing to consumers.

The optimized beverage was further evaluated in **objective 2**, titled "**Quality evaluation and comparative physicochemical and sensory studies of optimized chickpea beverage**." The study was divided into three parts. In the first part, an evaluation of the protein quality of the optimized chickpea beverage was done. The protein scoring method, in vitro protein digestibility, corrected amino acid score (PDCASS) was used to assess protein quality in the chickpea beverage. The PDCAAS scoring pattern ranges from 0 to 1 reflecting the protein's capacity to fulfill nutritional needs. The amino acid profiling of optimized chickpea beverage revealed methionine and cysteine as the limiting amino acids. The PDCAAS score for the optimized beverage was determined to be 0.89, signifying that it can meet around 89% of the essential amino acid needs of the reference population. Further, the optimized chickpea beverage demonstrated a significantly higher viscosity (27.36±4.8 mPa.s) compared to bovine milk (7.76±2.7 mPa.s) at a shear rate of 10 (1/sec) ($p < 0.05$). The beverage's particle composition, including protein aggregates and oil droplets, contributed to its distinct properties. It displayed a well-controlled unimodal distribution of particle sizes below 100 μm, leading to improved stability, smoothness and mouthfeel. The second part of the study aimed at a comparative nutritional assessment of the chickpea beverage with other plant-based beverages. The results showed significant variations in protein content among the beverages, with the highest protein content observed in the optimized chickpea beverage (4.86±0.60%), followed by soy, and the lowest protein content in rice beverage

(0.15±0.02%). This high protein content in the chickpea beverage can be attributed to the superior solubility of chickpea proteins in water compared to soy proteins. Regarding carbohydrates, there was also a significant difference among the beverages, with rice beverage containing the highest carbohydrate content (11.04±0.51%). However, there were no significant differences in the ash content of the beverages. On the other hand, the almond beverage had the highest lipid content, which differed significantly from other beverages. The results emphasize the promising potential of chickpea-based beverages as a nutritious and protein-rich alternative to other plant-based choices. The optimized chickpea beverage showed outstanding performance by delivering a significant protein content while maintaining excellent solubility, enhancing its nutritional value and overall quality. This underscores the advantages of including chickpea-based beverages in diets as a viable and sustainable plant-based protein source.

Sensory evaluation plays a vital role in the preparation of a new beverage, offering invaluable insights and guidance throughout the development process. By assessing the sensory attributes such as taste, aroma, appearance, texture, and aftertaste, sensory evaluation helps to gauge consumer acceptance and preference. Understanding how consumers perceive and respond to the beverage allows for targeted improvements and refinements to align with their expectations. This evaluation serves as a quality control measure, ensuring that the final product consistently meets the desired sensory standards. Additionally, sensory evaluation aids in market positioning and differentiation by identifying the unique sensory characteristics of the beverage and leveraging them for effective branding and marketing strategies. Furthermore, it provides valuable consumer insights and enables adaptation to changing market trends, ensuring the beverage remains relevant and appealing. Overall, sensory evaluation is indispensable for creating a successful new beverage that not only meets consumer expectations but also stands out in a competitive market.

In recognition of the significance of sensory evaluation, a comprehensive and comparative sensory evaluation was conducted for the chickpea beverage in the third part of the study. This evaluation aimed to deeply understand the sensory attributes and consumer response to the beverage. Through rigorous assessment of appearance, mouthfeel, flavour, taste, after taste and overall acceptability, the sensory evaluation provided valuable insights into consumer acceptance and preference. The comparative results indicate that the almond beverage received the highest overall acceptability rating, followed closely by the chickpea beverage, while the soy beverage obtained the lowest rating.

Among other attributes, rice beverage received the highest score for appearance, whereas chickpea beverage obtained the lowest score in this attribute. In terms of mouthfeel, the chickpea beverage received the highest score among all the beverages evaluated, indicating its excellence in this attribute. Mouthfeel is recognized as a significant factor in enhancing the overall sensory experience of a beverage, contributing to texture, thickness, smoothness, and tactile sensations. The remarkable performance of the chickpea beverage in terms of mouthfeel signifies that it provided an exceptional and desirable sensory experience to the evaluators.

The sensory evaluation results further revealed positive ratings for the flavor and taste of the chickpea beverage, with the almond beverage scoring slightly higher. Additionally, the aftertaste of the chickpea beverage was also rated positively, albeit slightly outperformed by the almond beverage suggesting the chickpea beverage leaves a pleasant and satisfying lingering taste, contributing to the overall sensory experience. By considering the positive feedback received for different sensory attributes, such as mouthfeel, flavour, and taste, manufacturers can focus on refining and enhancing these aspects to strengthen the market appeal of the chickpea beverage.

In addition to the conventional processing of plant-based beverages, novel processing techniques, such as ultrasound and microwave, have emerged as promising methods to enhance the nutritional properties of plant-based beverages. These innovative technologies offer unique advantages in terms of extracting and improving the nutritional content of these beverages. Moreover, these novel processing techniques offer potential sustainability benefits, such as reduced energy consumption and lower environmental impact compared to traditional extraction methods. Therefore, **objective 3** of the thesis titled **"Effect of Microwave and Ultrasound extraction on protein and quality parameters of chickpea beverage"** aimed to evaluate how the application of microwave and ultrasound technologies can optimize the extraction process and improve various quality attributes of the beverage.

The results yielded that both microwave and ultrasound treatments led to a significant increase in the protein content of the beverage ($p < 0.05$). Microwave processing (optimized conditions: 65.8 °C and 6.7 minutes) resulted in a remarkable 47.78% increase in protein content, while ultrasound processing (optimized conditions: duty cycle of 50% and 13.5 minutes) showed a substantial 21.22% increase compared to conventional methods.

In addition to protein content, other important beverage characteristics like protein solubility, In vitro protein digestibility (IVPD), and Trypsin inhibitor (TI) activity were also assessed and compared at optimized conditions, and significant improvements were observed ($p < 0.05$). These findings indicate that microwave and ultrasound treatments not only enhance protein content but also positively impact the functional properties and nutritional value of the chickpea beverage. While both microwave and ultrasound techniques were found to be superior to conventional processing, there were differences in their effectiveness. Microwave processing showed slightly better results in enhancing the nutritional properties of the beverages compared to ultrasound.

However, ultrasound was gentler in its approach, making it a viable option for processing sensitive compounds while still achieving notable improvements. Apart from enhancing the nutritional properties, microwave and ultrasound techniques also offer improved storage stability compared to conventional processing. These innovative methods better retain the beverage's characteristics and functional properties, including bioactive compounds. This ensures that the beverage maintains its quality and efficacy over time, making microwave and ultrasound processing advantageous for producing plant-based beverages with enhanced shelf life and sustained bioactive content. Therefore **objective 4** of the study **"Storage studies of chickpea beverage: Effects on the physicochemical and functional properties and microbial analysis"** was focused on evaluating the effects of conventional, ultrasound, and microwave processing techniques on the stability and quality of chickpea beverage during a 21-day refrigerated storage period. Both ultrasound and microwave processing significantly improved the yield of bioactive compounds compared to conventional processing. Ultrasound processing increased total phenolic compounds by 27.11% and total flavonoid content by 29.16%, while microwave processing showed a remarkable increase of 47.32% in total phenolic compounds and 58.34% in total flavonoid content. During the storage period, all beverages experienced a decline in bioactive compounds and antioxidant activities. However, beverages processed with ultrasound and microwave techniques retained the total phenolic compounds more effectively, with a decrease of only 18.71% and 17.79%, respectively, compared to conventional processing. In addition, the microbial study demonstrated that microwave and ultrasound-processed beverages had lower total bacterial and fungal counts throughout the storage period. The lower total bacterial and fungal counts observed in the microwave and ultrasound-processed beverages indicate improved microbial safety during storage. Importantly, the different processing techniques did not significantly alter the pH and

acidity values of the chickpea beverage. In conclusion, the study reveals that the application of ultrasound and microwave processing techniques efficiently retains the chickpea beverage's bioactive compounds and nutritional content, while also maintaining low microbial counts. This not only prevents spoilage and ensures food safety but also significantly prolongs the beverage's shelf life.

Proper management of food waste is vital for sustainable food systems and the health of our planet. Food processing waste contains valuable ingredients like dietary fiber, antioxidants, and organic acids that can be repurposed to create healthy and sustainable products. Okara, a by-product of plant-based beverage (soymilk) processing, is a rich source of protein, fiber, and nutrients. By converting okara into dried flour, it can be used to enhance the nutritional composition and functional properties of various food products. Therefore, **objective 5** of the study titled "**Physicochemical, microscopical and functional properties of chickpea okara flour extracted by different processing methods"** focuses on comparing the physicochemical and functional properties of chickpea okara obtained from conventional, microwave, and ultrasound processing methods. Understanding the potential of such by-products can lead to the development of nutritious and environmentally friendly food products while ensuring the eco-friendliness and circularity of the plant-based beverage sector.

The study compared the physicochemical, functional, and microstructural properties of chickpea okara flours obtained through conventional, microwave, and ultrasound processing methods. Significant differences were observed in moisture content, carbohydrate content, water-holding capacity, emulsifying properties, and protein solubility among the different processing techniques. Ultrasonic processing resulted in higher moisture content, water-holding capacity, emulsifying activity, and protein solubility compared to conventional and microwave processing. Microwave

treatment, on the other hand, showed higher oil holding capacity and emulsion stability index. The microstructural analysis revealed that microwave treatment caused significant destruction to the surface of the okara particles, indicating the breakdown of cell walls. The results highlight the impact of different processing methods on the composition and properties of chickpea okara flour, which has significant implications for its potential applications in various food formulations.

The importance of this research lies in understanding how various processing methods affect the properties of chickpea okara flour. By optimizing the processing technique, food manufacturers can tailor the characteristics of the flour to suit specific applications in food products. The findings offer insights into utilizing okara as an ingredient to enhance the nutritional content, sensory attributes, and overall quality of food formulations. Additionally, the study contributes to reducing food waste and promoting sustainability in the food industry by efficiently utilizing by-products. The knowledge gained from this research can pave the way for innovative and eco-friendly approaches to incorporating okara into food products, ultimately leading to a more resource-efficient and environmentally conscious food system.

This thesis significantly emphasizes the potential of exploring new plant sources, such as chickpeas, to develop innovative plant-based beverages that boast excellent nutritional properties and sensory appeal. The comprehensive research conducted in this thesis sheds light on the importance of utilizing chickpeas as a valuable and sustainable ingredient for beverage development. Furthermore, the research delves into the impact of innovative extraction techniques, namely microwave and ultrasound, on the protein content and overall quality of the chickpea beverage. This exploration highlights the potential of non-thermal technologies in preserving nutrients and improving product safety. Additionally, the thesis explores the utilization of by-products, specifically chickpea okara flour, in developing new products, exemplifying waste

upcycling and sustainability. The research showcases the repurposing of the by-product as valuable and nutritious ingredients that can be incorporated into various nutritious food formulations while minimizing food waste.

Conclusion

In conclusion, this thesis focused on exploring the potential of *Cicer arietinum* (chickpea) as a versatile and sustainable source of functional food ingredients and functional nutrition. The research encompassed several studies, including the optimization of extraction parameters for preparing chickpea-based beverages using response surface methodology, physicochemical characterization, sensory evaluation of the optimized beverage, and the effects of microwave and ultrasound extraction on its protein and quality parameters. Additionally, storage studies were conducted to investigate the changes in the chickpea beverage's physicochemical and functional properties and microbial analysis over time. Furthermore, the physicochemical, microscopical, and functional properties of chickpea okara flour obtained through different processing methods were evaluated.

The optimized chickpea beverage exhibited favorable physicochemical, sensory, and nutritional attributes, making it a promising candidate for further product development. Moreover, the utilization of ultrasound and microwave techniques showcased their ability to enhance the yield of bioactive compounds and microbial safety, contributing to the preservation and nutritional value of the beverage. The study on chickpea okara flour revealed its potential as a valuable ingredient with unique functional properties for improving the nutritional composition and sensory attributes of various food products.

Through this thesis, we have shed light on the importance of sustainable food production, waste reduction, and the development of nutritious and appealing plant-based food options. The future recommended studies outlined herein offer exciting opportunities to explore the vast potential of chickpea-based products and expand their applications in the food industry. This research

contributes to the ongoing efforts to create a more sustainable and healthier food system, benefitting both human health and the environment.

Future work recommendations

The future work recommendations regarding this thesis include:

1. **Evaluating the Health Benefits and Nutritional Impact**

   Extending the research on the optimized chickpea beverage, it would be interesting to conduct in-depth studies on its health benefits and nutritional impact. Here health benefits focus on the positive effects a food product has on specific health outcomes, while nutritional impact emphasizes its contribution to overall nutritional intake and dietary patterns. Investigating the bioavailability of bioactive compounds and antioxidants in the beverage through in vivo studies or human clinical trials may help in assessing its potential effects on specific health outcomes, such as antioxidant status, inflammation, and cardiovascular health. This would provide valuable evidence of the beverage's health-promoting properties and contribute to its positioning as a functional and nutritious plant-based beverage.

2. **Consumer Behavior and Market Analysis**

   Conducting consumer behaviour studies, to understand the factors influencing consumers' preferences and purchase intentions towards the chickpea beverage, may help in evaluating the market potential and consumer acceptance of the product in different demographic segments. Further, the identification of potential barriers to adoption and analysis of the competitive landscape and market trends for plant-based beverages would help to identify opportunities for successful product positioning and market penetration.

3. **Process Optimization and Scaling-Up**

Focusing on optimizing the production process of the chickpea beverage on a larger scale could help in investigating the feasibility of commercial-scale production while maintaining the product's quality and nutritional attributes. Further, conducting comprehensive cost-effectiveness analyses to gauge the economic viability of large-scale production while also identifying and addressing potential barriers that may hinder the scaling-up process may lay the groundwork for successfully transitioning the chickpea beverage from a laboratory-scale concept to a sustainable and economically viable commercial product.

4. **Value-Added Product Development**

   Utilizing the chickpea beverage as a base for developing value-added products beyond traditional beverages, for example, exploring the formulation of plant-based (such as chickpea) dairy alternatives, like frozen desserts, yogurts or functional food products enriched with specific nutrients and better sensory profiles can help to expand the product line and cater to diverse consumer needs.

5. **Applications of Chickpea Okara Flour**

   Further exploring the potential of chickpea okara flour in different food formulations and investigating its functionality in gluten-free products, meat analogs, and other plant-based food alternatives can pave the way for meaningful upcycling practices and foster sustainability efforts with a positive environmental impact, helping in moving toward a more circular and eco-conscious approach to food production and consumption.

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