Extraction of phenolic compounds from wheat by developing a rapid and reliable microwave-assisted method

By

Kemashalini Kirusnaruban

A Thesis Submitted to Faculty of Graduate Studies of the University of Manitoba

in Partial Fulfillment of the Requirements of Degree of

MASTER OF SCIENCE

Department of Food and Human Nutritional Sciences

University of Manitoba

Winnipeg

Dedication

I dedicate this thesis to my dearest mother Indradevi, for her boundless love and remarkable dedication that have shaped me into the person I am today, my loving husband Kirusnaruban and my little prince Aathiran, for their affection and enduring love. I would like to dedicate this work to free education of Sri Lanka.

Contents

ABSTRACT

Phenolic compounds (PC) are vital secondary plant metabolites with recognized health benefits, including antioxidant and anti-inflammatory properties. These compounds help plants manage environmental stress and contribute to their resilience, with a higher concentration found in the outer layers of wheat, particularly in the bran. Current extraction methods are time-consuming and often involve the use of acids, alkalis, and organic solvents, which pose environmental concerns. Therefore, efficient, green, and rapid methods for extracting these phenolic compounds are essential, especially in the food industry, which has an increasing demand for sustainable processes.

We hypothesize that Microwave-Assisted Extraction (MAE) will serve as a superior methodology for phenolic compound extraction from wheat, offering improved yield, faster processing, and enhanced environmental sustainability compared to conventional extraction (CE).

This study aims to validate MAE as a green and efficient alternative to CE for quantifying phenolic compounds in Canada Western Red Spring wheat kernels, utilizing whole grains and flour. Additionally, MAE was applied to wheat kernels with varying degrees of pearling to assess its effectiveness across different wheat layers. Two solvent systems were tested: 80% ethanol (v/v) and water, with MAE conditions including temperatures between 140°C and 180°C and extraction times of 5, 10, and 15 minutes. A solid-to-liquid ratio of 1:9 was used for whole kernels, and 1:99 for flour fractions, to prevent starch gelatinization.

Results showed that MAE outperformed CE in terms of TPC yield, with water as a solvent leading the highest TPC of 5.41 ± 0.10 mg/g DW ($p < 0.05$) at 170°C for 10 minutes, compared to 80% ethanol extracts at 3.52 ± 0.24 mg/g DW ($p < 0.05$). CE yielded the lowest TPC at 2.78 ± 0.18 mg/g DW ($p < 0.05$). Among individual phenolic acids, gallic acid was found to be the most prominent in all MAE extracts.

Additionally, phenolic acid extraction was evaluated in six pearled fractions of wheat subjected to different pearling time, ranging from 50 to 450 seconds. Shorter pearling times resulted in higher phenolic acid concentration. Also, TPC values were found to be higher in the outer bran layers, supporting the conclusion that PCs are more concentrated in these parts of the wheat grain.

A significant negative correlation was observed between pearling time and phenolic acid content, emphasizing the importance of optimizing pearling times for enhanced phenolic yields.

It must be stressed that for the first time, a method for extracting phenolic acids is proposed using water as a solvent and extracting the compounds directly from the wheat kernel.

This study highlights the efficiency and sustainability of MAE for phenolic extraction, offering a faster, greener alternative to traditional methods. The findings provide insights into enhancing the phenolic content of wheat-based products, with implications for functional food development and wheat breeding.

Keywords: Microwave-assisted extraction (MAE), Conventional extraction (CE), Total phenolic content (TPC), Gallic acid, Wheat, Green extraction

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisor, Dr. C.M. Rosell, for her invaluable professional and financial support through the NSERC Engage Grant (RGPIN-2023-04289) and Sustainable CAP, as well as her personal motivation and scientific guidance throughout this project. Her acceptance of me as a graduate student in her research team is deeply appreciated.

I am also grateful to Dr. Thomas Netticadan and Dr. Michael Eskin for their valuable contributions as members of my thesis supervisory committee. Their insightful suggestions and advice have been instrumental to the success of this project and the development of my thesis. A special thanks to Dr. Michael Eskin for his extensive efforts in the revision and improvement of the manuscripts, which were crucial for producing high-quality publications.

I would like to acknowledge Cereal Canada for providing CWRS wheat grain samples, as well as for their assistance with the pearling machine. I am very much thankful to Dr. Nicola Gasparre for his insights into chemistry and phenolic acids during his tenure as a post-doctoral researcher. His guidance in laboratory skills, statistical analysis, and scientific writing helped me build my confidence in research. My sincere appreciation also goes to Dr. Ruchira Nandasiri for his training and support in HPLC-DAD analysis of wheat extracts his continuous support on his expertise of phenolic compounds.

I am thankful for the technical support provided by the staff of the Richardson Centre for Food Technology and Research (RCFTR). Additionally, I extend my gratitude to all my colleagues in Dr. Rosell's research team for fostering an enjoyable research environment and helping during challenging times.

Finally, I wish to express my heartfelt thanks to my husband, amma, and other family members for their unwavering encouragement and support throughout my program.

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANN- Artificial Neural Networks

CCD - Central Composite Design

DW- Dry Weight

EAE - Enzyme Assisted Extraction

FAE - Ferulic Acid Equivalence

GAE - Gallic Acid Equivalence

HPLC - High Performance Liquid Chromatography

LLE - Liquid -Liquid Extraction

MAE - Microwave-Assisted Extraction

PC - Phenolic Compounds

PF - Pearled Fraction

PLE - Pressurized Fluid Extraction

RFF – Refined Flour Fraction

RKF – Refined Kernel Fraction

RPM – Revolution Per Minute

RSM - Response Surface Methodology

SEM - Scanning Electron Microscope

SFE - Supercritical Fluid Extraction

SLE - Solid-Liquid Extraction

TPC - Total Phenol Content

UAE - Ultrasound Assisted Extraction

CHAPTER 1

Introduction, objectives and working plan

1.1 Background

Phenolic compounds are a diverse group of secondary metabolites produced by plants to adapt to environmental stressors, including biotic factors like pathogens and abiotic factors such as UV radiation and drought. Structurally, they contain at least one hydroxyl group attached to an aromatic ring and are categorized into various groups, including phenolic acids, flavonoids, tannins, and lignans (**Figure 1.1**). Beyond their biological roles in plants, phenolic compounds have gained significant attention for their health-promoting properties, particularly their antioxidant, anti-inflammatory, and anticancer activities (Masisi et al., 2016). These bioactive properties make them valuable components in the development of functional foods and nutraceuticals, contributing to human health by combating oxidative stress and inflammation.

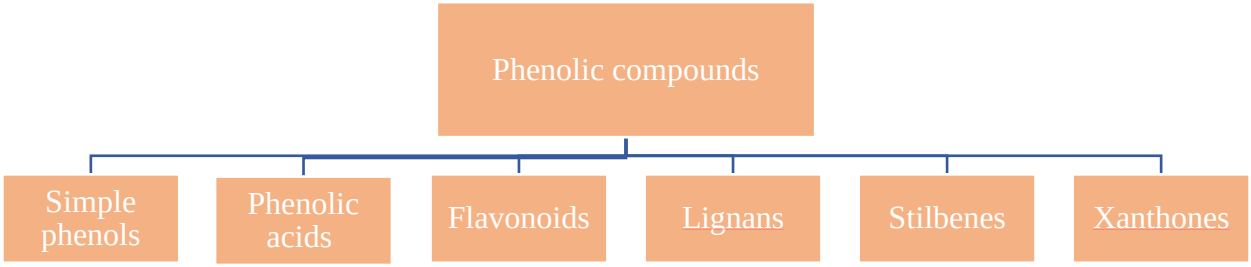


Figure 1.1: Classification of phenolic compounds

Wheat (*Triticum aestivum* L.), one of the most widely cultivated cereal crops of the *Poaceae* family, plays a crucial role in global human nutrition. In 2023, worldwide wheat production reached 781 million tons, with Canada ranking as the seventh-largest producer, contributing approximately 33.8 million tons (Agriculture and Agri-food Canada, 2023; FAO, 2023). A wheat

kernel comprises three primary components: the outer bran, the starchy endosperm, and the germ (Onipe et al., 2015) (**Figure 1.2**). The bran is rich in fiber and bioactive compounds, along with vitamins, and minerals, while the germ is the reproductive part, containing fats, antioxidants, and other essential nutrients. The endosperm, which constitutes the largest portion of the kernel, is primarily composed of starch, accounting for approximately 71% of the kernel's total mass. In addition, wheat contains 14% protein, 9.8% fiber, 1.9% lipids, 1.6% minerals, and 1.7% other components, including phenolic compounds (Tullio et al., 2020).

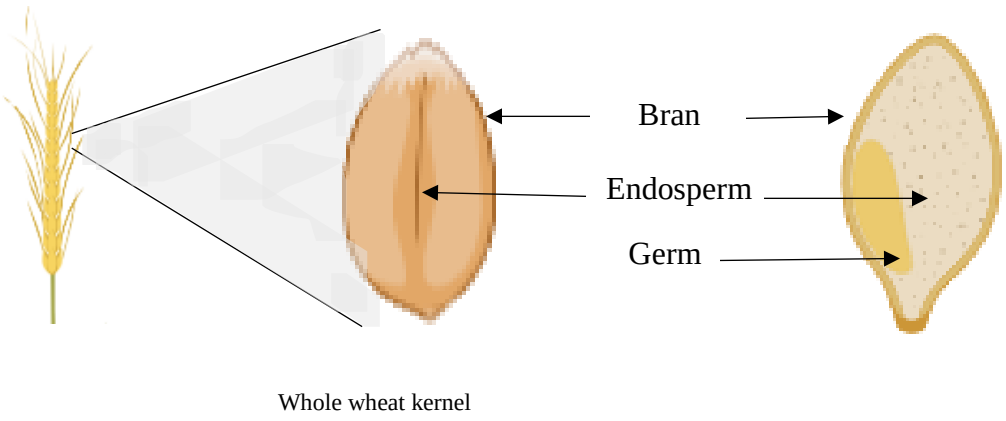


Figure 1.2: Anatomical structure of the wheat kernel

Phenolic compounds in wheat are predominantly concentrated in the outer layers, particularly the bran, where they exist in free, conjugated, and bound forms (Li et al., 2008). Free phenolics are readily available, conjugated phenolics are linked to sugars or organic acids, and bound phenolics are covalently attached to cell wall polysaccharides, contributing to the structural integrity of the wheat kernel and its antioxidant properties. The bran is particularly rich in bound phenolics, while the wheat germ contains a variety of bioactive compounds that further enhance the functional potential of wheat-based products (Liyana-Pathirana & Shahidi, 2005; Onipe et al., 2015; Wang et al., 2013) . With rising consumer demand for natural bioactive compounds, particularly in functional foods, phenolic compounds extracted from whole wheat has gained significant interest

in the food industry. Whole wheat extraction is crucial, as it maximizes the utilization of the entire grain, thereby capturing the full spectrum of beneficial phenolic compounds present in both the bran and germ. This approach not only aligns with the growing trend towards holistic health benefits but also supports sustainable practices by reducing food waste. However, to meet this demand, extraction techniques must be efficient and eco-friendly, addressing the need for sustainable methods that minimize environmental impact while maximizing phenolic yields (Usman et al., 2023).

Conventional methods of extracting phenolic compounds, particularly solvent extraction, are widely used but present significant challenges. These methods typically involve soaking plant materials in organic solvents, such as methanol, ethanol, or acetone, to dissolve the phenolic compounds over extended periods (Oufnac et al., 2007; Zhou & Yu, 2004). This extraction process can take several hours or even days, requiring substantial labor and the use of large quantities of solvents, which can be hazardous to the environment and pose health risks (Alara et al., 2018a Alara et al., 2018b). Additionally, the recovery with those techniques might result in low recovery rates of phenolics due to thermal degradation or incomplete extraction from the wheat matrix, as high temperatures and prolonged exposure to solvents can lead to the breakdown of sensitive compounds (Usman et al., 2023).

To address these limitations, there is an increasing emphasis on developing green extraction methods that aim to reduce solvent consumption, lower energy requirements, and enhance extraction efficiency, all while adhering to principles of environmental sustainability. Green extraction technologies, such as microwave-assisted extraction (MAE) (Teslić et al., 2019), ultrasound-assisted extraction (UAE) (Chen et al., 2022), and supercritical fluid extraction (SFE) (Pinelo et al., 2007), have emerged as promising alternatives to traditional methods. These

innovative approaches offer faster processing times and higher yields while minimizing solvent use, thereby aligning with the growing demand for more sustainable practices in the food industry (Hromadkova et al., 2008; Sparr Eskilsson & Bjorklund, 2000).

MAE has been widely studied for its effectiveness in extracting bioactive compounds, particularly phenolics. MAE utilizes electromagnetic radiation to heat solvents in contact with the sample, which accelerates the extraction process (Jiang et al., 2018). Rapid heating disrupts the plant matrix, improving the release of bound phenolic compounds while using less solvent and energy than conventional extraction (CE) methods (Chen et al., 2022). The ability of MAE to reduce extraction time and to prevent the degradation of heat-sensitive compounds makes it a promising tool for the food and nutraceutical industries (Abdel-Aal et al., 2014; Camel, 2000; Sparr Eskilsson & Bjorklund, 2000).

MAE proved to be an efficient method for extracting phenolic compounds from grains, demonstrating its ability to enhance phenolic yields in shorter times (Chiremba et al., 2012; Teslić et al., 2019). Chiremba *et al*. (2012) explored the extraction of bound phenolic acids from maize and sorghum using MAE and reported significantly reduced extraction times compared to CE. Similarly, Halee *et al*. (2020) investigated the extraction of phenolics from black rice bran and optimized MAE conditions using Response Surface Methodology (RSM). Their findings demonstrated that MAE performed efficiently under optimized conditions, yielding high levels of phenolics compared to CE.

Few studies have focused on extracting phenolic compounds from wheat using MAE, especially from bran and germ, demonstrating significantly higher yields with MAE compared to CE. For instance, Chen *et al*. (2022) compared different extraction techniques for bound phenolics from

Jizi439 black wheat bran, showing that MAE yielded 8,340.7 μg GAE/g of total bound phenolics, substantially higher than the 5,688.9 μg GAE/g obtained with CE. Similarly, Oufnac *et al*. (2007) compared the extraction of phenolic compounds from wheat bran using CE and MAE, showing that MAE produced 467.5 μg catechin equivalents/g of wheat bran, nearly double the amount obtained through CE. Teslić *et al*. (2019) investigated the extraction of phenolics from wheat germ, using Artificial Neural Networks (ANN) and RSM to optimize MAE conditions. Their findings revealed that MAE performed well, producing higher phenolic yields compared to CE and UAE.

The choice of solvent is a critical factor in optimizing the extraction of phenolic compounds. Ethanol, as a food-grade solvent, is widely used due to its efficacy in solubilizing both polar and non-polar compounds. Water, recognized as a green and sustainable solvent, also offers advantages in terms of environmental safety and cost-effectiveness. Ethanol-water mixtures often yield the best results due to their ability to dissolve a broader range of phenolic compounds (Galanakis et al., 2013).

While numerous studies have focused on extracting total phenolic compounds (Kim et al., 2006; Li et al., 2008) and total bound phenolics (Chen et al., 2022; Zhang et al., 2018) from whole wheat flour, wheat germ, and bran using CE, few have specifically investigated MAE of phenolic compounds from wheat germ and bran (Chen et al., 2022; Oufnac et al., 2007; Teslić et al., 2019). As a result, a comprehensive method for reliably extracting phenolic compounds from whole wheat using MAE is still lacking. Despite of extensive research on MAE for phenolic extraction from various plant sources, no studies have specifically targeted wheat kernels and their milling fractions. Most existing studies have concentrated on wheat bran or wheat germ, leaving a significant gap in understanding how MAE can be optimized for extracting phenolic acids from whole wheat kernels, whole wheat flour, and their respective fractions. Furthermore, the

exploration of green solvents in MAE, particularly the use of water for wheat phenolic extraction, has not been thoroughly investigated, underscoring the importance of this research.

This study aims to address these gaps by developing a novel, comprehensive method that leverages MAE to efficiently extract and quantify individual phenolic acids from wheat, comparing extraction efficiency across kernel and flour fractions. By focusing on optimizing extraction techniques for wheat and enhancing its functional and nutritional properties, this research will provide valuable insights into the field, facilitate the selection of wheat varieties for breeders, and promote sustainable practices in phenolic extraction.

1.2 Research Hypothesis and Objectives

1.2.1 Hypothesis:

The application of MAE under optimized conditions (temperature, time, solvent type) can efficiently extract higher amounts of phenolic compounds from wheat compared to CE method.

1.2.2 Objectives:

I. General Objective:

To develop a rapid, reliable, and environmentally friendly method for extracting phenolic compounds from wheat using MAE.

II. Specific Objectives:

a. To investigate the effect of key parameters (temperature, time, solvent) on the efficiency of phenolic compound extraction from wheat kernel and flour using MAE.

b. To compare the phenolic compound yields obtained from wheat through conventional extraction methods with those from MAE.

c. To assess the distribution of phenolic compounds in various pearling fractions of wheat applying MAE.

1.3 Work plan

The study is organized into the following chapters:

Chapter 1: The introduction provides background information and context regarding phenolic compounds in wheat, emphasizing their significance for extraction. This chapter also reviews the broader field of study, highlights previous research, and identifies gaps that the current study aims to address.

Chapter 2: The literature review summarizes existing research on phenolic compounds in whole wheat and its fractions, emphasizing their structure and distribution. It compares novel extraction methods, particularly MAE, with conventional extraction techniques. Additionally, this chapter highlights significant gaps in current knowledge that underscore the necessity for further research in this area.

Chapter 3: This chapter explores the MAE of total phenolic compounds and individual phenolic acids from wheat, focusing on optimizing the time, temperature, and solvent conditions for extracting phenolic acids from both wheat kernels and flour.

Chapter 4: This investigation examines the distribution of phenolic acids throughout the wheat kernel across different pearling fractions, obtained by pearling at various time intervals of 50,100, 150, 250, 350, and 450 seconds.

Chapter 5: The final chapter summarizes the key findings and identifies potential avenues for future research based on the study's outcomes.

A schematic and graphical representation of the research performed in this thesis is presented in **Figure 1.3**.and **Figure 1.4**

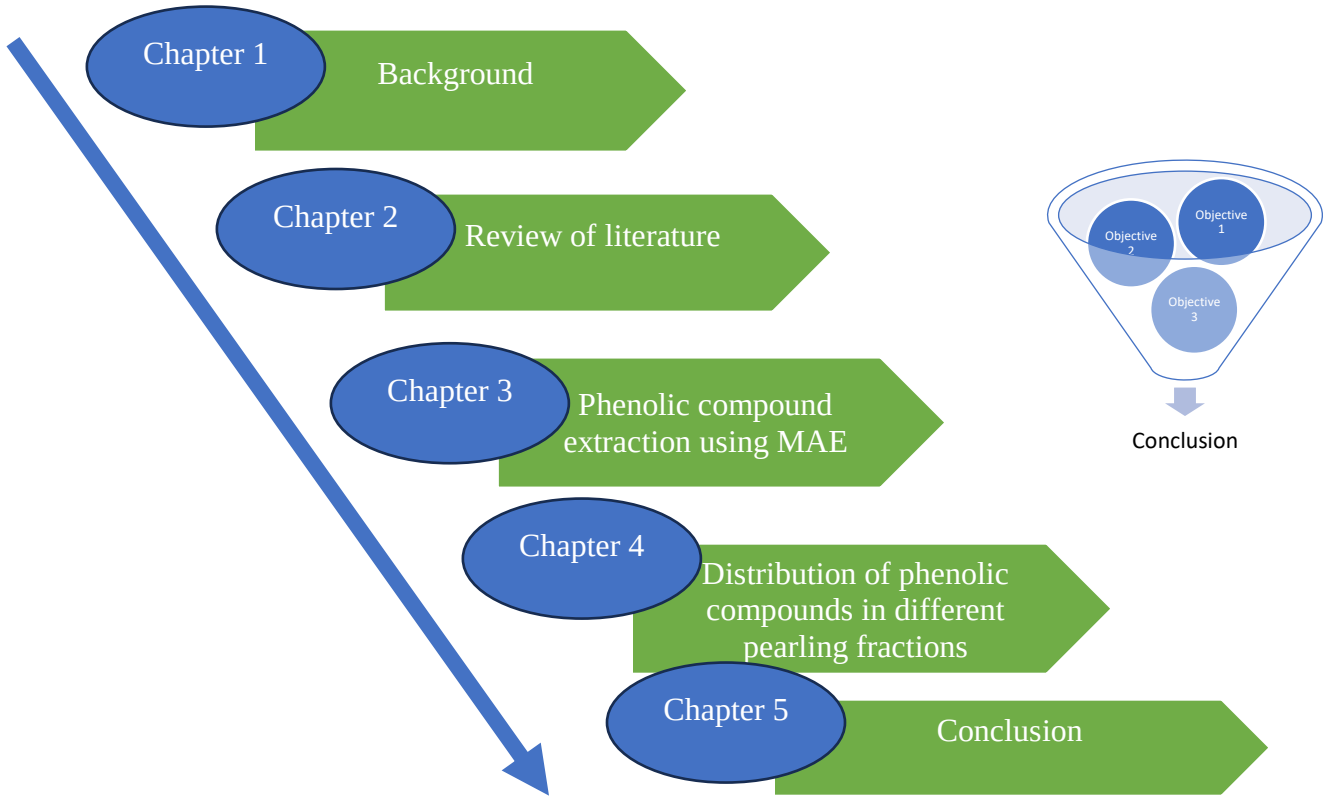


Figure 1.3: Schematic representation of different stages of research in this study

Objective 1 To study impact of solvent, temperature and time on MAE of phenolic compounds from wheat

Objective 2 To compare phenolic compound extraction from wheat using CE and MAE

Objective 3 To investigate the distribution of phenolic compounds throughout the kernel using pearling

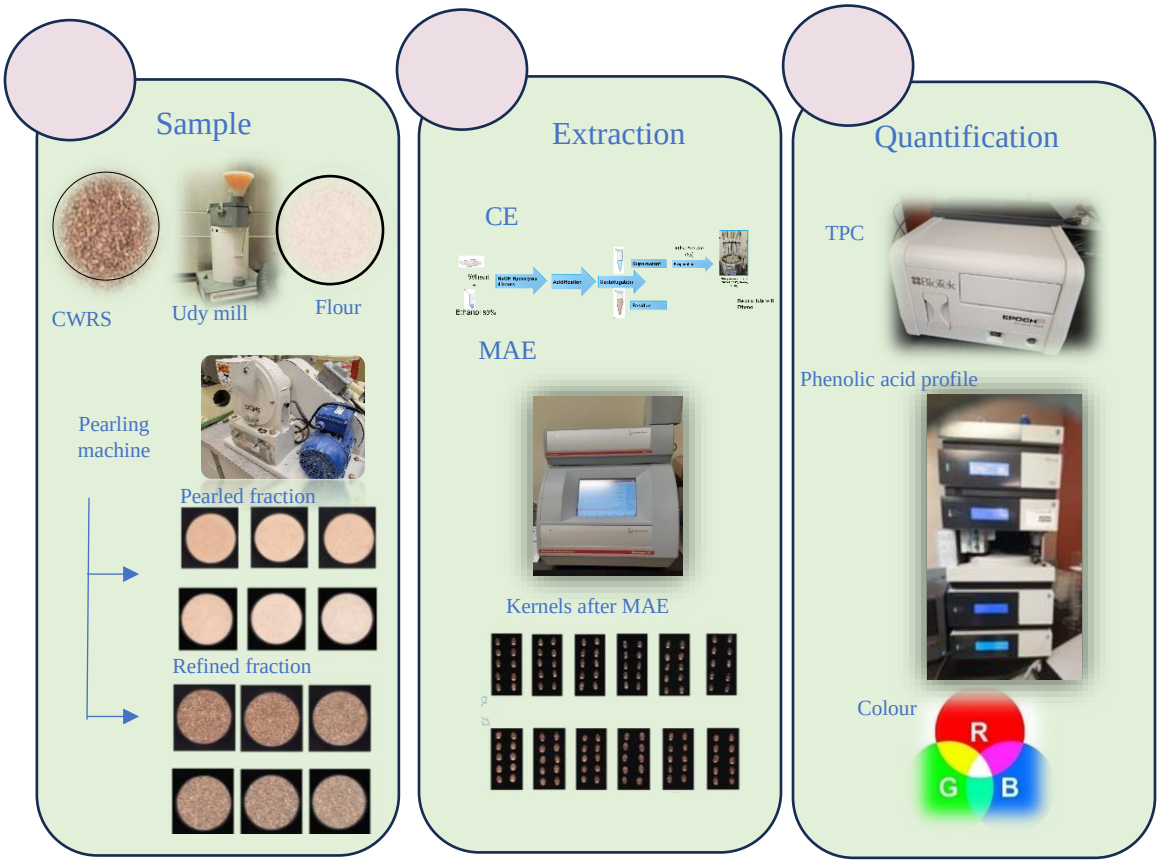


Figure 1.4: Graphical representation of overall study

1.4 References

Abdel-Aal, E.-S. M., Akhtar, H., Rabalski, I., & Bryan, M. (2014a). Accelerated, Microwave-Assisted, and Conventional Solvent Extraction Methods Affect Anthocyanin Composition from Colored Grains. *Journal of Food Science*, *79*(2), C138–C146. https://doi.org/10.1111/1750-3841.12346

Abdel-Aal, E.-S. M., Akhtar, H., Rabalski, I., & Bryan, M. (2014b). Accelerated, Microwave-Assisted, and Conventional Solvent Extraction Methods Affect Anthocyanin Composition from Colored Grains. *Journal of Food Science*, *79*(2), Article 2. https://doi.org/10.1111/1750-3841.12346

Alara, O. R., Abdurahman, N. H., & Olalere, O. A. (2018). Optimization of microwave-assisted extraction of flavonoids and antioxidants from Vernonia amygdalina leaf using response surface methodology. *Food and Bioproducts Processing*, *107*, 36–48. https://doi.org/10.1016/j.fbp.2017.10.007

Alara, O. R., Abdurahman, N. H., & Ukaegbu, C. I. (2018). Soxhlet extraction of phenolic compounds from Vernonia cinerea leaves and its antioxidant activity. *Journal of Applied Research on Medicinal and Aromatic Plants*, *11*, 12–17. https://doi.org/10.1016/j.jarmap.2018.07.003

Camel, V. (2000). Microwave-assisted solvent extraction of environmental samples. *TrAC Trends in Analytical Chemistry*, *19*(4), 229–248. https://doi.org/10.1016/S0165-9936(99)00185-5

Agriculture and Agri-Food Canada. (2023, January 24). Canada: Outlook for principal field crops, 2023-01-20. Retrieved from https://agriculture.canada.ca/en/sector/crops/reports-statistics/canada-outlook-principal-field-crops-2023-01-20

Chen, X., Sun, K., Zhuang, K., & Ding, W. (2022). Comparison and Optimization of Different Extraction Methods of Bound Phenolics from Jizi439 Black Wheat Bran. *Foods (Basel, Switzerland)*, *11*(10), 1478. https://doi.org/10.3390/foods11101478

Chiremba, C., Rooney, L. W., & Beta, T. (2012). Microwave-Assisted Extraction of Bound Phenolic Acids in Bran and Flour Fractions from Sorghum and Maize Cultivars Varying in Hardness. *Journal of Agricultural and Food Chemistry*, *60*(18), Article 18. https://doi.org/10.1021/jf300279t

FAO Cereal Supply and Demand Brief | World Food Situation | Food and Agriculture Organization of the United Nations. (n.d.). Retrieved April 5, 2023, from https://www.fao.org/worldfoodsituation/csdb/en/

Galanakis, C. M., Goulas, V., Tsakona, S., Manganaris, G. A., & Gekas, V. (2013). A Knowledge Base for The Recovery of Natural Phenols with Different Solvents. *International Journal of Food Properties*, *16*(2), 382–396. https://doi.org/10.1080/10942912.2010.522750

Halee, A. (2020). OPTIMISATION OF THE MICROWAVE-ASSISTED EXTRACTION OF NATURAL ANTIOXIDANTS FROM DEFATTED BLACK RICE BRAN OF ORYZA SATIVA L. CV. HOMNIN. *Journal of Microbiology, Biotechnology and Food Sciences*, *9*(6), 1134–1140. https://doi.org/10.15414/jmbfs.2020.9.6.1134-1140

Hromádková, Z., Košťálová, Z., & Ebringerová, A. (2008). Comparison of conventional and ultrasound-assisted extraction of phenolics-rich heteroxylans from wheat bran. *Ultrasonics Sonochemistry*, *15*(6), 1062–1068. https://doi.org/10.1016/j.ultsonch.2008.04.008

Jiang, H., Liu, Z., & Wang, S. (2018). Microwave processing: Effects and impacts on food components. *Critical Reviews in Food Science and Nutrition*, *58*(14), Article 14. https://doi.org/10.1080/10408398.2017.1319322

Kim, K.-H., Tsao, R., Yang, R., & Cui, S. W. (2006). Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chemistry*, *95*(3), 466–473. https://doi.org/10.1016/j.foodchem.2005.01.032

Li, L., Shewry, P. R., & Ward, J. L. (2008). Phenolic Acids in Wheat Varieties in the HEALTHGRAIN Diversity Screen. *Journal of Agricultural and Food Chemistry*, *56*(21), 9732–9739. https://doi.org/10.1021/jf801069s

Liyana-Pathirana, C., & Shahidi, F. (2005). Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food Chemistry*, *93*(1), Article 1. https://doi.org/10.1016/j.foodchem.2004.08.050

Masisi, K., Beta, T., & Moghadasian, M. H. (2016). Antioxidant properties of diverse cereal grains: A review on in vitro and in vivo studies. *Food Chemistry*, *196*, 90–97. https://doi.org/10.1016/j.foodchem.2015.09.021

Onipe, O. O., Jideani, A. I. O., & Beswa, D. (2015). Composition and functionality of wheat bran and its application in some cereal food products. *International Journal of Food Science & Technology*, *50*(12), 2509–2518. https://doi.org/10.1111/ijfs.12935

Oufnac, D. S., Xu, Z., Sun, T., Sabliov, C., Prinyawiwatkul, W., & Godber, J. S. (2007). Extraction of Antioxidants from Wheat Bran Using Conventional Solvent and Microwave-Assisted Methods. *Cereal Chemistry*, *84*(2), Article 2. https://doi.org/10.1094/CCHEM-84-2-0125

Pinelo, M., Ruiz-Rodríguez, A., Sineiro, J., Señoráns, F. J., Reglero, G., & Núñez, M. J. (2007). Supercritical fluid and solid–liquid extraction of phenolic antioxidants from grape pomace: A comparative study. *European Food Research and Technology*, *226*(1), 199–205. https://doi.org/10.1007/s00217-006-0526-3

Sparr Eskilsson, C., & Björklund, E. (2000). Analytical-scale microwave-assisted extraction. *Journal of Chromatography A*, *902*(1), 227–250. https://doi.org/10.1016/S0021-9673(00)00921-3

Teslić, N., Bojanić, N., Rakić, D., Takači, A., Zeković, Z., Fišteš, A., Bodroža-Solarov, M., & Pavlić, B. (2019). Defatted wheat germ as source of polyphenols—Optimization of microwave-assisted extraction by RSM and ANN approach. *Chemical Engineering and Processing - Process Intensification*, *143*, 107634. https://doi.org/10.1016/j.cep.2019.107634

Tullio, V., Gasperi, V., Catani, M., & Savini, I. (2020). The Impact of Whole Grain Intake on Gastrointestinal Tumors: A Focus on Colorectal, Gastric and Esophageal Cancers. *Nutrients*, *13*, 81. https://doi.org/10.3390/nu13010081

Usman, M., Nakagawa, M., & Cheng, S. (2023). Emerging Trends in Green Extraction Techniques for Bioactive Natural Products. *Processes*, *11*(12), Article 12. https://doi.org/10.3390/pr11123444

Zhang, J., Ding, Y., Dong, H., Hou, H., & Zhang, X. (2018). Distribution of Phenolic Acids and Antioxidant Activities of Different Bran Fractions from Three Pigmented Wheat Varieties. *Journal of Chemistry*, *2018*, e6459243. https://doi.org/10.1155/2018/6459243

Zhou, K., & Yu, L. (2004). Effects of extraction solvent on wheat bran antioxidant activity estimation. *LWT - Food Science and Technology*, *37*(7), Article 7. https://doi.org/10.1016/j.lwt.2004.02.008

CHAPTER 2

State of the art

2.1 Phenolic acids in wheat

The phenolic compounds in wheat are primarily located in the bran, germ, and aleurone layer of the grain, with lower concentrations in the endosperm (Liyana-Pathirana & Shahidi, 2007). The bran and germ fractions, which are the outer layers of the wheat kernel, contain the highest amounts of phenolics. Phenolic compounds are the secondary metabolites produced by plants to withstand stress conditions. Based on the chemical structures, phenolic compounds are grouped as phenolic acids, flavonoids, lignin, tannin, and stilbenes. Wheat consists of phenolic acids and flavonoids as major phenolic compounds (Gan et al., 2019; Sharma & Bhaskar, 2021). Among them, phenolic acids are reported to be in higher proportion. Phenolic acid are categorized into two groups hydroxybenzoic acid and hydroxycinnamic acid: studies revealed that phenolics such as *p*-hydroxybenzoic acid, protocatechuic, vanillic, syringic, and gallic acids of hydroxybenzoic derivatives and *p*-coumaric, caffeic, ferulic, and sinapic acids of hydroxycinnamic acid derivatives are highly present in wheat (Hernandez et al., 2011; Li et al., 2008; Moore et al., 2005). **Figure 2.1** and **Figure 2.2** illustrate the basic structures of hydroxybenzoic and hydroxycinnamic acids, while **Table 2.1** and **Table 2.2** summarize the specific substituent groups for each phenolic acid derivative.

i) Hydroxybenzoic Acids (HBAs): These are simpler phenolic acids with a C6-C1 structure. Major HBAs include:

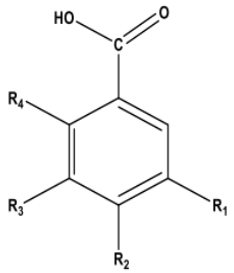


Table 2.1: Substituent patterns of hydroxybenzoic acids

Phenolic acid name	R1	R2	R3	R4
Gallic acid	OH	OH	OH	H
***p*-Hydroxybenzoic acid**	H	OH	H	H
Vanillic acid	OCH_3	OH	H	H
Syringic acid	OCH_3	H	OCH_3	H
Salicylic acid	H	H	H	OH

Figure 2.1: General structure of hydroxybenzoic acids and examples of key compounds Source: Giada (2013)

ii) Hydroxycinnamic Acids (HCAs): These are more abundant phenolic acids and have a C6-C3 structure. Major HCAs include:

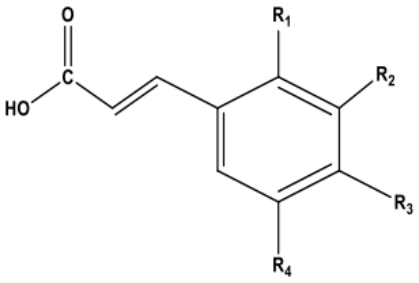


Table 2.2: Substituent patterns of hydroxycinnamic acids

Phenolic acid name	R1	R2	R3	R4
Ferulic acid	H	OCH_3	OH	H
***p*-Coumaric acid**	H	H	OH	H
Sinapic acid	H	OCH_3	OH	OCH_3
Caffeic acid	H	OH	OH	H

Figure 2.2: General structure of hydroxycinnamic acids and examples of key compounds Source: Giada (2013)

Phenolics in wheat exist in three forms: free, conjugated, and bound, with the bound form being the most abundant, followed by conjugated and free forms (Li et al., 2008). Liyana-Pathirana & Shahidi (2005) found that bound phenolics were more prevalent (2.1 mg FAE/g whole wheat), being concentrated in the bran. In fact, bound phenolic concentration was 11.3 mg FAE/g in hard wheat bran and 12.2 mg FAE/g in soft wheat bran, while flour contained 0.33 mg FAE/g and 0.46 mg FAE/g for hard and soft wheat bran, respectively. Free phenolic content ranged from 0.14 to

0.98 mg FAE/g across different milling fractions of hard and soft wheat. Their study demonstrated that bound phenolics are present in greater quantities than conjugated and free forms, with higher concentrations in the outer bran layer compared to the inner endosperm.

Various factors influence phenolic content, including wheat variety, year of cultivation, geographical location, and seasonal conditions (Fernandez-Orozco et al., 2010; Li et al., 2008). For instance, Moore *et al*. (2005) identified the VA97W-024 wheat variety as having the highest TPC at 0.8 mg GAE/g of grain, while the MV5-46 variety had the lowest at 0.4 mg GAE/g. In a study carried out by Okarter *et al.* (2010), bound phenolics in six whole wheat varieties ranged from 5.82 to 6.62 μmol GAE/g, constituting 53.8% to 69.7% of the total phenolic content. Luthria *et al*. (2015), reported that phenolic acid content in whole wheat varies from 200 to 1200 mg/g DW. Fernandez-Orozco *et al*. (2010), in the HEALTHGRAIN project reported the diversity screen of 130 winter, 20 spring, and 10 durum wheat varieties. The study revealed total phenolic content ranging from 728 to 900 μg/g DW across genotypes, with yearly variations from 869 to 1171 μg/g DW and location-based differences from 707 to 900 μg/g DW. Additionally, Li *et al*. (2008) reported that the total phenolic content in wheat varieties averaged 664, 612, and 699 μg/g DW for winter, spring, and durum wheat, respectively. These variations highlight the impact of intrinsic factors on phenolic compound content of wheat flour.

2.2 Distribution of phenolic acids in wheat kernels

Pearling, a widespread technique in cereal processing, involves the sequential removal of the outer layers of grains, leading to distinct fractions that exhibit varying nutritional profiles. In the case of wheat, this process generates several pearling fractions, with the bran fraction standing out for its nutritional importance, constituting roughly 25% of the grain's weight (Neves et al., 2006). However, bran is not a homogeneous structure; it includes outer grain layers as well as parts of the

starchy endosperm and aleurone layer (Koutinas et al., 2005). Structurally, wheat bran consists of multiple layers: the pericarp (4-5%), testa (1%), and aleurone (6-9%) (Hemery et al., 2007).

Phenolic compounds distribution in the wheat kernel is uneven, with the outer layers (bran and germ) containing significantly higher amounts than the endosperm. In fact, Zhang *et al*. (2018), showed that total phenolic extraction from defatted bran of blue, black, and purple wheat decreased from the outer to the inner fractions, with values of 7737.17 to 4387.99 μg GAE/g, 8269.97 to 4647.64 μg GAE/g, and 8012.64 to 3942.18 μg GAE/g, respectively. Adom *et al.* (2005) found that bran and germ have 15 to 18 times more phenolics (2867-3120 μmol GAE/100 g) than the endosperm (176-195 μmol GAE/100 g). Similarly, Wang *et al.* (2013) reported that phenolic acid content in flour ranged from 54 μg/g (60% extraction rate) to 695 μg/g (100% extraction rate). Phenolic acid types also vary across wheat fractions. Phenolic compounds, particularly phenolic acids such as ferulic acid, *p*-coumaric acid, and sinapic acid, are especially abundant in the outer layers (Laddomada et al., 2015). These bioactive compounds are present in higher concentrations in the bran and aleurone layers, while their levels diminish as pearling proceeds into the inner grain layers. Research has shown that the bran and germ fractions contribute about 83% of the total phenolic content in whole wheat, correlating with their strong antioxidant properties compared to more refined fractions (Adom et al., 2005; Liyana-Pathirana & Shahidi, 2007). Zhang *et al.* (2018) reported that bran contains gallic acid, ferulic acid, and salicylic acid as major phenolics, with ferulic acid representing 16.81% to 23.01% of total detectable phenolics.

Table 2.3 summarizes literature reports that quantified individual phenolic acids in various wheat fractions. Although research has shed light on the overall phenolic content of bran fractions, the distribution of individual phenolic acids across different pearling stages remains insufficiently explored.

Table 2.3: Phenolic acid profiles reported for different wheat fractions

No	Source	Method	Phenolic acid	Phenolic acids content (µg/g DW)	Reference
1	Wheat bran	HPLC	Ferulic acid	2094.1	Chen et al. (2022)
			Vanillic acid	91.1	
			Caffeic acid,	19.8	
			Syringic acid	91.1	
			p-Coumaric acid	47.1	
			Protocatechuic acid	18.8	
			Apigenin	8.8	
2	Blue wheat outer bran	HPLC	Hydroxybenzoic acid	12.81	Zhang et al. (2018)
			Vanillic acid	19.47	
			Syringic acid	33.99	
			p-Coumaric acid	112.31	
			Ferulic acid	2467.47	
			Isoferulic acid	277.21	
			Salicylic acid	535.45	
3	Whole wheat meal	Semimicro separation scale RP-HPLC	*p*-Hydroxybenzoic acid	5	Nicoletti et al. (2013)
			Vanillic acid	18.01	
			Syringic acid	1.83	
			p-Coumaric acid	25.33	
			Ferulic acid	795.75	
4	Wheat bran	Capillary Electrophoresis UV-Vis diode array detector (DAD)	Ferulic acid	532.4	Vaher et al. (2010)
			p-Coumaric acid	17.7	
			Syringic acid	37.9	
			Vanillic acid	32.1	
			Sinapic acid	272.1	
5	Wheat grain		Ferulic acid	154	

		Capillary Electrophoresis UV-Vis diode array detector (DAD)	*p*-Coumaric acid	4.2	Vaher et al. (2010)
			Syringic acid	20.9	
			Vanillic acid	23.3	
			Sinapic acid	182.3	
6	Wheat flour	HPLC	Sinapic acid	182.3	Wang et al. (2008)
			Caffeic	64	
			Chlorogenic	6	
			Syringic	16	
			Gentisic	2	
			Coumaric	49	
7	Whole grain	HPLC	Coumaric	49	Liyana-Pathirana & Shahidi (2007)
			p-Coumaric acid	1.56	
			Ferulic acid	6.26	
			Sinapic acid	18.41	
8	Wheat bran	HPLC	Vanillic acid	32.85	Liyana-Pathirana & Shahidi (2007)
			p-Coumaric acid	6.93	
			Ferulic acid	33.86	
			Sinapic acid	123.13	
9	Wheat flour	HPLC	Vanillic acid	1.64	Liyana-Pathirana & Shahidi (2007)
			p-Coumaric acid	0.28	
			Ferulic acid	2.0	
			Sinapic acid	3.2	

2.3 Phenolic acids extraction methods

The structural differences among various phenolic compounds influence their chemical and physical properties, which in turn affect their extraction efficiency (Vermerris & Nicholson, 2006).

The extraction and separation of phenolic compounds largely depend on hydrogen bonding. The presence of intramolecular hydrogen bonds can reduce the solubility of phenolics in alcohol, thereby hindering purification and extraction efficiency.

Apart from the chemical structure of the phenolic compounds, several factors influence the extraction of phenolic compounds from any matrix, including particle size, solvent type, solvent-to-sample ratio, extraction temperature, and extraction duration. The solvent should be selected based on the type and quantity of phenolic compounds being targeted (Camel, 2000). Organic solvents, particularly ethanol and methanol, are commonly used for phenolic extraction (Luque-García, 2005).

Traditional methods of phenolic extraction, such as solvent extraction, often focus on extracting free or easily extractable phenolic compounds while overlooking the bound phenolics, which are covalently linked to cell wall components like cellulose, hemicellulose, and lignin (Alara et al., 2021). These methods typically involve the use of organic solvents such as methanol, ethanol, or acetone to dissolve extractable phenolics under ambient or heated conditions (Kim et al., 2006). However, they are often inefficient in breaking the bonds between bound phenolics and the plant matrix. This limitation can result in an underestimation of the total phenolic content, as a significant portion of phenolics remains trapped in the plant material (Routray & Orsat, 2013). Furthermore, these methods can involve long extraction times, high solvent consumption, and the use of potentially toxic organic solvents, which pose environmental and health risks (Shams et al., 2015). Pérez-Jiménez & Saura-Calixto (2005) have emphasized that conventional techniques may not fully release these non-extractable phenolic compounds, pointing to the need for improved extraction methods to obtain a more accurate assessment of total phenolic content.

To address these limitations, novel extraction techniques have been developed, such as MAE, UAE, PLE, SFE, EAE (enzyme-assisted extraction) and ASE (accelerated solvent extraction). These innovative technologies aim to reduce the final cost of extraction by minimizing energy consumption and extraction time (Alara et al., 2021). For example, Nandasiri *et al*. (2019) found that the extraction of phenolics from canola meal using ASE method depended on the solvent's type and polarity. Similarly, ASE has been utilized for extracting alkylresorcinols from wheat (Holt et al., 2012), and lignin from nutshells (Klein et al., 2010). UAE has also been employed for phenolic compounds from herbal leaves such as *Lavandula stoechas* L. (Ez-zoubi et al., 2021) and charantin from bitter melon (Lee & Yoon, 2022). Likewise, MAE has been effectively used to extract anthocyanins from grape skins (Curko et al., 2019).

2.4 Microwave assisted extraction of phenolic compounds

Among the novel extraction techniques, MAE stands out for overcoming many of the limitations associated with conventional methods for extracting phenolic compounds (Chen et al., 2022; Oufnac et al., 2007; Teslić et al., 2019). MAE provides superior and more consistent thermal transfer than traditional extraction techniques, as the heating is generated by an electromagnetic source, which uniformly penetrates the entire extraction system (Mandal et al., 2015). The heat produced during MAE is generated via two key mechanisms: ionic conduction, which results from the resistance of the medium to ion flow, and dipole rotation, which arises from the alternating movement of polar molecules. These processes facilitate the rupture of cell walls, leading to the release of phenolic compounds from plant matrices (Llompart et al., 2019; Nandasiri et al., 2023). The degree to which microwave energy is absorbed depends on the nature of the solvent and the sample matrix, with polar molecules and ionic solutions being particularly efficient at absorbing microwave energy (Veggi et al., 2013).

The advantages of MAE are well documented, including rapid extraction times, reduced solvent consumption, higher yields of phenolics, and simplified sample preparation (Camel, 2000; Chiremba et al., 2012; Liazid et al., 2007). The efficiency of MAE in extracting phenolic compounds is influenced by several factors, including the nature, size, and viscosity of the sample; the temperature and pressure during extraction; the type and volume of solvent; extraction time; and microwave power (Eskilsson & Bjorklund, 2000).

For optimal results, an ethanol-water mixture is commonly recommended as the solvent (Osorio-Tobón, 2020). Temperature control is crucial, higher temperatures generally increase the solubility of target compounds, improving extraction efficiency (Camel, 2000). For example, Nandasiri *et al*. (2023) demonstrated the efficiency of MAE for extracting canolol from canola meal using low volume of solvent. However, phenolic acids may degrade at temperatures above 150°C. Nandasiri *et al.* (2023) showed that optimum canolol extraction using MAE with methanol at 151°C for 15.43 minutes and ethanol at 170°C for 19.31 minutes, highlighting the variability in optimal conditions. Rose & Inglett (2010) reported that MAE of esterified ferulic acid from corn bran yielded 123 mg/100 g ferulic acid after 5 minutes, increasing up to 281 mg/100 g after 10 minutes at a constant temperature of 200°C. Liazid *et al*. (2007) observed that phenolic extraction could be performed at up to 100°C for 20 minutes without degradation. Therefore, the solvent's microwave absorption properties, the solubility of the target compounds, and the interaction between the solvent and matrix must also be considered in MAE (Bagade & Patil, 2021).

Although considerable research has been conducted on MAE for extracting phenolic compounds from canola and a variety of cereals (yellow corn, wheat, barley, oats and rice) (Nandasiri et al., 2023; Ndolo & Beta, 2014; Setyaningsih et al., 2015), no universal microwave extraction conditions have yet been established for the extraction of different phenolic compounds.

2.5 Extraction of phenolic compounds from wheat

The extraction of phenolic compounds from wheat has been the focus of many studies, due to the recognized antioxidant and health benefits of these bioactive compounds (Liu, 2007; Luthria et al., 2015). Despite extensive research on the health-promoting effects of phenolics, limited attention has been paid to optimizing extraction methods specifically for wheat (Chen et al., 2022; Liyana-Pathirana & Shahidi, 2005). As efficiency of phenolic compound extraction is influenced by a variety of factors, including solvent choice, extraction time, temperature, and sample preparation, proper optimization of these factors is crucial for improving the yield and quality of phenolic compounds extracted from wheat.

Particle size reduction through grinding plays an important role, as smaller particles provide a larger surface area, increasing exposure to extraction solvents (Llompart et al., 2019). Brewer *et al*. (2014) confirmed that finer wheat bran particles yielded higher amounts of phenolic acids during conventional extraction, further underscoring the importance of optimizing sample preparation.

Conventional methods, such as Soxhlet extraction, have been widely used but present several limitations, including long extraction times and the use of large volumes of harmful solvents. Solvent choice influences the extraction of specific phenolic compounds. Zhao *et al.* (2006) reported that 80% acetone extracts from barley showed the highest antioxidant activities in CE, while Oufnac *et al*. (2007) demonstrated that methanol yielded higher extraction rates and phenolic content from wheat bran compared to acetone and hexane in CE. Further, Yu *et al.* (2003) reported that Soxhlet extraction of total phenolic content from the bran of hard red winter wheat using ethanol for 15 hours yielded only 2.29 - 3.24 mg GAE/g of bran, highlighting the inefficiency of this approach. This method's low recovery rates and selectivity further emphasize the need for

more efficient alternatives. Similarly, Li *et al.* (2008) conducted CE on 175 wheat flour samples and reported an average total phenolic content of 658 μg/g across all wheat genotypes. In another study, Kim *et al*. (2006) analyzed the phenolic acid profile of four different types of wheat bran using methanol and subsequent hydrolysis for phenolic extraction, and Zhou & Yu (2004) suggested that 50% acetone was the most effective solvent for extracting phenolic antioxidants from wheat bran. Moreover, Verma *et al*. (2009) explored phenolic acid extraction from the bran of six wheat varieties using CE and found that syringic acid was undetectable in base-hydrolyzed samples but was present in the acid-hydrolyzed fraction, suggesting that acidic hydrolysis is necessary to liberate this phenolic compound. Furthermore, caffeic acid, which was present in the acid-labile fraction, was not detected in the alkali fraction, highlighting the limitations of CE. These results indicate that conventional methods may fail to provide a complete profile of phenolic acids and may even lead to the degradation of sensitive phenolics due to the harsh solvents and conditions used in the extraction process. Because of that some pre-treatment, like steam explosion at 215°C for 120 seconds was reported by Liu *et al.* (2016) to increase phenolic acid extraction. The total free phenolic acids and conjugated phenolic acids reached 6671.8 and 2578.6 μg GAE/g of bran, respectively representing approximately 39-fold and seven-fold increases compared to the untreated sample. This finding underscores the inefficiency of CE as a standalone method, as it requires additional pre-treatment steps to achieve substantial phenolic yields. The need for such pre-treatments further highlights the limitations of conventional methods.

MAE has proven to be a more efficient technique for extracting phenolic compounds from wheat. Oufnac *et al*. (2007) demonstrated that MAE of hydrophilic antioxidants from wheat bran yielded over 467.5 μg catechin equivalents per gram, nearly double that obtained through conventional methods. More recently, Chen *et al.* (2022) compared several extraction methods for bound phenolics from Jizi439 black wheat bran and found that MAE yielded 8,340.7 μg GAE/g of total

bound phenolics, significantly surpassing the 5,688.9 μg GAE/g obtained via traditional methods. MAE conditions, such as temperature, power, and time, significantly impact the phenolic yield. Abdel-Aal *et al*. (2014) demonstrated that optimal anthocyanin yields were obtained from blue wheat under MAE conditions of 70°C, 300 W, and 10 minutes. Additionally, Oufnac *et al*. (2007) showed that antioxidants from wheat bran could be efficiently extracted using MAE at 120°C. Likewise, Chen *et al*. (2022) found that 2 minutes with 420 W was the optimal extraction time for bound phenolics from Jizi439 black wheat bran, demonstrating the potential for MAE to reduce extraction times while enhancing phenolic yields.

In the case of MAE, again solvents have a great impact. Teslic *et al.* (2019) also noted that a 50:50 ethanol-to-water mixture was particularly effective for MAE of phenolics. Water enhances the polarity of organic solvents, leading to greater microwave energy absorption, higher sample temperature, and improved phenolic extraction (Chen et al., 2022; Oufnac et al., 2007; Teslić et al., 2019). Similarly, Zhu *et al.* (2011) evaluated ethanol mixtures (30%, 50%, 70%, and 100%) for antioxidant properties of defatted wheat germ extracts and found that 50% ethanol yielded the highest total phenolic content (15.95 mg GAE/g).

Despite their widespread use, CE methods are associated with several drawbacks, including prolonged extraction times, low recovery rates of target compounds, and the need for large volumes of environmentally unfriendly solvents (Teslic et al., 2019). As, Yu *et al*. (2003) have noted, these limitations make modern, green extraction methods like MAE, more appealing for efficient, high-yield phenolic extraction from wheat.

2.6 References

Abdel-Aal, E.-S. M., Akhtar, H., Rabalski, I., & Bryan, M. (2014). Accelerated, Microwave-Assisted, and Conventional Solvent Extraction Methods Affect Anthocyanin Composition from Colored Grains. *Journal of Food Science*, *79*(2), C138–C146. https://doi.org/10.1111/1750-3841.12346

Adom, K. K., Sorrells, M. E., & Liu, R. H. (2005). Phytochemicals and Antioxidant Activity of Milled Fractions of Different Wheat Varieties. *Journal of Agricultural and Food Chemistry*, *53*(6), Article 6. https://doi.org/10.1021/jf048456d

Alara, O. R., Abdurahman, N. H., & Ukaegbu, C. I. (2021). Extraction of phenolic compounds: A review. *Current Research in Food Science*, *4*, 200–214. https://doi.org/10.1016/j.crfs.2021.03.011

Bagade, S. B., & Patil, M. (2021). Recent Advances in Microwave Assisted Extraction of Bioactive Compounds from Complex Herbal Samples: A Review. *Critical Reviews in Analytical Chemistry*, *51*(2), 138–149. https://doi.org/10.1080/10408347.2019.1686966

Brewer, L. R., Kubola, J., Siriamornpun, S., Herald, T. J., & Shi, Y.-C. (2014). Wheat bran particle size influence on phytochemical extractability and antioxidant properties. *Food Chemistry*, *152*, 483–490. https://doi.org/10.1016/j.foodchem.2013.11.128

Camel, V. (2000). Microwave-assisted solvent extraction of environmental samples. *TrAC Trends in Analytical Chemistry*, *19*(4), 229–248. https://doi.org/10.1016/S0165-9936(99)00185-5

Chen, X., Sun, K., Zhuang, K., & Ding, W. (2022). Comparison and Optimization of Different Extraction Methods of Bound Phenolics from Jizi439 Black Wheat Bran. *Foods (Basel, Switzerland)*, *11*(10), 1478. https://doi.org/10.3390/foods11101478

Chiremba, C., Rooney, L. W., & Beta, T. (2012). Microwave-assisted extraction of bound phenolic acids in bran and flour fractions from sorghum and maize cultivars varying in hardness.

Journal of Agricultural and Food Chemistry, *60*(18), 4735–4742. https://doi.org/10.1021/jf300279t

Curko, N., Verica, D.-U., Valinger, D., Tomašević, M., & Kovačević Ganić, K. (2019). Microwave-Assisted Extraction of Different Groups of Phenolic Compounds from Grape Skin Pomaces: Modeling and Optimization. *Polish Journal of Food and Nutrition Sciences*, *69*, 235–246. https://doi.org/10.31883/pjfns/109423

Ez zoubi, Y., Fadil, M., Bousta, D., El Ouali Lalami, A., Lachkar, M., & Farah, A. (2021). Ultrasound-Assisted Extraction of Phenolic Compounds from Moroccan *Lavandula stoechas* L.: Optimization Using Response Surface Methodology. *Journal of Chemistry*, *2021*, e8830902. https://doi.org/10.1155/2021/8830902

Fernandez-Orozco, R., Li, L., Harflett, C., Shewry, P. R., & Ward, J. L. (2010). Effects of Environment and Genotype on Phenolic Acids in Wheat in the HEALTHGRAIN Diversity Screen. *Journal of Agricultural and Food Chemistry*, *58*(17), 9341–9352. https://doi.org/10.1021/jf102017s

Gan, R.-Y., Chan, C.-L., Yang, Q.-Q., Li, H.-B., Zhang, D., Ge, Y.-Y., Gunaratne, A., Ge, J., & Corke, H. (2019). 9—Bioactive compounds and beneficial functions of sprouted grains. In H. Feng, B. Nemzer, & J. W. DeVries (Eds.), *Sprouted Grains* (pp. 191–246). AACC International Press. https://doi.org/10.1016/B978-0-12-811525-1.00009-9

Giada, M. (2013). Food Phenolic Compounds: Main Classes, Sources and Their Antioxidant Power. In *Oxidative Stress and Chronic Degenerative Diseases—A Role for Antioxidants* (pp. 87–112). https://doi.org/10.5772/51687

Hemery, Y., Rouau, X., Lullien-Pellerin, V., Barron, C., & Abecassis, J. (2007). Dry processes to develop wheat fractions and products with enhanced nutritional quality. *Journal of Cereal Science*, *46*(3), 327–347. https://doi.org/10.1016/j.jcs.2007.09.008

Hernández, L., Afonso, D., Rodríguez, E. M., & Díaz, C. (2011). Phenolic compounds in wheat grain cultivars. *Plant Foods for Human Nutrition (Dordrecht, Netherlands)*, *66*(4), 408–415. https://doi.org/10.1007/s11130-011-0261-1

Holt, M. D., Moreau, R. A., DerMarderosian, A., McKeown, N., & Jacques, P. F. (2012). Accelerated solvent extraction of alkylresorcinols in food products containing uncooked and cooked wheat. *Journal of Agricultural and Food Chemistry*, *60*(19), 4799–4802. https://doi.org/10.1021/jf3001422

Hromádková, Z., Košťálová, Z., & Ebringerová, A. (2008). Comparison of conventional and ultrasound-assisted extraction of phenolics-rich heteroxylans from wheat bran. *Ultrasonics Sonochemistry*, *15*(6), 1062–1068. https://doi.org/10.1016/j.ultsonch.2008.04.008

Kim, K.-H., Tsao, R., Yang, R., & Cui, S. W. (2006). Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chemistry*, *95*(3), 466–473. https://doi.org/10.1016/j.foodchem.2005.01.032

Klein, A. P., Beach, E. S., Emerson, J. W., & Zimmerman, J. B. (2010). Accelerated Solvent Extraction of Lignin from Aleurites moluccana (Candlenut) Nutshells. *Journal of Agricultural and Food Chemistry*, *58*(18), 10045–10048. https://doi.org/10.1021/jf1019856

Koutinas, A. A., Wang, R., Campbell, G. M., & Webb, C. (2005). A Whole Crop Biorefinery System: A Closed System for the Manufacture of Non-food Products from Cereals. In *Biorefineries-Industrial Processes and Products* (pp. 165–191). John Wiley & Sons, Ltd. https://doi.org/10.1002/9783527619849.ch8

Laddomada, B., Caretto, S., & Mita, G. (2015). Wheat Bran Phenolic Acids: Bioavailability and Stability in Whole Wheat-Based Foods. *Molecules*, *20*(9), Article 9. https://doi.org/10.3390/molecules200915666

Lee, J., & Yoon, K. (2022). Ultrasound-Assisted Extractions for Improving the Recovery of Phenolics and Charantin from Bitter Melon and for Increasing the Antioxidant, Antidiabetic and Anti-Obesity Activities of Its Extracts. *Polish Journal of Food and Nutrition Sciences*, *72*, 141–150. https://doi.org/10.31883/pjfns/149434

Li, L., Shewry, P. R., & Ward, J. L. (2008). Phenolic Acids in Wheat Varieties in the HEALTHGRAIN Diversity Screen. *Journal of Agricultural and Food Chemistry*, *56*(21), 9732–9739. https://doi.org/10.1021/jf801069s

Liazid, A., Palma, M., Brigui, J., & Barroso, C. G. (2007). Investigation on phenolic compounds stability during microwave-assisted extraction. *Journal of Chromatography A*, *1140*(1), 29–34. https://doi.org/10.1016/j.chroma.2006.11.040

Liu, L., Zhao, M., Liu, X., Zhong, K., Tong, L., Zhou, X., & Zhou, S. (2016). Effect of steam explosion-assisted extraction on phenolic acid profiles and antioxidant properties of wheat bran. *Journal of the Science of Food and Agriculture*, *96*(10), Article 10. https://doi.org/10.1002/jsfa.7532

Liu, R. H. (2007). Whole grain phytochemicals and health. *Journal of Cereal Science*, *46*(3), 207–219. https://doi.org/10.1016/j.jcs.2007.06.010

Liyana-Pathirana, C. M., & Shahidi, F. (2007). The antioxidant potential of milling fractions from breadwheat and durum. *Journal of Cereal Science*, *45*(3), 238–247. https://doi.org/10.1016/j.jcs.2006.08.007

Liyana-Pathirana, C., & Shahidi, F. (2005). Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food Chemistry*, *93*(1), 47–56. https://doi.org/10.1016/j.foodchem.2004.08.050

Llompart, M., Garcia-Jares, C., Celeiro, M., & Dagnac, T. (2019a). Extraction | Microwave-Assisted Extraction☆. In P. Worsfold, C. Poole, A. Townshend, & M. Miró (Eds.),

Encyclopedia of Analytical Science (Third Edition) (pp. 67–77). Academic Press. https://doi.org/10.1016/B978-0-12-409547-2.14442-7

Luque-García, J. L. (2005). EXTRACTION | Microwave-Assisted Solvent Extraction. In P. Worsfold, A. Townshend, & C. Poole (Eds.), *Encyclopedia of Analytical Science (Second Edition)* (pp. 584–591). Elsevier. https://doi.org/10.1016/B0-12-369397-7/00693-2

Luthria, D. L., Lu, Y., & John, K. M. M. (2015). Bioactive phytochemicals in wheat: Extraction, analysis, processing, and functional properties. *Journal of Functional Foods*, *18*, 910–925. https://doi.org/10.1016/j.jff.2015.01.001

Mandal, S., Mandal, V., & Das, A. (2015). *Classification of Extraction Methods, in: Essentials of Botanical Extraction*.

Moore, J., Hao, Z., Zhou, K., Luther, M., Costa, J., & Yu, L. (Lucy). (2005). Carotenoid, Tocopherol, Phenolic Acid, and Antioxidant Properties of Maryland-Grown Soft Wheat. *Journal of Agricultural and Food Chemistry*, *53*(17), 6649–6657. https://doi.org/10.1021/jf050481b

Nandasiri, R., Eskin, N. A. M., & Thiyam-Höllander, U. (2019). Antioxidative Polyphenols of Canola Meal Extracted by High Pressure: Impact of Temperature and Solvents. *Journal of Food Science*, *84*(11), 3117–3128. https://doi.org/10.1111/1750-3841.14799

Nandasiri, R., Fadairo, O., Nguyen, T., Zago, E., Anas, M. U. M., & Eskin, N. A. M. (2023). Optimization of Canolol Production from Canola Meal Using Microwave Digestion as a Pre-Treatment Method. *Foods*, *12*(2), Article 2. https://doi.org/10.3390/foods12020318

Ndolo, V., & Beta, T. (2014). Comparative Studies on Composition and Distribution of Phenolic Acids in Cereal Grain Botanical Fractions. *CEREAL CHEMISTRY*, *91*(5), 522–530. https://doi.org/10.1094/CCHEM-10-13-0225-R

Neves, M. A. D., Kimura, T., Shimizu, N., & Shiiba, K. (2006). Production of alcohol by simultaneous saccharification and fermentation of low-grade wheat flour. *Brazilian Archives of Biology and Technology*, *49*(3), 481–490. https://doi.org/10.1590/S1516-89132006000400017

Nicoletti, I., Martini, D., De Rossi, A., Taddei, F., D'Egidio, M. G., & Corradini, D. (2013). Identification and Quantification of Soluble Free, Soluble Conjugated, and Insoluble Bound Phenolic Acids in Durum Wheat (Triticum turgidum L. var. Durum) and Derived Products by RP-HPLC on a Semimicro Separation Scale. *Journal of Agricultural and Food Chemistry*, *61*(48), 11800–11807. https://doi.org/10.1021/jf403568c

Okarter, N., Liu, C.-S., Sorrells, M. E., & Liu, R. H. (2010). Phytochemical content and antioxidant activity of six diverse varieties of whole wheat. *Food Chemistry*, *119*(1), 249–257. https://doi.org/10.1016/j.foodchem.2009.06.021

Osorio-Tobón, J. F. (2020). Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds. *Journal of Food Science and Technology*, *57*(12), 4299–4315. https://doi.org/10.1007/s13197-020-04433-2

Oufnac, D. S., Xu, Z., Sun, T., Sabliov, C., Prinyawiwatkul, W., & Godber, J. S. (2007). Extraction of Antioxidants from Wheat Bran Using Conventional Solvent and Microwave-Assisted Methods. *Cereal Chemistry*, *84*(2), 125–129. https://doi.org/10.1094/CCHEM-84-2-0125

Pérez-Jiménez, J., & Saura-Calixto, F. (2005). Literature data may underestimate the actual antioxidant capacity of cereals. *Journal of Agricultural and Food Chemistry*, *53*(12), 5036–5040. https://doi.org/10.1021/jf050049u

Rose, D. J., & Inglett, G. E. (2010). Production of feruloylated arabinoxylo-oligosaccharides from maize (Zea mays) bran by microwave-assisted autohydrolysis. *Food Chemistry*, *119*(4), 1613–1618. https://doi.org/10.1016/j.foodchem.2009.09.053

Routray, W., & Orsat, V. (2013). Preparative Extraction and Separation of Phenolic Compounds. In K. G. Ramawat & J.-M. Mérillon (Eds.), *Natural Products: Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes* (pp. 2013–2045). Springer. https://doi.org/10.1007/978-3-642-22144-6_55

Setyaningsih, W., Saputro, I., Palma, M., & Barroso, C. (2015). Optimisation and validation of the microwave-assisted extraction of phenolic compounds from rice grains. *FOOD CHEMISTRY*, *169*, 141–149. https://doi.org/10.1016/j.foodchem.2014.07.128

Shams, K., Abdel-Azim, N., Saleh, I., Hegazy, M. E., El-Missiry, M., & Hammouda, F. (2015). Green technology: Economically and environmentally innovative methods for extraction of medicinal & aromatic plants (MAP) in Egypt. *Journal of Chemical and Pharmaceutical Research*, *7*.

Sharma, M., & Bhaskar, P. (2021). Phenolic compounds in wholegrains of wheat: A review. *The Applied Biology & Chemistry Journal*, 8–17. https://doi.org/10.52679/tabcj.2021.0003

Sparr Eskilsson, C., & Björklund, E. (2000). Analytical-scale microwave-assisted extraction. *Journal of Chromatography A*, *902*(1), 227–250. https://doi.org/10.1016/S0021-9673(00)00921-3

Teslić, N., Bojanić, N., Rakić, D., Takači, A., Zeković, Z., Fišteš, A., Bodroža-Solarov, M., & Pavlić, B. (2019). Defatted wheat germ as source of polyphenols—Optimization of microwave-assisted extraction by RSM and ANN approach. *Chemical Engineering and Processing - Process Intensification*, *143*, 107634. https://doi.org/10.1016/j.cep.2019.107634

Vaher, M., Matso, K., Levandi, T., Helmja, K., & Kaljurand, M. (2010). Phenolic compounds and the antioxidant activity of the bran, flour and whole grain of different wheat varieties. *Procedia Chemistry*, *2*(1), 76–82. https://doi.org/10.1016/j.proche.2009.12.013

Veggi, P. C., Martinez, J., & Meireles, M. A. A. (2013). Fundamentals of Microwave Extraction. In F. Chemat & G. Cravotto (Eds.), *Microwave-assisted Extraction for Bioactive Compounds: Theory and Practice* (pp. 15–52). Springer US. https://doi.org/10.1007/978-1-4614-4830-3_2

Verma, B., Hucl, P., & Chibbar, R. (2009). Phenolic acid composition and antioxidant capacity of acid and alkali hydrolysed wheat bran fractions. *Food Chemistry*, *116*, 947–954. https://doi.org/10.1016/j.foodchem.2009.03.060

Vermerris, W., & Nicholson, R. (2006). Biosynthesis of Phenolic Compounds. In W. Vermerris & R. Nicholson (Eds.), *Phenolic Compound Biochemistry* (pp. 63–149). Springer Netherlands. https://doi.org/10.1007/978-1-4020-5164-7_3

Wang, J., Sun, B., Cao, Y., Tian, Y., & Li, X. (2008). Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran. *Food Chemistry*, *106*(2), 804–810. https://doi.org/10.1016/j.foodchem.2007.06.062

Wang, L., Yao, Y., He, Z., Wang, D., Liu, A., & Zhang, Y. (2013). Determination of phenolic acid concentrations in wheat flour produced at different extraction rates. *Journal of Cereal Science*, *57*(1), 67–72. https://doi.org/10.1016/j.jcs.2012.09.013

Yu, L., Perret, J., Harris, M., Wilson, J., & Haley, S. (2003). Antioxidant Properties of Bran Extracts from "Akron" Wheat Grown at Different Locations. *Journal of Agricultural and Food Chemistry*, *51*(6), 1566–1570. https://doi.org/10.1021/jf020950z

Zhang, J., Ding, Y., Dong, H., Hou, H., & Zhang, X. (2018). Distribution of Phenolic Acids and Antioxidant Activities of Different Bran Fractions from Three Pigmented Wheat Varieties. *Journal of Chemistry*, *2018*, e6459243. https://doi.org/10.1155/2018/6459243

Zhao, H., Dong, J., Lu, J., Chen, J., Li, Y., Shan, L., Lin, Y., Fan, W., & Gu, G. (2006). Effects of Extraction Solvent Mixtures on Antioxidant Activity Evaluation and Their Extraction

Capacity and Selectivity for Free Phenolic Compounds in Barley (Hordeum vulgare L.). *Journal of Agricultural and Food Chemistry*, *54*(19), 7277–7286. https://doi.org/10.1021/jf061087w

Zhou, K., & Yu, L. (2004). Effects of extraction solvent on wheat bran antioxidant activity estimation. *LWT - Food Science and Technology*, *37*(7), Article 7. https://doi.org/10.1016/j.lwt.2004.02.008

Zhu, K.-X., Lian, C.-X., Guo, X.-N., Peng, W., & Zhou, H.-M. (2011). Antioxidant activities and total phenolic contents of various extracts from defatted wheat germ. *Food Chemistry*, *126*(3), 1122–1126. https://doi.org/10.1016/j.foodchem.2010.11.144

CHAPTER 3

Green extraction of wheat phenolic acids using microwave-assisted extraction

Abstract

Phenolic acids are important secondary metabolites in wheat, concentrated mainly in the wheat bran. These compounds exist in free, conjugated, and bound forms. Traditional extraction methods using organic solvents like ethanol and acetone are labor-intensive procedures. The aim of this study was to study phenolic acids extraction with Microwave-Assisted Extraction (MAE) using water as a green solvent. In addition, the extraction of phenolic acids directly from wheat kernels was explored. MAE conditions such as solvent type (water vs 80% (v/v) ethanol), temperature (140, 160, 170, and 180°C), and extraction time (2, 5, 10, and 15 minutes) were tested and phenolic acids extraction compared to that following CE.

The findings revealed that MAE could effectively extract phenolic acids directly from wheat kernels, though the yield was lower compared to flour due to the smaller surface area of the kernels. Water proved to be a more efficient solvent for extracting phenolic compounds (5.41 ± 0.25 mg/g DW) compared to the amount of total phenolic compounds (3.52 ± 0.24 mg/g DW) extracted with 80% (v/v) ethanol, especially at 170°C for 10 minutes. Flour samples consistently exhibited higher total phenolic content (TPC) than kernels, attributed to the greater surface area available for extraction. Kernel extracts obtained using water and 80% ethanol at 170°C for 15 minutes yielded 2.21 ± 0.22 mg/g DW and 0.96 ± 0.03 mg/g DW, respectively.

Overall, this study demonstrates the potential of MAE as a rapid, sustainable method for phenolic extraction, enhancing wheat's health benefits and supporting future wheat breeding initiatives.

Keywords: Kernel, Flour, Total Phenolic Content, Solvent extraction, HPLC.

3.1 Introduction

As vital dietary phytochemicals, phenolic compounds are central to many health-promoting effects, providing antioxidant, anti-inflammatory, and antimutagenic benefits (Masisi et al., 2016). Beyond human health, these compounds are integral to plant resilience, enhancing the plant's ability to withstand environmental stressors and supporting its physiological functions (Balasundram et al., 2006). In the context of wheat, phenolic compounds not only contribute to human nutrition but also play a crucial role in plant physiology, helping plants manage environmental stress and enhance their resilience (Lin et al., 2016). Wheat contains various phenolic acids, which are primarily derivatives of hydroxybenzoic and hydroxycinnamic acids, particularly concentrated in the cell walls of the outer layers like the bran (Dykes, 2007; Laddomada et al., 2015; Wang et al., 2008).

Previous studies have primarily concentrated on the total phenolic content in the flour or bran and its distribution in wheat by analyzing the bran (Beta et al., 2005; Liu, 2007; Shahidi et al., 2006). However, due to the different nature of the phenolic compounds, CE methods are time consuming and employ organic solvents like methanol, ethanol, and acetone to isolate phenolics at sub-boiling temperatures (Kim et al., 2006), and often requiring multiple cycles of solvent use (Alara, Abdurahman, & Ukaegbu, 2018; Luthria et al., 2015). While organic solvent-based methods are effective, there is a growing demand for greener and more sustainable extraction technologies, which have been explored to a limited extent in the context of wheat phenolics. Previous research has been focused on extracting phenolic compounds from whole wheat flour or bran using organic solvents (Kim et al., 2006; Liyana-Pathirana & Shahidi, 2006; Zhou & Yu, 2004). However, the environmental concerns associated with these solvents highlight the need for greener extraction methods. While water-based extraction methods have been studied for phenolic compounds

(Maran et al., 2014; Martino et al., 2006; Poadang et al., 2017), no studies have yet explored water as a solvent for wheat phenolic extraction.

Microwave-assisted extraction (MAE) has emerged as a promising alternative to conventional methods due to its ability to reduce solvent consumption, lesser time and cost while utilizing electromagnetic waves to enhance extraction efficiency (Bansod et al., 2023). MAE uses polar and ionizable molecules, such as water, to generate heat through molecular agitation in response to microwave radiation (Tao et al., 2020). While MAE has shown success in extracting phenolic compounds from many plant materials, its use in whole wheat kernels and flour has not been widely explored. Wheat's phenolic acids, particularly those in the outer layers, are difficult to extract because they are tightly bound to cell wall polysaccharides (Adom et al., 2005; Dykes, 2007). However, some studies reported that MAE can effectively extract phenolic compounds from wheat bran and colored wheat flour without degrading phenolic acids (Abdel-Aal et al., 2014; Oufnac et al. 2007; Chen et al., 2022). Specifically, Abdel-Aal *et al.* (2014) investigated effects of MAE on anthocyanin extraction efficiency from blue wheat flour using an acetone/water (80/20, v/v) solvent system at 70°C, 300 W, and 10 minutes, confirming that MAE outperformed the commonly used CE. Oufnac *et al*. (2007) extracted 489.5 μg catechin equivalent/g from wheat bran by using MAE with methanol at temperatures ranging from 60°C to 120°C for 20 minutes at 500 W, compared to only 241.3 μg catechin equivalent/g using CE. Similarly, Chen *et al.* (2022) compared various techniques (MAE,UAE and CE) for extracting bound phenolics from Jizi439 black wheat bran and found that MAE at 420 W for 120 seconds using 2 M NaOH as the solvent, produced higher total bound phenolics than CE.

Despite these advances, the studies primarily explored temperatures below 120°C and focused on using non-green solvents. Additionally, no research has optimized MAE conditions for extracting

phenolic compounds from whole wheat kernels or flour, which contain different distributions and binding states of phenolics compared to bran and pigmented wheat. The need remains to optimize MAE conditions such as solvent type, extraction temperature, and extraction time for these components to achieve the best results.

This study aims to address this gap by developing a MAE procedure for extracting phenolic compounds from whole wheat, including kernels and flour. Water and ethanol are employed as solvents to compare extraction yields and efficiencies with conventional methods. By doing so, this research intends to expand the applicability of MAE in wheat processing and contribute to a more comprehensive understanding of phenolic extraction.

3.2 Materials and Methods

3.2.1 Materials

Canada Western Red Spring (CWRS) class commercial wheats were kindly provided by Cereals Canada (Winnipeg, Canada). Ethanol (HPLC grade), Folin-Ciocalteu's (FC) reagent, sodium hydroxide, sodium carbonate, hydrochloric acid were purchased from Fisher Scientific Canada Ltd. (Ottawa, ON, Canada). Phenolic acid standards including gallic acid, hydroxybenzoic acid, chlorogenic acid, vanillic acid, syringic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, hydroxycinnamic acid, and salicylic acid (HPLC-grade) were obtained from Cayman Chemicals (Michigan, USA). Water used in the HPLC analysis was purified using a Milli-Q purification system (Billerica, MA, USA).

3.2.2 Flour production and moisture content

CWRS wheat kernels provided by Agricultural Agri-Food Canada (Winnipeg, Canada) were ground using a cyclone mill (Udy Corporation, Fort Collins, CO, USA) equipped with 0.5 mm sieve. Obtained samples were stored in -20°C for further analysis.

The moisture content of wheat flours was assessed using a moisture analyzer (Denver instrument IR35, Denver, CL, USA). The analysis was performed on three replicates to calculate the average moisture content.

3.2.3 Conventional extraction of phenolic compounds

Phenolic compounds were extracted following the method outlined by Tian *et al.* (2021) with slight adjustments. Briefly, wheat flour (0.1 g ± 0.001 g) was mixed with 0.9 mL of 80% (v/v) ethanol for 20 minutes. Subsequently, 0.4 mL of 6 M NaOH was added into the mixture, which was then subjected to continuous mixing for 3 hours in the dark while sonicating using ultrasonic bath (250HT, New York, USA). Acidification of the sample was achieved by adding 0.1 mL of 12 M HCl until reaching a pH of 2. The resulting mixture underwent centrifugation at 6,000 x *g* for 10 minutes. The supernatant was then extracted three times with ethyl acetate. The combined organic phase was evaporated to dryness using a Vacufuge plus (Eppendorf, Hamburg, Germany).

3.2.4 Microwave-assisted extraction of phenolic compounds from wheat

Preliminary tests were performed with different ethanol concentrations (100, 80, 50, and 40% (v/v)) and 80% (v/v) ethanol was selected based on the total phenolics content obtained. Additionally, water was selected as an eco-friendly green extraction medium. Whole kernels or flour was subjected to different MAE conditions. The solid-to-liquid ratio was 1:99 and 1:9 for wheat flour and kernels, respectively. Temperatures tested included 140, 160, 170, and 180°C based on previously reported conditions (Nandasiri et al., 2023). The MAE treatment time varied

between 2 and 15 minutes (2, 5, 10, and 15 minutes) (Setyaningsih et al., 2015). Microwave-assisted extraction was performed using the Monowave 400 system (Anton Paar, Graz, Austria) to extract phenolic compounds from wheat kernels and whole wheat flour. Codes were: kernel (K) or flour (F) extracted with water (w) or 80% (v/v) ethanol (e).

Following MAE treatment, samples were centrifuge at 6,000 × *g* for 15 minutes at 4°C using a centrifuge (corning LSE, New York, US). The supernatant was taken out using a Pasteur pipette and evaporated to dryness using a Vacufuge plus (Eppendorf, Hamburg, Germany) then stored at -20°C for subsequent analysis.

3.2.5 Color assessment

After the MAE extraction of phenolic compounds from kernels, their color was measured using a Minolta Spectrophotometer CM-3500 (Mahwah, NJ, USA). The color parameters analyzed were *L**, *a**, and *b** in the CIELAB color space. *L** represents lightness on a scale from 0 to 100, where 0 signifies black and 100 signifies white. The *a** value indicates the color's red (positive)/green (negative) chromaticity. The *b** value measures the color's yellow/blue chromaticity: positive values indicate a shift towards yellow, while negative values denote a shift towards blue.

3.2.6 Determination of total phenolic content

Dried extracts were reconstituted with 2 mL of 100% (v/v) HPLC-grade methanol and filtered through a 0.45 µm filter. TPC were measured according to Singleton & Rossi (1965) with slight modification. Briefly, an aliquot of sample 0.2 mL was mixed with 0.8 mL of 0.2 N Folin-Ciocalteu reagent in a 2 mL micro tube. The mixture was vortexed and left to react for 3 minutes to develop color. Then, 1 mL of sodium carbonate (Na_2CO_3) solution (7.5%, w/w) was added. The reaction mixture was kept in the dark for 30 minutes and then the absorbance of the blue-colored complex was measured at 765 nm in a microplate spectrophotometer (BioTek Epoch 2, Santa

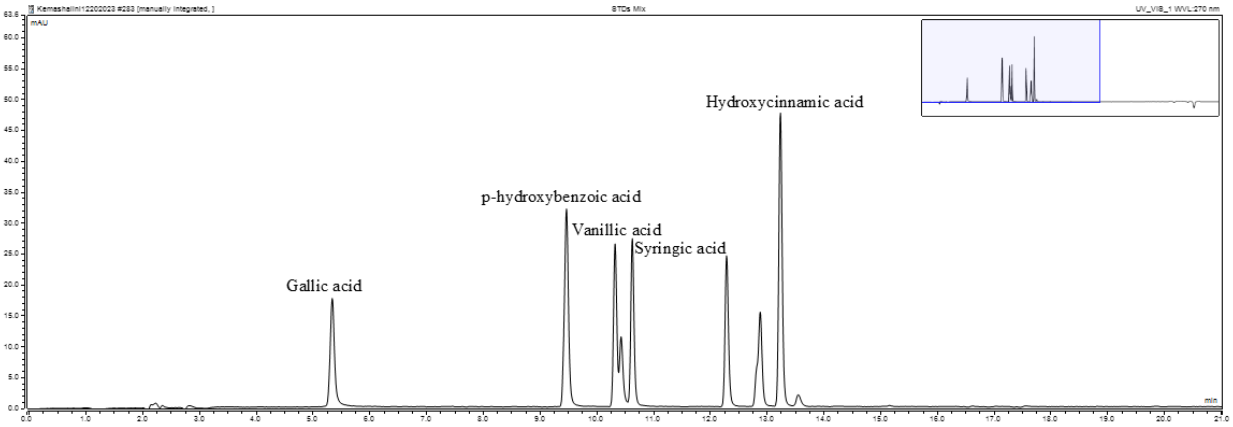
Clara, USA). Ethanol 80% (v/v) was used as the blank and the standard curve was prepared using gallic acid (0.1 mg/mL to 1 mg/mL) in ethanol (80%). The total phenolic content was expressed as micrograms of Gallic acid equivalent (GAE) per millilitre of solution. The total phenolic contents in wheat samples were subsequently calculated from these data.

3.2.7 HPLC analysis of phenolic acid

Phenolic acids were analyzed by using High-Performance Liquid Chromatography (HPLC) following the method described by Shamanin *et al*. (2022) with a few modifications. The HPLC Ultimate 3000 instrument (Dionex, Sunnyvale, Torrance, CA, USA) equipped with Diode Array Detection (HPLC-DAD) was used. A Kinetex® Biphenyl C18 100 Å RP column (2.6 mm, 150 × 4.6 mm, Phenomenex, Torrance, CA, USA) was used with a flow rate of 1 mL/min and an injection volume of 20 μL. The column was maintained at 30°C. The separation was achieved using a gradient elution system with water (0.1% (v/v) acetic acid) as solvent A and acetonitrile (0.1% (v/v) acetic acid) as solvent B. The gradient elution program proceeded as follows: 0% to 10% B (0 to 5 min), 10% to 50% B (5 to 20 min), 50% to 100% B (20 to 30 min), 100% to 10% B (30 to 32 min), and 10% to 0% B (32 to 35 min). Chromatograms were obtained at 270 nm and 320 nm using Chromeleon software Version 7.2 SR4 (Dionex Canada Ltd., Oakville, ON, Canada).

Phenolic compounds, including gallic acid, hydroxy benzoic acid, chlorogenic acid, vanillic acid, syringic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, hydroxy cinnamic acid, and salicylic acid were used for obtaining calibration curves (**Figure 3.1**). The detection limits ranged from 0.005 mg/mL to 0.1 mg/mL.

a)



b)

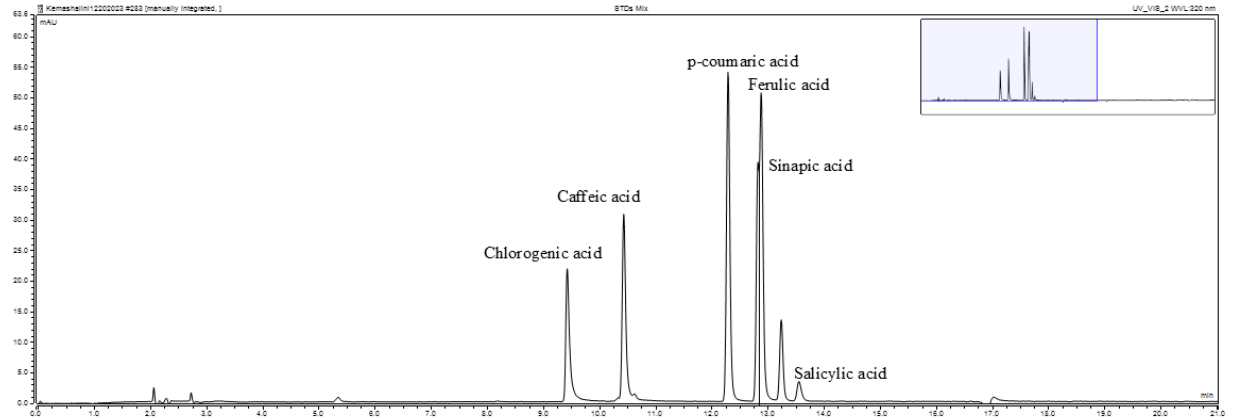


Figure 3.1: HPLC chromatogram of eleven phenolic acid standards at two different wavelengths a) 270 nm b) 320 nm. Gallic acid, hydroxy benzoic acid, vanillic acid, syringic acid, and hydroxy cinnamic acid showed the highest absorption peaks at 270nm while chlorogenic acid, caffeic acid, p-coumaric acid, sinapic acid, ferulic acid, and salicylic acid showed the highest absorption peaks at 320nm.

3.2.8 Statistical analysis

The experiments were conducted in triplicate, and the results are presented as the mean ± standard deviation. Statistical analysis was conducted using Statgraphics Centurion XVII software (Statistical Graphics Corporation, Rockville, MD, USA). Multivariate analysis of variance (MANOVA) was employed to identify significant variations among the different variables with a confidence level of 95%. Upon detecting significant effects, Tukey's test was utilized to determine honest significant differences (HSD).

3.3 Results and Discussion

3.3.1 Color measurement

Color of the kernels after subjected to MAE treatment was measured as a first indicator of the phenolic compounds' extraction. **Figure 3.2** shows the appearance of the kernels after the MAE extraction. It was readily evident that kernels extracted with 80% (v/v) ethanol were darker and thinner, particularly when extracted at 180°C. Conversely kernels extracted with water had a swollen aspect, adopting a more rounded appearance, but become thinner when increasing the treatment temperature. Therefore, kernels appearance indicates that the extraction mechanism when using 80% (v/v) ethanol or water in the MAE might be unlike, affecting differently their microstructure.

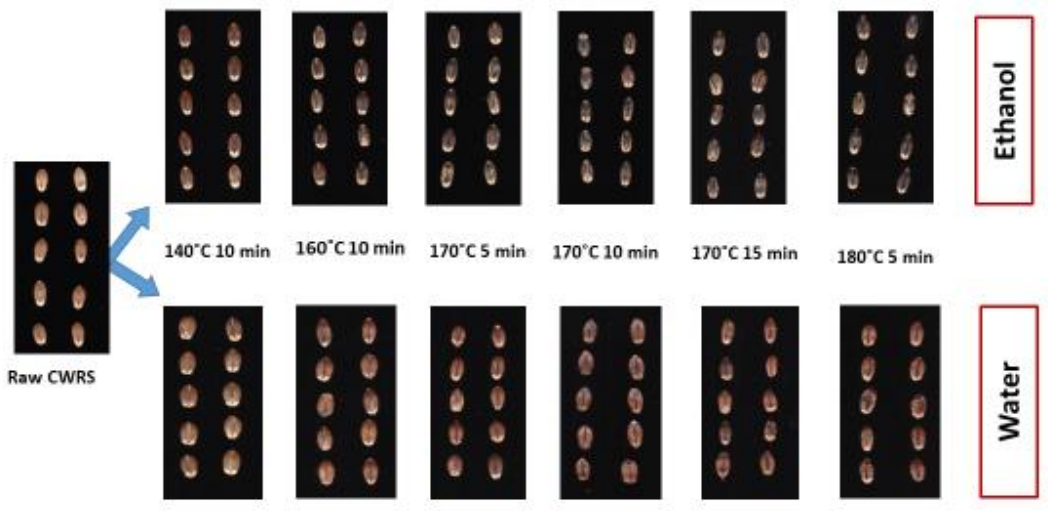


Figure 3.2: Kernels after MAE using 80% (v/v) ethanol and water as solvents under various temperature and time conditions. Each treatment is labeled by temperature (°C) followed by time (minutes). For example, "140°C 10 min" indicates kernels were subjected to MAE at 140°C for 10 minutes.

Color of wheat kernels after MAE is given in **Table 3.1**. Wheat kernels exhibited *L**, *a**, and *b** values of 55.56, 5.59, and 20.43, respectively. After microwave treatment *L** (lightness) and *b** values decreased, while the *a** value increased. The thermal degradation of pigments, such as carotenoids and chlorophyll, during microwave treatment significantly contributes to color changes (Kumar et al., 2019). The *L** values of microwave treated wheat kernels extracted with water significantly ($p < 0.05$) decreased as temperature increased from 140 to 180°C and the extraction time extended from 5 to 15 minutes, aligned with previous research on thermal treatments (Dhua et al., 2021). A similar trend was observed with 80% (v/v) ethanol, but less reduction in *L** value was observed. The decrease in lightness can be attributed to the Maillard reaction and caramelization processes, which occur at high temperatures and lead to the formation of brown pigments, as described by Gili *et al.* (2018). The Maillard reaction is a well-known non-enzymatic browning process that occurs between amino acids and reducing sugars, contributing to

the darker color of wheat kernels (Rahman, 2007). Dhua *et al.* (2021) analyzed the effect of sand, pan, and microwave roasting on different wheat varieties (yellow, purple, and black wheat) and observed that microwave roasting significantly reduced the L^* values ($p < 0.05$) compared to control in all wheat varieties. Conversely, Warchalewski *et al.* (1998) investigated the color changes in wheat grains subjected to microwave irradiation for varying durations (15 to 180 seconds) at a high power of 1250 W. Their study reported an increase in L^* values at lower temperatures (64-98°C). This observation contrasts with the findings in MAE treatments where L^* values decreased significantly at higher temperatures. The increase in lightness at lower temperatures in Warchalewski's study suggested that the exposure durations and lower temperature range were not sufficient to promote Maillard reactions or caramelization, processes that typically occur at elevated temperatures. Additionally, when subjected to gamma ionizing irradiation at selected doses between 0.05 and 10 kGy, the changes in L^* values were not substantial. This highlights that higher temperatures and prolonged exposure, as used in the MAE process, are key factors driving non-enzymatic browning and the resulting darker color in wheat kernels.

The increase in a^* values amid microwave treated kernels indicates a shift towards a more reddish hue. This effect was particularly evident when extending the treatment time at 170°C, regardless of the solvent used for the extraction. Meanwhile, the b^* values exhibited a decrease when comparing microwave-treated kernels with raw kernels, and the reduction was more accentuated when using water as extracting solvent. Water, as a highly polar solvent, effectively extracts more hydrophilic compounds, like anthocyanins, which can lead to different color outcomes than ethanol. In contrast, ethanol, as a polar solvent, is known to solubilize a broader range of phytochemicals, like carotenoids, including lutein, β-carotene, and zeaxanthin, that influence the

wheat color (Lachman et al., 2017). The ability of ethanol to extract a more diverse array of pigments might lead to lighter-colored extracts while also affecting pigment stability during thermal treatment, as indicated in studies by Brenna & Berardo (2004) and Gili *et al*. (2018). This solvent-related variability suggests that the choice of solvent profoundly influences both the quantity of extracted phenolic compounds and the color profile of the extracts (Usman et al., 2023).

Table 3.1: Color of wheat kernel after microwave extraction

Solvent	**Temperature (°C)**	**Time (min)**	***L****	***a****	***b****
Control			55.6 ± 0.7^{g}	5.6 ± 0.3^{a}	21.0 ± 0.6^{h}
Water	140	10	33.6 ± 1.1^{de}	7.7 ± 0.5^{bcde}	16.8 ± 0.7^{fg}
Water	160	10	30.3 ± 0.7^{bc}	8.8 ± 1.1^{e}	14.1 ± 0.3^{de}
Water	170	5	29.4 ± 0.5^{bc}	6.2 ± 0.3^{abc}	16.1 ± 0.3^{ef}
Water	170	10	28.2 ± 0.4^{ab}	7.2 ± 1.0^{abcde}	13.2 ± 0.7^{bcd}
Water	170	15	26.4 ± 0.5^{a}	8.3 ± 0.5^{de}	9.5 ± 0.2^{a}
Water	180	5	25.4 ± 0.5^{a}	7.8 ± 0.2^{bcde}	11.9 ± 1.7^{bc}
80% Ethanol	140	10	42.4 ± 1.3^{f}	6.7 ± 0.5^{abcd}	18.5 ± 0.6^{g}
80% Ethanol	160	10	40.9 ± 0.1^{f}	7.3 ± 0.5^{abcde}	16.0 ± 0.1^{ef}
80% Ethanol	170	5	36.2 ± 1.9^{e}	6.0 ± 0.1^{ab}	17.4 ± 0.6^{fg}
80% Ethanol	170	10	35.6 ± 0.6^{e}	7.8 ± 0.2^{cde}	14.2 ± 0.4^{de}
80% Ethanol	170	15	31.8 ± 1.0^{cd}	8.8 ± 0.8^{e}	11.3 ± 0.7^{ab}
80% Ethanol	180	5	30.3 ± 1.1^{bc}	7.1 ± 0.7^{abcde}	13.7 ± 0.4^{cd}

*Differences in L^*, a^*, and b^* value of kernels after different MAE conditions are highlighted by different letters at $p < 0.05$. Results are expressed as mean values ± standard deviations.

3.3.2 Total phenolic content of wheat extracts

The TPC of wheat flour extracts under different MAE conditions and CE is presented in **Table 3.2**. CE method yielded a TPC of 2.78 ± 0.18 mg/g GAE DW, which was similar to that previously reported for winter wheat (2.14 ± 3.38 mg/g GAE DW) by Wang *et al*. (2020). In wheat kernels, the highest TPC was observed at 170°C for 15 minutes using water (2.21 ± 0.22 mg/g GAE DW). These TPC values were significantly ($p < 0.05$) lower than that from conventional extraction. Regarding the solvent impact, higher TPC were obtained when using water as an extracting solvent for kernels. Likely, the high polarity of water enables better penetration into the plant matrix and solubilizes a broader range of phenolic compounds (Mandal et al., 2007). Water provides a more gradual and consistent heating process, mitigating the risk of thermal damage and making it a more effective solvent for extracting phenolics (Routray & Orsat, 2013). While ethanol's high dielectric properties promote rapid heating in MAE, combining ethanol with water improves the overall heating capacity. However, prolonged microwave exposure in ethanol-based solvents can lead to overheating, causing the degradation of phenolic compounds. The kernels have a dense and rigid cell wall structure, which restricts solvent penetration and phenolic diffusion. This denser structure of kernels necessitates longer extraction time, which was evident at 170°C displaying higher extraction at 15 min. Similarly, Teslić *et al*. (2019), who optimized MAE for defatted wheat germ, reported that the ideal extraction time was 14.48 and 15 minutes, identified through response surface methodology (RSM) and artificial neural network (ANN) approaches, respectively. Similarly, Alifakı *et al*. (2022) reported that a 15-minute MAE extraction yielded the highest TPC when extracting phenolics from cranberries.

The significantly higher TPC levels in wheat flour compared to wheat kernels across all tested conditions ($p < 0.05$) highlight the influence of matrix structure on extraction efficiency. Flour facilitates faster and more efficient extraction of phenolic compounds. Likely, the increased

surface area and cell wall disruption in flour facilitated more efficient extraction, which contributed to the higher TPC values compared to wheat kernels. This phenomenon is supported by the findings of Xiaokang *et al.* (2020), who demonstrated that microwave energy effectively disrupts plant cell walls. Their use of Scanning Electron Microscopy (SEM) revealed that MAE leads to structural damage in the cell walls, enhancing the release of phenolic compounds. The results from wheat flour extraction indicate that MAE is significantly more effective than CE for phenolic extraction.

When comparing solvents, water was found to outperform 80% (v/v) ethanol in extracting phenolic compounds from flour across all tested conditions. The highest TPC was observed when extracting at 170°C for 10 min (5.41 ± 0.25 mg/g GAE DW) and 15 min (5.77 ± 0.67 mg/g GAE DW), using water as the solvent. Although there is no direct data for wheat or other cereals, Tomasi *et al.* (2023) highlighted water's effectiveness as a green solvent for extracting tannins from Eucalyptus bark using MAE, although specific data on other phenolic compounds were not provided. Poadang *et al.* (2017) also successfully extracted phenolic compounds from pineapple peel using distilled water, reporting a TPC of 4.21 ± 0.036 mg/g DW. Similarly, Maran *et al*. (2014) employed aqueous solvent extraction to obtain phenolic compounds from Indian jamun fruit pulp, achieving a TPC of 1,332.36 mg GAE/100 g. In the case of the flour, the extraction time increased the TPC, but after 10 min there was not significant effect, indicating that 10 min was optimal for flour. This finding is consistent with studies by Martino *et al*. (2006), which showed that a 10-minute extraction yielded higher levels of phenolic compounds from *Melilotus officinalis* (sweet yellow clover), indicating that prolonged exposure beyond this point may lead to degradation rather than increased extraction.

Although the optimal temperature for the extraction was 170°C for kernel and flour, the optimal extraction time for flour was 10 minutes, whereas kernels required 15 minutes to achieve the highest TPC, indicating that different matrices require tailored extraction parameters. These results agrees with those reported by Tsubaki *et al.* (2010), who demonstrated that 170°C was the optimal temperature for extracting phenolic compounds from oolong tea residue among the tested range (110-230°C), with a maximum extractability of 74%. The study indicated that temperatures beyond this point, or extending the extraction time too long, led to a decrease in phenolic yield, likely due to thermal degradation of the compounds.

Table 3.2: Effect of solvent, temperature and time on total phenolic content of extracts

Type	Solvent	Temperature (°C)	Time (min)	TPC (mg/g) GAE DW
Control	80% Ethanol			2.78 ± 0.18 ^{jk}
Kernel	Water	140	10	0.29 ± 0.02 ^{a}
Kernel	Water	160	10	1.67 ± 0.1 ^{efg}
Kernel	Water	170	5	0.85 ± 0.06 ^{bcd}
Kernel	Water	170	10	1.40 ± 0.1 ^{def}
Kernel	Water	170	15	2.21 ± 0.22 ^{ghi}
Kernel	Water	180	5	1.28 ± 0.03 ^{de}
Kernel	80% Ethanol	140	10	0.39 ± 0.02 ^{ab}
Kernel	80% Ethanol	160	10	0.55 ± 0.01 ^{abc}
Kernel	80% Ethanol	170	5	0.72 ± 0.01 ^{abc}
Kernel	80% Ethanol	170	10	0.8 ± 0.01 ^{bc}
Kernel	80% Ethanol	170	15	0.96 ± 0.03 ^{cd}
Kernel	80% Ethanol	180	5	0.66 ± 0.03 ^{abc}
Flour	Water	140	10	1.93 ± 0.06 ^{fgh}
Flour	Water	160	10	2.12 ± 0.07 ^{ghi}
Flour	Water	170	5	2.45 ± 0.05 ^{hij}
Flour	Water	170	10	5.41 ± 0.25 ^{m}

Flour	Water	170	15	5.77 ± 0.67 [m]
Flour	Water	180	5	3.12 ± 0.16 [kl]
Flour	80% Ethanol	140	10	1.75 ± 0.02 [efg]
Flour	80% Ethanol	160	10	2.83 ± 0.1 [jk]
Flour	80% Ethanol	170	5	2.51 ± 0.04 [ij]
Flour	80% Ethanol	170	10	3.52 ± 0.24 [l]
Flour	80% Ethanol	170	15	3.14 ± 0.3 [kl]
Flour	80% Ethanol	180	5	2.84 ± 0.11 [jk]

*Differences in TPC extracted with different MAE conditions and CE are highlighted by different letters at $p < 0.05$.

Results have been analyzed using a multiple factor variance analysis, considering as factors the solvent used for the extraction, the type of sample (kernel or flour), the temperature for the extraction and the time. **Table 3.3** shows the main and interaction effect of different parameters on TPC of wheat extracted using MAE while **Table 3.4** shows the least square means of the extracts.

Table 3.3: Main and interaction effects of different factors on TPC of extracts

Source	**Sum of Squares**	**Df**	**Mean Square**	***F*-Ratio**	***P*-Value**
Solvent	3.43786E6	1	3.43786E6	26.78	0.0000
Type	2.77237E7	1	2.77237E7	215.94	0.0000
Temperature	1.81352E7	3	6.04507E6	47.08	0.0000
Time	1.32436E7	2	6.62181E6	51.58	0.0000
Solvent *Temperature	2.7959E6	3	931967.	7.26	0.0004
Solvent *Time	5.56271E6	2	2.78135E6	21.66	0.0000
Type *Temperature	7.63536E6	3	2.54512E6	19.82	0.0000
Type *Time	4.43909E6	2	2.21955E6	17.29	0.0000
Residual	6.80453E6	53	128387.		
Total (corrected)	1.46982E8	71			

All factors exhibited significant interaction effects at $p < 0.05$.

Table 3.4: Least squares mean for TPC of extracts

Level	*Count*	*Mean* (ug/g) DM	*Std. Error*	*Lower Limit*	*Upper Limit*
Grand mean	72	1893.93			
Solvent					
Water	36	2220.64	129.03	1962.87	2478.41
80% (v/v) Ethanol	36	1567.21	129.03	1309.45	1824.98
Type					
Kernel	36	827.217	129.03	569.451	1084.98
Flour	36	2960.64	129.03	2702.87	3218.4
Temperature (°C)					
140	12	784.303	235.575	313.688	1254.92
160	12	1486.69	235.575	1016.08	1957.31
170	36	2479.39	105.352	2268.93	2689.86
180	12	2825.32	235.575	2354.7	3295.93
Time (min)					
5	24	1047.04	182.475	682.499	1411.57
10	36	2200.0	129.03	1942.23	2457.77
15	12	2434.74	223.486	1988.28	2881.21

The rightmost two columns show 95.0% confidence intervals for each of the means.

3.3.3 Quantification of phenolic acids in wheat using HPLC

The profile of the phenolic compounds extracted with different MAE conditions from whole wheat kernel is shown in **Figure 3.3** and from whole wheat flour is shown in **Figure 3.4**. The sum of phenolic acids quantified by HPLC for MAE extracts obtained at 170°C and 10 to15 minutes exceed that from conventional methods (**Figure 3.4**).

The optimal extraction conditions for phenolic acids vary depending on the compound. For instance, gallic acid, chlorogenic acid, vanillic acid, syringic acid, and sinapic acid exhibit highest yields at 170°C for 15 minutes, while hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and salicylic acid showed maximum efficiency at 170°C for 10 minutes. Flour samples

consistently show higher extraction of phenolic acids than wheat samples. Additionally, water generally led to higher extraction yields compared to 80% (v/v) ethanol for most phenolic acids, including gallic acid, hydroxybenzoic acid, chlorogenic acid, vanillic acid, syringic acid, sinapic acid, ferulic acid, and salicylic acid. The efficiency of extraction conditions can be attributed to the polarity of phenolic acids and the choice of solvent. Phenolic acids exhibit varying degrees of polarity, with some being more polar than others. Water, known for its high polarity with a strong dipole moment of approximately 1.85 Debye, is particularly effective at extracting polar compounds like phenolic acids (Li & Du, 2011). Consequently, phenolic acids with higher polarity, such as gallic acid, hydroxybenzoic acid, chlorogenic acid, vanillic acid, syringic acid, sinapic acid, ferulic acid, and salicylic acid, showed superior extraction yields when water is used as the solvent. This is due to the strong interactions between the polar functional groups of these phenolic acids and the polar solvent molecules, facilitating their extraction. In contrast, 80% (v/v) ethanol, while moderately polar due to its hydroxyl group, has a slightly lower dipole moment of around 1.69 Debye (Li & Du, 2011). As a result, it may be somewhat less effective at extracting highly polar compounds, explaining why water tends to yield higher extraction efficiencies for most phenolic acids.

However, despite water's higher polarity, the extraction efficiency for each phenolic acid also depends on the individual compound's polarity. LogP values (available in databases such as PubChem and https://foodb.ca) offer valuable insights into this. These values highlight how highly polar phenolic acids (with low LogP values) are more efficiently extracted by water, while less polar compounds may show better solubility in ethanol or a water-ethanol mixture, providing a clearer understanding of solvent interactions with phenolic acids.

The gallic acid consistently exhibited the highest extraction yields across all MAE treatment conditions, ranging from 1802.56 to 92.02 µg/g DW. This contrasts with previous findings that ferulic acid is typically the most abundant phenolic acid in wheat, accounting for over 70% of its polyphenols (Adom et al., 2005; Liyana-Pathirana & Shahidi, 2006; Zhang et al., 2012). The variability in ferulic acid concentration observed in this study could be attributed to thermal degradation or structural changes caused by microwave treatment (Abdel-Aal et al., 2014; Camel, 2000; Chen et al., 2022). Specifically, ferulic acid is predominantly bound to the cell wall matrix in wheat, often in esterified or cross-linked forms with arabinoxylans and lignin (Aguedo et al., 2014). Tuyet-Lam *et al*. (1992) highlighted the necessity of reaching a specific threshold temperature to break these ether bonds and Chiremba *et al*. (2012) employed a microwave temperature of 190°C to ensure the breakdown of ether bonds. The microwave heating mechanism in MAE, which involves volumetric heating, may not efficiently disrupt these complex bonds within the short exposure time. Likewise, Liazid *et al*. (2007) reported the different thermal stability of 22 phenolic compounds from various families, including benzoic acids, cinnamic acids, and flavonols, under MAE conditions, being stable up to 100°C. However, significant degradation of specific compounds like epicatechin, resveratrol, and myricetin began at 125°C. This suggests that achieving an optimal temperature is critical not only for breaking bonds but also for preserving the integrity of sensitive phenolic compounds. Therefore, the lower yield of ferulic acid in this study could be attributed to a combination of insufficient temperature to break ether linkages and the potential risk of degradation at higher temperatures. The findings emphasize the delicate balance between efficient bond breaking and compound stability, underscoring the importance of fine-tuning MAE conditions to maximize the recovery of specific phenolic acids like ferulic acid.

Gallic acid is a hydrophilic compound containing multiple hydroxyl groups, highly soluble in polar solvents like water and ethanol. Nevertheless, Galanakis *et al*. (2013) have predicted that although phenolic compounds are generally solubilized more readily in polar protic solvents, some phenolics like gallic acid, cinnamic and coumaric acids, show a preference for water, dichloromethane and acetone respectively. Regarding the temperature, extraction of gallic acid increased with the temperature. The elevated temperatures in MAE likely enhanced the solubility of gallic acid, contributing to its higher extraction efficiency in both water and ethanol as solvents. In fact, Srinivas *et al*. (2010) reported that the aqueous solubility of gallic acid varies between 12.6 g/L at 25.6°C and 2870 g/L at 142.7°C.

Comparing TPC with the sum of quantified phenolic acids measured via HPLC, TPC generally exhibits higher values. This discrepancy is likely due to TPC encompassing all oxidative compounds in the sample, including protein molecules contributing to total phenolic content (Everette et al., 2010). It is important to note that HPLC analysis only quantified 11 phenolic acids. Apart from the compounds identified there are other thermo-generative compounds produced during MAE, potentially contributing to the higher TPC. In fact, Nandasiri *et al*. (2023) observed that the microwave treatment to canola meal convert the sinapic acid to canolol and its other thermos generative compounds.

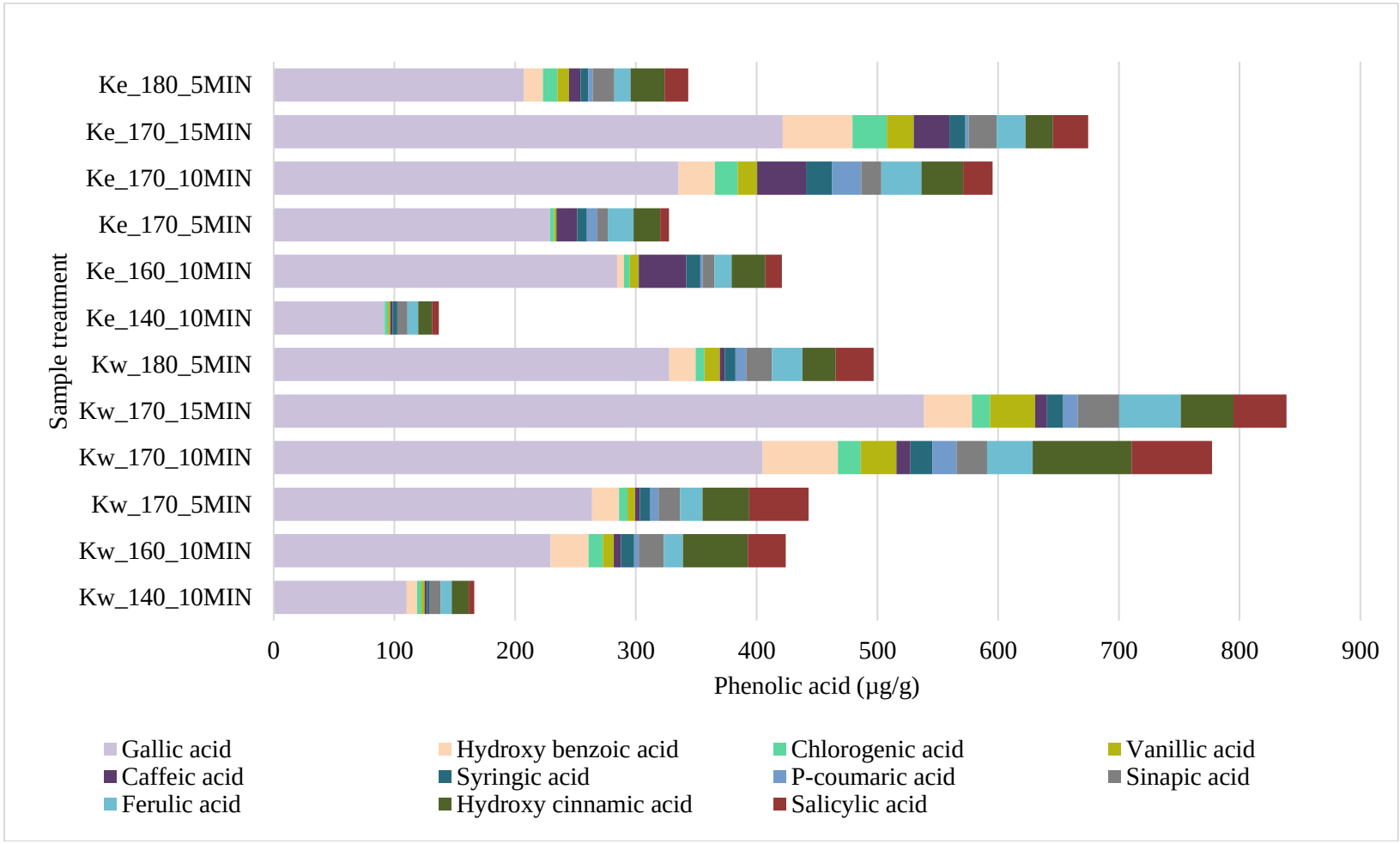


Figure 3.3: Phenolic acid profiles from wheat kernel (K) extracted using MAE with water (w) and 80% (v/v) ethanol (e) as solvents. Treatments are denoted in the following format: sample type (K), solvent (w/e), temperature (°C), and extraction time (minutes). For example, "Fe_180_5 Min" represents flour extracted with ethanol at 180°C for 5 minutes.

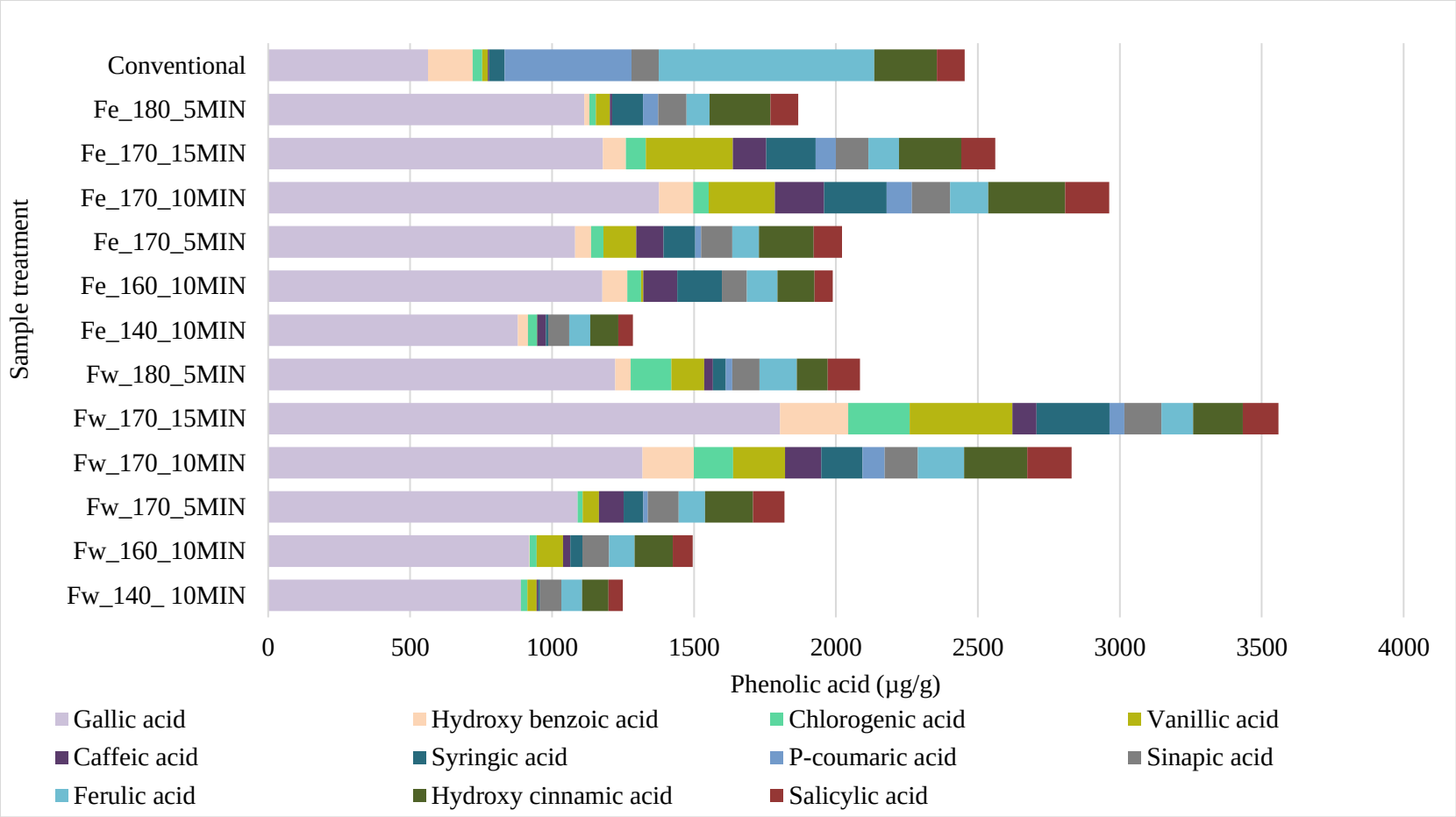
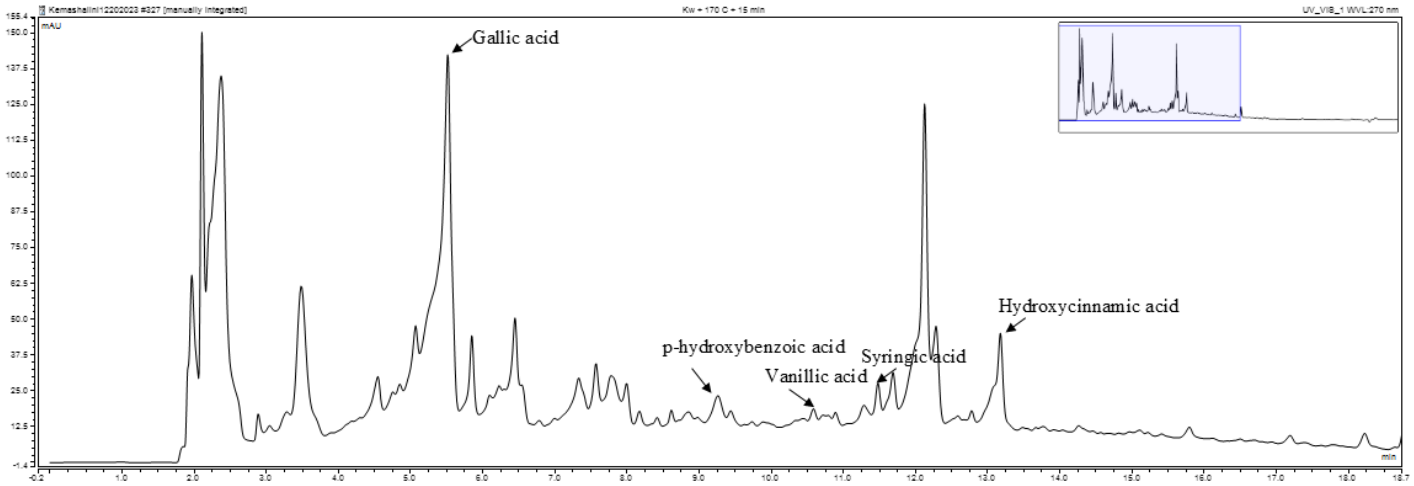


Figure 3.4: Phenolic acid profiles from wheat flour (F) extracted using MAE and CE with water (w) and 80% (v/v) ethanol (e) as solvents. Treatments are denoted in the following format: sample type (F), solvent (w/e), temperature (°C), and extraction time (minutes). For example, "Fe_180_5 Min" represents flour extracted with ethanol at 180°C for 5 minutes.

Chromatogram of phenolic acids extracted by MAE from wheat kernel and flour shown in **Figure 3.5** and **Figure 3.6** respectively.

a)



b)

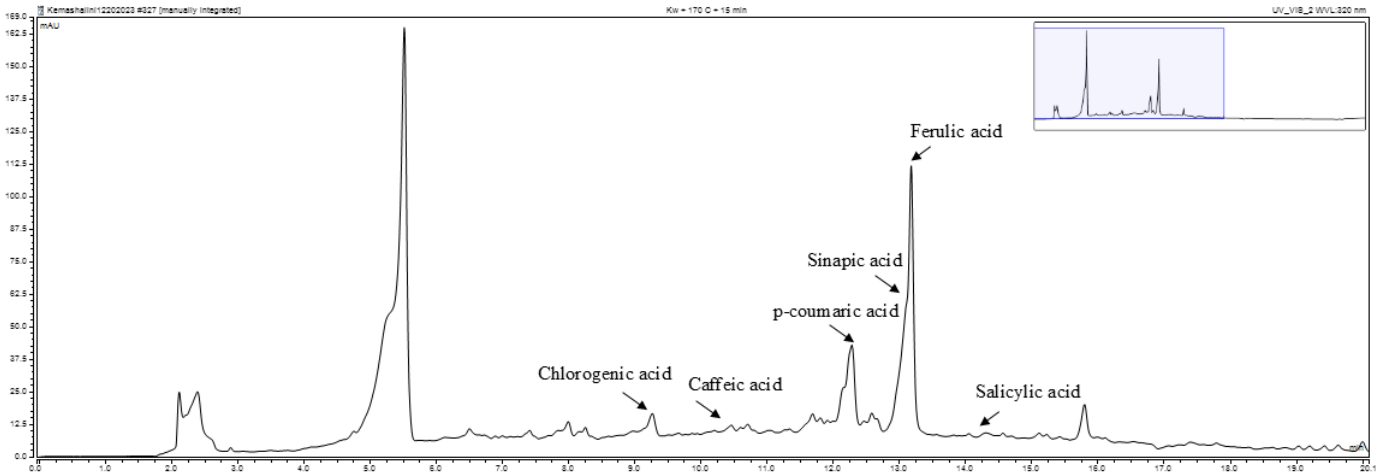
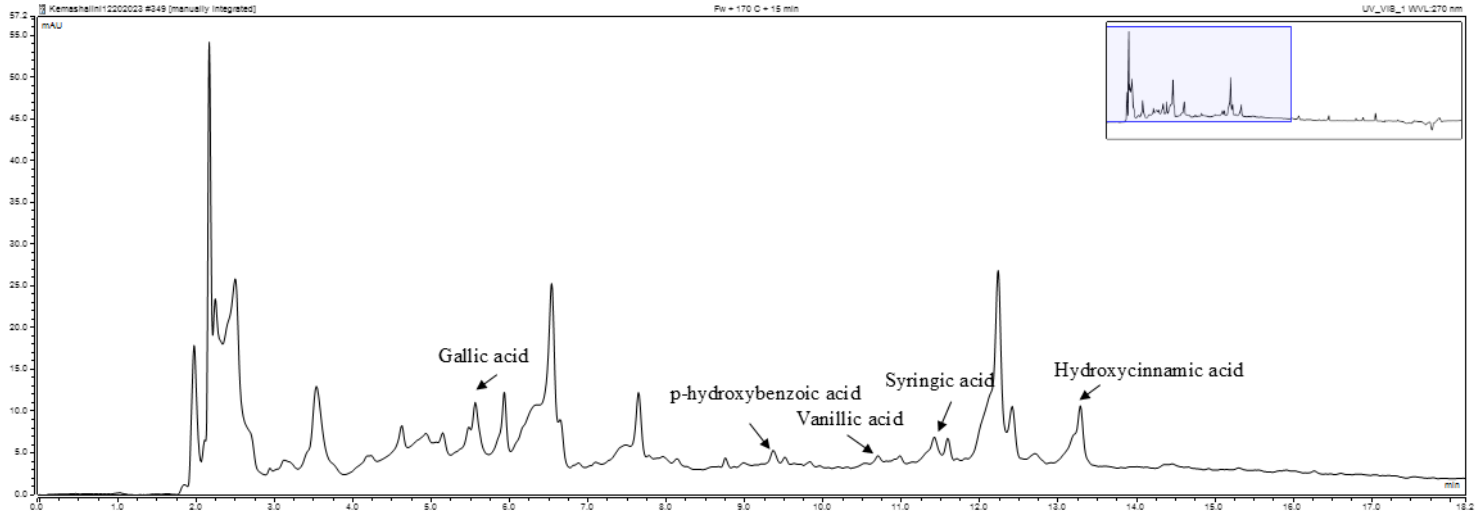


Figure 3.5: HPLC chromatogram of MAE extracts from wheat kernel using 80% (v/v) ethanol as the solvent at 170°C for 15 minutes. The chromatograms display phenolic compound peaks detected at wavelengths of 270 nm (a) and 320 nm (b).

a)



b)

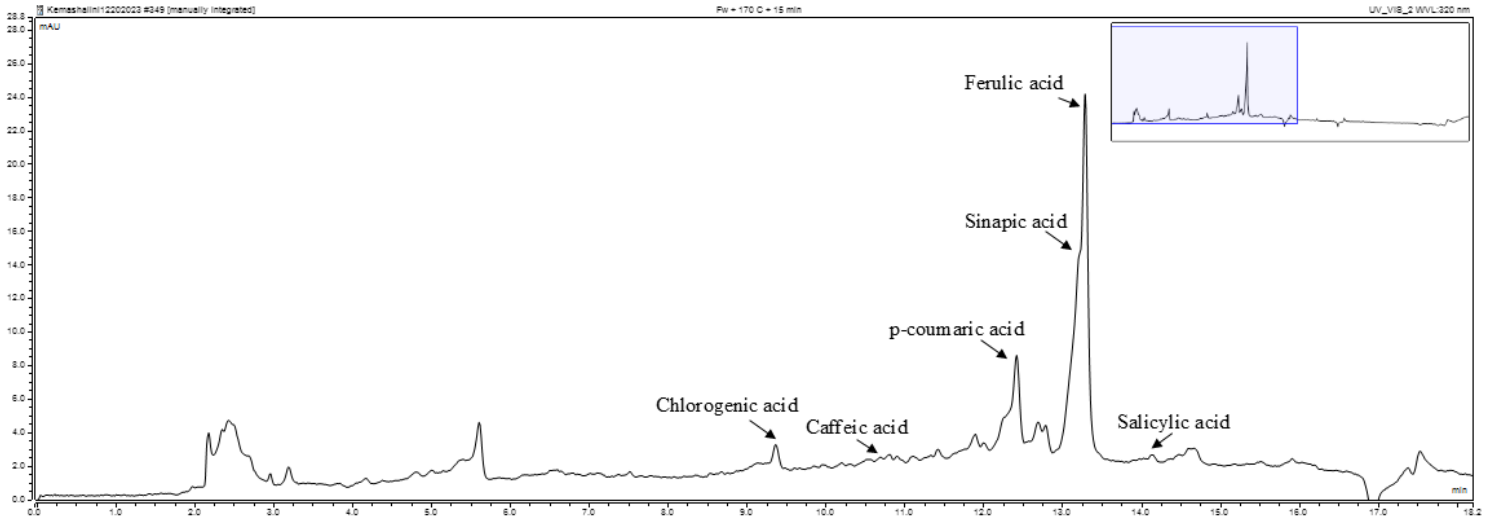


Figure 3.6: HPLC chromatogram of MAE extracts from wheat flour using water as the solvent at 170°C for 15 minutes. The chromatograms display phenolic compound peaks detected at wavelengths of 270 nm (a) and 320 nm (b).

3.4 Conclusions

MAE proves to be a more efficient method for extracting phenolic compounds from wheat compared to CE techniques. The efficiency of the MAE method is highly dependent on several factors, including extraction temperature, time, solvent choice, and the type of sample. Generally, higher temperatures and longer extraction times resulted in increased TPC, with wheat flour samples consistently yielding higher values than wheat kernels. The study also highlighted differences in solvent efficiency, with water outperforming 80% (v/v) ethanol at higher temperatures and longer extraction times in terms of phenolic yields. The optimal conditions for extracting phenolic compounds from wheat kernel or flour were 170°C for 15 min or 10 min, respectively.

Additionally, the application of MAE caused noticeable color changes in wheat kernels, likely due to Maillard browning reactions, with 80% (v/v) ethanol extraction leading to more pronounced browning compared to water.

The study's results contribute valuable insights into the optimization of extraction protocols for phenolic compounds and emphasize the need for further research to understand the complex interactions between phenolic acids and varying extraction conditions. This understanding is critical for the advancement of sustainable and efficient extraction techniques and for deepening the analysis of phenolic compounds in wheat and other plant matrices.

Author contributions

The authors contributed to the chapter "Green extraction of wheat phenolic acids using microwave-assisted extraction" as follows: Kemashalini Kirusnaruban was responsible for writing the original draft, investigation, methodology, formal analysis, and data curation. Dr. Nicola Gasparre contributed to conceptualization, investigation, supervision, formal analysis, writing - review and editing, and funding acquisition. Dr. Ruchira Nandasiri provided supervision, conducted formal analysis, and contributed to writing - review and editing as well as funding acquisition. Dr. Michael Eskin assisted with writing - review and editing. Dr. Cristina M. Rosell contributed to conceptualization, investigation, supervision, writing - review and editing, and funding acquisition.

3.5 References

Abdel-Aal, E.-S. M., Akhtar, H., Rabalski, I., & Bryan, M. (2014). Accelerated, Microwave-Assisted, and Conventional Solvent Extraction Methods Affect Anthocyanin Composition from Colored Grains. *Journal of Food Science*, *79*(2), C138–C146. https://doi.org/10.1111/1750-3841.12346

Adom, K. K., Sorrells, M. E., & Liu, R. H. (2005). Phytochemicals and Antioxidant Activity of Milled Fractions of Different Wheat Varieties. *Journal of Agricultural and Food Chemistry*, *53*(6), 2297–2306. https://doi.org/10.1021/jf048456d

Aguedo, M., Fougnies, C., Dermience, M., & Richel, A. (2014). Extraction by three processes of arabinoxylans from wheat bran and characterization of the fractions obtained. *Carbohydrate Polymers*, *105*, 317–324. https://doi.org/10.1016/j.carbpol.2014.01.096

Alara, O. R., Abdurahman, N. H., & Ukaegbu, C. I. (2018). Soxhlet extraction of phenolic compounds from Vernonia cinerea leaves and its antioxidant activity. *Journal of Applied Research on Medicinal and Aromatic Plants*, *11*, 12–17. https://doi.org/10.1016/j.jarmap.2018.07.003

Alifakı, Y. Ö., Şakıyan, Ö., & Isci, A. (2022). Extraction of phenolic compounds from cranberrybush (Viburnum opulus L.) fruit using ultrasound, microwave, and ultrasound-microwave combination methods. *Journal of Food Measurement and Characterization*, *16*(5), 4009–4024. https://doi.org/10.1007/s11694-022-01498-9

Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry*, *99*(1), 191–203. https://doi.org/10.1016/j.foodchem.2005.07.042

Bansod, S. P., Parikh, J. K., & Sarangi, P. K. (2023). Pineapple peel waste valorization for extraction of bio-active compounds and protein: Microwave assisted method and Box Behnken design optimization. *Environmental Research*, *221*, 115237. https://doi.org/10.1016/j.envres.2023.115237

Beta, T., Nam, S., Dexter, J. E., & Sapirstein, H. D. (2005). Phenolic Content and Antioxidant Activity of Pearled Wheat and Roller-Milled Fractions. *Cereal Chemistry*, *82*(4), 390–393. https://doi.org/10.1094/CC-82-0390

Brenna, O. V., & Berardo, N. (2004). Application of Near-Infrared Reflectance Spectroscopy (NIRS) to the Evaluation of Carotenoids Content in Maize. *Journal of Agricultural and Food Chemistry*, *52*(18), 5577–5582. https://doi.org/10.1021/jf0495082

Camel, V. (2000). Microwave-assisted solvent extraction of environmental samples. *TrAC Trends in Analytical Chemistry*, *19*(4), 229–248. https://doi.org/10.1016/S0165-9936(99)00185-5

Chen, X., Sun, K., Zhuang, K., & Ding, W. (2022). Comparison and Optimization of Different Extraction Methods of Bound Phenolics from Jizi439 Black Wheat Bran. *Foods*, *11*(10), 1478. https://doi.org/10.3390/foods11101478

Chiremba, C., Rooney, L. W., & Beta, T. (2012). Microwave-Assisted Extraction of Bound Phenolic Acids in Bran and Flour Fractions from Sorghum and Maize Cultivars Varying in Hardness. *Journal of Agricultural and Food Chemistry*, *60*(18), Article 18. https://doi.org/10.1021/jf300279t

Dhua, S., Kheto, A., Singh Sharanagat, V., Singh, L., Kumar, K., & Nema, P. K. (2021). Quality characteristics of sand, pan and microwave roasted pigmented wheat (*Triticum aestivum*). *Food Chemistry*, *365*, 130372. https://doi.org/10.1016/j.foodchem.2021.130372

Dykes. (2007). Phenolic Compounds in Cereal Grains and Their Health Benefits. *Cereal Foods World*. https://doi.org/10.1094/CFW-52-3-0105

Everette, J. D., Bryant, Q. M., Green, A. M., Abbey, Y. A., Wangila, G. W., & Walker, R. B. (2010). Thorough Study of Reactivity of Various Compound Classes toward the Folin−Ciocalteu Reagent. *Journal of Agricultural and Food Chemistry*, *58*(14), 8139–8144. https://doi.org/10.1021/jf1005935

Galanakis, C. M., Goulas, V., Tsakona, S., Manganaris, G. A., & Gekas, V. (2013). A Knowledge Base for The Recovery of Natural Phenols with Different Solvents. *International Journal of Food Properties*, *16*(2), 382–396. https://doi.org/10.1080/10942912.2010.522750

Gili, R. D., Penci, M. C., Torrez Irigoyen, M. R., Giner, S. A., & Ribotta, P. D. (2018). Effect of Wheat Germ Heat Treatment by Fluidised Bed on the Kinetics of Lipase Inactivation. *Food and Bioprocess Technology*, *11*(5), 1002–1011. https://doi.org/10.1007/s11947-018-2069-6

Kim, K.-H., Tsao, R., Yang, R., & Cui, S. W. (2006). Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chemistry*, *95*(3), 466–473. https://doi.org/10.1016/j.foodchem.2005.01.032

Kumar, M., Dahuja, A., Sachdev, A., Kaur, C., Varghese, E., Saha, S., & Sairam, K. V. S. S. (2019). Valorisation of black carrot pomace: Microwave assisted extraction of bioactive phytoceuticals and antioxidant activity using Box–Behnken design. *Journal of Food Science and Technology*, *56*(2), 995–1007. https://doi.org/10.1007/s13197-018-03566-9

Lachman, J., Martinek, P., Kotíková, Z., Orsák, M., & Šulc, M. (2017). Genetics and chemistry of pigments in wheat grain – A review. *Journal of Cereal Science*, *74*, 145–154. https://doi.org/10.1016/j.jcs.2017.02.007

Laddomada, B., Caretto, S., & Mita, G. (2015). Wheat Bran Phenolic Acids: Bioavailability and Stability in Whole Wheat-Based Foods. *Molecules*, *20*(9), Article 9. https://doi.org/10.3390/molecules200915666

Li, C. P., & Du, M. (2011). ChemInform Abstract: Role of Solvents in Coordination Supramolecular Systems. *Chemical Communications (Cambridge, England)*, *47*, 5958–5972. https://doi.org/10.1039/c1cc10935a

Liazid, A., Palma, M., Brigui, J., & Barroso, C. G. (2007). Investigation on phenolic compounds stability during microwave-assisted extraction. *Journal of Chromatography A*, *1140*(1), Article 1. https://doi.org/10.1016/j.chroma.2006.11.040

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H., & Chen, S. (2016). An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. *Molecules (Basel, Switzerland)*, *21*(10), 1374. https://doi.org/10.3390/molecules21101374

Liu, R. H. (2007). Whole grain phytochemicals and health. *Journal of Cereal Science*, *46*(3), 207–219. https://doi.org/10.1016/j.jcs.2007.06.010

Liyana-Pathirana, C. M., & Shahidi, F. (2006). Importance of Insoluble-Bound Phenolics to Antioxidant Properties of Wheat. *Journal of Agricultural and Food Chemistry*, *54*(4), 1256–1264. https://doi.org/10.1021/jf052556h

Luthria, D. L., Lu, Y., & John, K. M. M. (2015). Bioactive phytochemicals in wheat: Extraction, analysis, processing, and functional properties. *Journal of Functional Foods*, *18*, 910–925. https://doi.org/10.1016/j.jff.2015.01.001

Mandal, V., Mohan, Y., & Hemalatha, S. (2007). Microwave Assisted Extraction – An Innovative and Promising Extraction Tool for Medicinal Plant Research. *Pharmacognosy Reviews, 1*(1).

Maran, J. P., Sivakumar, V., Thirugnanasambandham, K., & Sridhar, R. (2014). Extraction, multi-response analysis, and optimization of biologically active phenolic compounds from the pulp of Indian jamun fruit. *Food Science and Biotechnology*, *1*(23), 9–14. https://doi.org/10.1007/s10068-014-0002-y

Martino, E., Ramaiola, I., Urbano, M., Bracco, F., & Collina, S. (2006). Microwave-assisted extraction of coumarin and related compounds from *Melilotus officinalis* (L.) Pallas as an alternative to Soxhlet and ultrasound-assisted extraction. *Journal of Chromatography A, 1125*(2), 147–151. https://doi.org/10.1016/j.chroma.2006.05.032

Masisi, K., Beta, T., & Moghadasian, M. H. (2016). Antioxidant properties of diverse cereal grains: A review on in vitro and in vivo studies. *Food Chemistry*, *196*, 90–97. https://doi.org/10.1016/j.foodchem.2015.09.021

Nandasiri, R., Fadairo, O., Nguyen, T., Zago, E., Anas, M. U. M., & Eskin, N. A. M. (2023). Optimization of Canolol Production from Canola Meal Using Microwave Digestion as a Pre-Treatment Method. *Foods*, *12*(2), Article 2. https://doi.org/10.3390/foods12020318

Oufnac, D. S., Xu, Z., Sun, T., Sabliov, C., Prinyawiwatkul, W., & Godber, J. S. (2007). Extraction of Antioxidants from Wheat Bran Using Conventional Solvent and Microwave-Assisted Methods. *Cereal Chemistry*, *84*(2), 125–129. https://doi.org/10.1094/CCHEM-84-2-0125

Poadang, S., Yongvanich, N., & Phongtongpasuk, S. (2017). Synthesis, Characterization, and Antibacterial Properties of Silver Nanoparticles Prepared from Aqueous Peel Extract of

Pineapple, Ananas comosus. *Chiang Mai University Journal of Natural Sciences*, *16*(2). https://doi.org/10.12982/cmujns.2017.0010

Rahman, M. S. (Ed.). (2007). *Handbook of Food Preservation* (2nd ed.). CRC Press. https://doi.org/10.1201/9781420017373

Routray, W., & Orsat, V. (2013). Preparative Extraction and Separation of Phenolic Compounds. In K. G. Ramawat & J.-M. Mérillon (Eds.), *Natural Products: Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes* (pp. 2013–2045). Springer. https://doi.org/10.1007/978-3-642-22144-6_55

Setyaningsih, W., Saputro, I. E., Palma, M., & Barroso, C. G. (2015). Optimisation and validation of the microwave-assisted extraction of phenolic compounds from rice grains. *Food Chemistry*, *169*, 141–149. https://doi.org/10.1016/j.foodchem.2014.07.128

Shahid, M., Yusuf, M., & Mohammad, F. (2015). *Plant phenolics: A Review on Modern extraction techniques* (pp. 265–287).

Shamanin, V. P., Tekin-Cakmak, Z. H., Gordeeva, E. I., Karasu, S., Pototskaya, I., Chursin, A. S., Pozherukova, V. E., Ozulku, G., Morgounov, A. I., Sagdic, O., & Koksel, H. (2022). Antioxidant Capacity and Profiles of Phenolic Acids in Various Genotypes of Purple Wheat. *Foods*, *11*(16), Article 16. https://doi.org/10.3390/foods11162515

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, *16*(3), 144–158. https://doi.org/10.5344/ajev.1965.16.3.144

Srinivas, K., King, J. W., Howard, L. R., & Monrad, J. K. (2010). Solubility of Gallic Acid, Catechin, and Protocatechuic Acid in Subcritical Water from (298.75 to 415.85) K. *Journal of Chemical & Engineering Data*, *55*(9), Article 9. https://doi.org/10.1021/je901097n

Tao, Y., Yan, B., Fan, D., Zhang, N., Ma, S., Wang, L., Wu, Y., Wang, M., Zhao, J., & Zhang, H. (2020). Structural changes of starch subjected to microwave heating: A review from the perspective of dielectric properties. *Trends in Food Science & Technology*, *99*, 593–607. https://doi.org/10.1016/j.tifs.2020.02.020

Teslić, N., Bojanić, N., Rakić, D., Takači, A., Zeković, Z., Fišteš, A., Bodroža-Solarov, M., & Pavlić, B. (2019). Defatted wheat germ as source of polyphenols—Optimization of microwave-assisted extraction by RSM and ANN approach. *Chemical Engineering and Processing - Process Intensification*, *143*, 107634. https://doi.org/10.1016/j.cep.2019.107634

Tian, W., Hu, R., Chen, G., Zhang, Y., Wang, W., & Li, Y. (2021). Potential bioaccessibility of phenolic acids in whole wheat products during *in vitro* gastrointestinal digestion and probiotic fermentation. *Food Chemistry*, *362*, 130135. https://doi.org/10.1016/j.foodchem.2021.130135

Tomasi, I. T., Santos, S. C. R., Boaventura, R. A. R., & Botelho, C. M. S. (2023). Microwave-Assisted Extraction of Polyphenols from Eucalyptus Bark—A First Step for a Green Production of Tannin-Based Coagulants. *Water*, *15*(2), Article 2. https://doi.org/10.3390/w15020317

Tsubaki, S., Sakamoto, M., & Azuma, J. (2010). Microwave-assisted extraction of phenolic compounds from tea residues under autohydrolytic conditions. *Food Chemistry*, *123*(4), 1255–1258. https://doi.org/10.1016/j.foodchem.2010.05.088

Tuyet Lam, T. B., Iiyama, K., & Stone, B. A. (1992). Cinnamic acid bridges between cell wall polymers in wheat and phalaris internodes. *Phytochemistry*, *31*(4), 1179–1183. https://doi.org/10.1016/0031-9422(92)80256-E

Usman, M., Nakagawa, M., & Cheng, S. (2023). Emerging Trends in Green Extraction Techniques for Bioactive Natural Products. *Processes*, *11*(12), Article 12. https://doi.org/10.3390/pr11123444

Wang, J., Sun, B., Cao, Y., Tian, Y., & Li, X. (2008). Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran. *Food Chemistry*, *106*(2), 804–810. https://doi.org/10.1016/j.foodchem.2007.06.062

Wang, X., Zhang, X., Hou, H., Ma, X., Sun, S., Wang, H., & Kong, L. (2020). Metabolomics and gene expression analysis reveal the accumulation patterns of phenylpropanoids and flavonoids in different colored-grain wheats (*Triticum aestivum* L.). *Food Research International*, *138*, 109711. https://doi.org/10.1016/j.foodres.2020.109711

Warchalewski, J. R., Gralik, J., Kusnierz, R., Zawirska-Wojtasiak, R., & Zabielski, J. (1998). The evaluation of wheat grain odor and color after gamma and microwave irradiation. *Electronic Journal of Polish Agricultural Universities*, *1*. https://www.osti.gov/etdeweb/biblio/20147416#fullrecord

Xiaokang, W., Lyng, J. G., Brunton, N. P., Cody, L., Jacquier, J.-C., Harrison, S. M., & Papoutsis, K. (2020). Monitoring the effect of different microwave extraction parameters on the recovery of polyphenols from shiitake mushrooms: Comparison with hot-water and organic-solvent extractions. *Biotechnology Reports*, *27*, e00504. https://doi.org/10.1016/j.btre.2020.e00504

Zhang, Y., Wang, L., Yao, Y., Yan, J., & He, Z. (2012). Phenolic acid profiles of Chinese wheat cultivars. *Journal of Cereal Science*, *56*(3), 629–635. https://doi.org/10.1016/j.jcs.2012.07.006

Zhou, K., & Yu, L. (2004). Effects of extraction solvent on wheat bran antioxidant activity estimation. *LWT - Food Science and Technology*, *37*(7), Article 7. https://doi.org/10.1016/j.lwt.2004.02.008

CHAPTER 4

Distribution of phenolic acids throughout the wheat kernel across different pearling fractions

Abstract

Wheat has an uneven distribution of phenolic acid through the kernel. This study investigates the phenolic acids distribution in wheat (*Triticum aestivum* L.) applying microwave-assisted extraction (MAE) to different pearling fractions. Three samples of commercial Canada Western Red Spring (CWRS) wheat kernels were pearled into six fractions, with pearling times ranging from 50 to 450 seconds, corresponding to 5%-45% removal of outer layers. Total phenolic content was assessed via the Folin-Ciocalteu method, while phenolic acid profiling was conducted using HPLC-DAD. Shorter pearling times preserved higher concentrations of Total Phenolic Content (TPC) and bioactive phenolic acids, particularly in the outer layers of the wheat. The 10% pearled fraction, achieved with 100 seconds of pearling, exhibited the highest TPC value of 9.29 ± 0.17 mg/g DW. Among all the phenolic compounds, gallic acid consistently showed the highest concentrations, ranging from 21.12 ± 0.04 mg/g DW in the initial pearling fractions to 10.59 ± 0.01 mg/g DW as pearling progressed. Multivariate analysis (MANOVA) confirmed a significant impact of pearling time on phenolic acid profiles, indicating that the phenolic composition of the kernel and flour fractions varies with the extent of pearling. The refined kernel fractions exhibited a more substantial reduction in the total sum of phenolic acids, ranging from 0.87 ± 0.08 mg/g DW to 0.20 ± 0.02 mg/g DW, highlighting the greater sensitivity of the outer layers to pearling. In contrast, the refined flour fractions displayed a higher overall sum of phenolic acids, ranging from 3.31 ± 0.02 mg/g DW to 1.38 ± 0.01 mg/g DW, indicating a relatively low reduction in phenolic content. Correlation analysis further indicated that the negative correlation between phenolic acid concentrations and pearling time. This study underscores the importance of optimizing pearling

times to enhance phenolic yields in wheat, offering insights into developing nutrient-rich wheat-based products with improved health benefits.

Keywords: Microwave-Assisted Extraction (MAE), Phenolic acids, Pearling fractions, Total Phenolic Content (TPC), Canada Western Red Spring (CWRS) wheat.

4.1 Introduction

Phenolic acids are well-known for their antioxidant and anti-inflammatory properties, which contribute to numerous potential health benefits. These bioactive compounds play a critical role in protecting cells from oxidative stress and inflammation, both of which are linked to chronic diseases (Bazzano et al., 2005; Liu, 2007). Wheat (*Triticum aestivum* L.), one of the most important cereal crops worldwide, is a vital source of nutrition for millions of people, providing proteins, carbohydrates, vitamins, and minerals (Wang et al., 2020). The outer layers of the wheat grain, especially the bran and aleurone, are particularly rich in phenolic acids, making them valuable for enhancing the nutritional profile of wheat-based foods (González-Thuillier et al., 2015; Hemery et al., 2007).

Despite the health-promoting potential of these compounds, milling removes most of the phenolic acids concentrated in the outer layers of the grain (Jiang et al., 2011). Conventional wheat processing techniques, such as pearling, remove these layers, leading to a significant loss of phenolic content in refined wheat products (Lu & Luthria, 2016). This creates a challenge for delivering the health benefits of phenolic acids to consumers, as many wheat-based foods are produced from refined flour that lacks these bioactive compounds (Bazzano et al., 2005; Liu, 2007). Understanding how wheat processing influences phenolic retention is key to improving the nutritional quality of wheat products.

Pearling is a mechanical process that removes the outer layers of wheat grain, significantly impacting the phenolic acid content. During pearling, the bran, pericarp, and aleurone layers are progressively removed, leaving behind the starchy endosperm, which contains much lower levels

of phenolic acids (Navarro et al., 2022). The degree of layer removal depends on pearling time, with longer durations resulting in more significant reductions in phenolic content.

Although much research has been done on phenolic acids in whole wheat, limited studies have examined how pearling time affects the composition of phenolic acids in distinct wheat fractions like bran, germ and endosperm (Adom & Liu, 2002). Most existing studies focus on total phenolic content rather than the specific phenolic acid profiles across the various pearling fractions (Adom & Liu, 2002; Beta et al., 2005; Liyana-Pathirana & Shahidi, 2006). This gap in knowledge highlights the need for further investigation into how different pearling times influence the retention of phenolic acids in wheat.

To analyze and quantify phenolic acids, conventional extraction methods, such as solvent-based techniques, have been widely used (Vaher et al., 2010; Zhao et al., 2006). However, these methods are time-consuming, require significant solvent volumes, and pose environmental concerns. In contrast, MAE offers a faster, more efficient, and potentially solvent-free approach for extracting phenolic acids. MAE uses microwave energy to rapidly heat and extract compounds, reducing both the time and solvents needed compared to traditional methods (Teslić et al., 2019). MAE has been increasingly recognized as a rapid and efficient tool for extracting bioactive compounds, including phenolic acids from plant matrices (Chiremba et al., 2012). Nevertheless, its application to wheat fractions produced at different pearling stages remains unexplored. Given the rising interest in optimizing phenolic extraction for functional food development and nutraceutical applications, it is crucial to identify the phenolic acid distribution in the outer layers to obtain enriched fractions.

This study aims to investigate the effect of different pearling times on the phenolic acid composition of wheat using MAE. By analyzing specific wheat fractions obtained through varying

degrees of pearling, the study will evaluate how pearling affects the retention of phenolic acids and how MAE can serve as an effective method for extraction. This research seeks to bridge the gap in understanding how both wheat processing and modern extraction techniques influence phenolic retention, with implications for the development of functional foods that retain the nutritional benefits of these compounds.

4.2 Materials and Methods

4.2.1 Materials

Three different commercial wheat samples belonging to the Canada Western Red Spring (CWRS) class were generously provided by Cereals Canada (Winnipeg, Canada). Ethanol, Folin-Ciocalteu (FC) reagent, were sourced from Fisher Scientific Canada Ltd. (Ottawa, ON, Canada). Phenolic acid standards including gallic acid, hydroxybenzoic acid, chlorogenic acid, vanillic acid, syringic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, hydroxycinnamic acid, and salicylic acid (HPLC-grade) were obtained from Cayman Chemicals (Michigan, USA). Water used in the HPLC analysis was purified using a Milli-Q purification system (Billerica, MA, USA).

4.2.2 Pearling process

A general flow diagram of the wheat processing and phenolic compounds analysis is displayed in **Figure 4.1**. The pearling procedure was performed using an abrasive-type pearling machine (Model TM-05C, Satake, Tokyo, Japan) to progressively separate wheat kernels into distinct fractions. The process involved the sequential removal of material at 5% intervals, with each pass representing a specific percentage reduction in the initial kernel weight. Initially, wheat kernels underwent pearling to remove 5% of their original weight, corresponding to a pearling time of 50 seconds, leaving 95% of the kernel weight. The second pass removed an additional 5%, reducing

the kernel weight to 90%, with a pearling time of 100 seconds. Similarly, further pearling processes were carried out at 150, 250, 350, and 450 seconds, corresponding to 85, 75, 65 and 55% of the initial kernel weight, respectively. The process was conducted at a constant speed of 750 rpm, with the pearling time carefully controlled to ensure consistency in fraction removal. Continuous monitoring was used to maintain accuracy, and the equipment was thoroughly cleaned after each pearling session to minimize contamination. A total of six pearled fractions (PF) as PF50, PF100, PF150, PF250, PF350 and PF450 and six refined kernel fractions (RKF50, RKF100, RKF150, RKF250, RKF350 and RKF450) were obtained, along with an un-pearled control sample (RKF0), for subsequent analysis. The pearled fractions, representing 0% to 45% of the initial kernel weight, were collected and analyzed to evaluate the efficiency of the pearling process.

4.2.3 Flour attainment

The wheat kernels from each of the six fractions, along with the un-pearled wheat kernels, were ground using a cyclone mill (Udy Corporation, Fort Collins, CO, USA) equipped with a 0.5 mm screen. The attained seven refined flour fractions (RFF) as RFF0, RFF50, RFF100, RFF150, RFF250, RFF350 and RFF450 samples were stored at -20°C for subsequent analysis. The pearled fractions were ground using a coffee grinder (Model 80301C, proctor silex, China) to homogenize the samples and stored at -20°C for future analysis.

4.2.4 Moisture content analysis

The moisture content of the wheat samples was determined using a moisture analyzer (Denver Instrument IR35, Denver, CL, USA). The samples were heated to 130°C for 4 minutes to measure moisture levels, with ten replicates performed to obtain the average moisture content.

4.2.5 Microwave-assisted extraction

Microwave-Assisted Extraction of phenolic compounds from pearled fractions, refined kernel fractions, and the refined flour fractions were performed using the Monowave 400 system (Anton Paar, Graz, Austria). The system's smart vent technology ensured consistent temperature and pressure control, with a magnetic stirrer providing uniform heat distribution. Water was used as the solvent for all samples. For the pearling fractions and flour, the extraction was conducted at 170°C for 10 minutes, while for the pearled kernels, the extraction time was extended to 15 minutes under the same temperature conditions.

Following extraction, the phenolic extracts were collected and centrifuged at 6,000 × g for 15 minutes at 4°C using a Corning LSE centrifuge (New York, USA). The supernatant was carefully extracted using a Pasteur pipette and stored at -20°C for subsequent analysis.

4.2.6 Determination of total phenolic content

The total phenolic content of the wheat fractions, including six pearled fractions and the seven refined kernel fractions and refined flour fraction, was measured following a modified procedure based on Singleton & Rossi (1965). A sample aliquot of 0.2 mL was mixed with 0.8 mL of 0.2 N Folin-Ciocalteu reagent in a 2 mL microcentrifuge tube, followed by vortexing and allowing the mixture to react for 3 minutes at room temperature to initiate color development. Afterward, 1 mL of sodium carbonate (Na_2CO_3) solution (7.5% w/w) was added to the mixture. The reaction was then kept in darkness for 30 minutes to allow for full color development. The absorbance of the resulting blue complex was measured at 765 nm using a microplate spectrophotometer (BioTek Epoch 2, Santa Clara, U.S.A.). Ethanol served as the blank, and the calibration curve was obtained using gallic acid standards prepared in 80% ethanol at a concentration of 0.1 mg/mL to 1 mg/mL

(R^2 = 0.9992). The TPC for each sample was calculated and expressed as micrograms of gallic acid equivalent (GAE) per milliliter of solution.

4.2.7 Analysis of phenolic acids

A modified high-performance liquid chromatography-diode array detection (HPLC-DAD) method was used to analyze the phenolic acid composition of pearled fractions, refined kernel fractions, and refined flour fractions, based on Shamanin *et al*. (2022).

The samples were filtered through 0.45-µm syringe filters and analyzed using a reversed-phase HPLC-DAD system (Ultimate 3000, Dionex, Sunnyvale, CA, USA) equipped with an online degasser, binary pump, autosampler, column heater, and diode array detector. A Kinetex® Biphenyl C18 100 Å RP column (2.6 µm, 150 × 4.6 mm, Phenomenex, Torrance, CA, USA) was used for separation, maintained at 30°C with a 1 mL/min flow rate and 20-µL injection volume. A gradient elution method was employed with solvent A (water with 0.1% acetic acid) and solvent B (acetonitrile with 0.1% acetic acid). The gradient profile was as follows: 0-10% B (0–5 min), 10-50% B (5–20 min), 50%-100% B (20-30 minutes), hold at 100% B until 32 minutes, then return to 10% B by 35 minutes. Chromatograms were recorded at 270 nm and 320 nm, with data analyzed using Chromeleon software (Version 7.2 SR4, Dionex Canada Ltd., Oakville, ON, Canada).

Phenolic standards, including gallic acid, hydroxybenzoic acid, chlorogenic acid, vanillic acid, syringic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, hydroxycinnamic acid, and salicylic acid, were used to generate external calibration curves (**Figure 3.1)**. These standards showed high linearity with R^2 values between 0.9762 and 0.9994, ensuring accurate quantification of phenolic acids in the wheat samples. Overall methodology is shown as flow diagram in **Figure 4.1**.

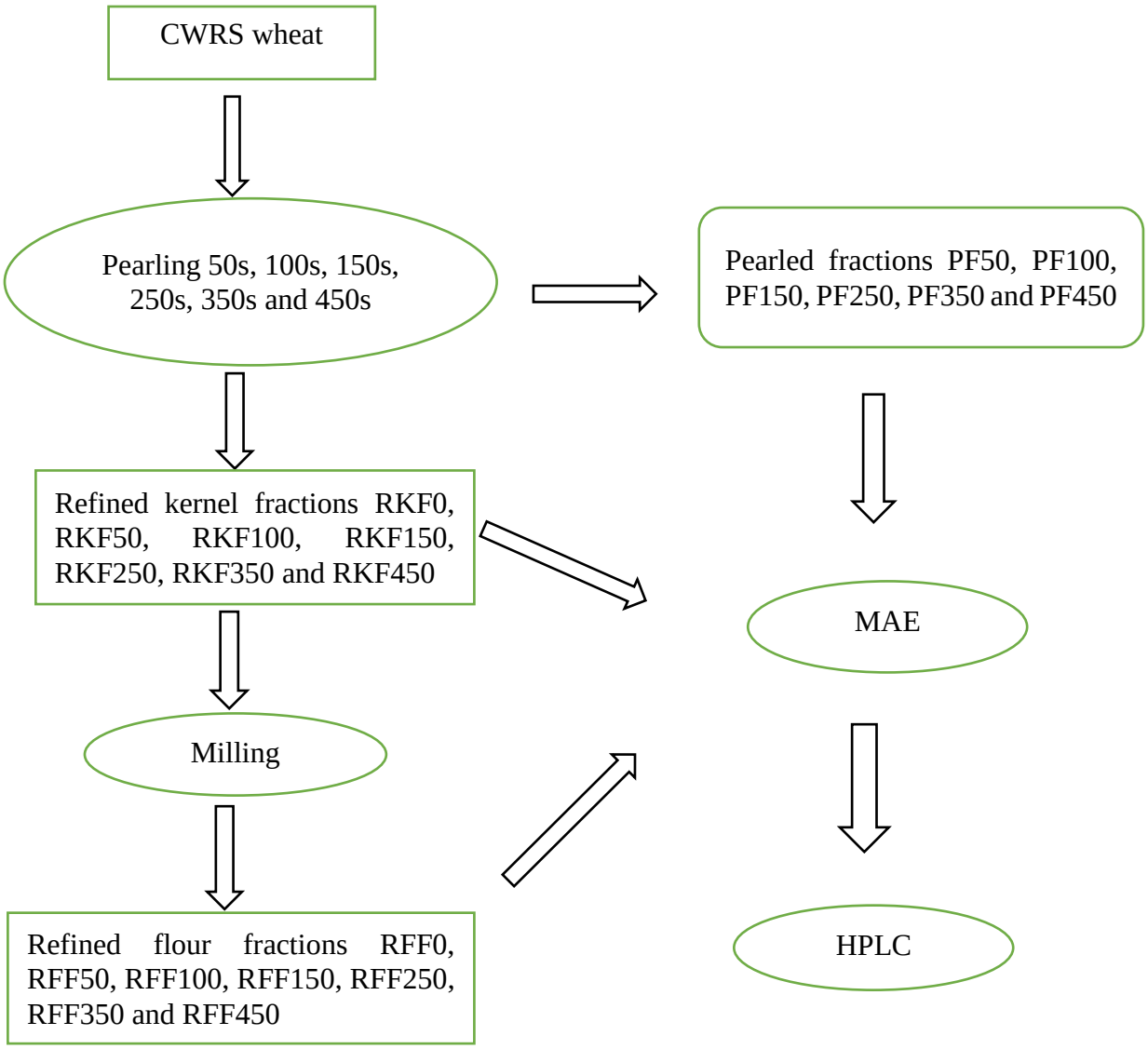


Figure 4.1: Flow diagram of the wheat pearling process and subsequent phenolic extraction

4.2.8 Statistical Analysis

All analyses were conducted using R Studio software (version 4.4.1, R Core Team, Vienna, Austria), and data were expressed as means ± standard deviation from triplicate measurements. To evaluate the effect of pearling time on the phenolic acid composition and TPC in both pearled fractions and refined fractions (kernel and flour), a Multivariate Analysis of Variance (MANOVA) was applied. This analysis allowed for the simultaneous assessment of the impact of pearling time

on multiple dependent variables (phenolic acids). Pillai's trace was used as the test statistic to determine if there were any statistically significant multivariate effects of pearling time on the phenolic acid profiles.

For individual phenolic acids, a one-way Analysis of Variance (ANOVA) was conducted to investigate whether pearling time had a significant effect on the concentration of each phenolic acid in both pearled and refined fractions. Post-hoc comparisons were performed using Tukey's Honest Significant Difference (HSD) test to identify specific pearling times with significantly different phenolic acid concentrations ($p < 0.05$). Additionally, pairwise t-tests applied to further investigate differences in phenolic concentrations between different pearling times.

Pearson's correlation analysis was employed to examine the relationship between pearling time and the concentration of individual phenolic acids across pearled and refined fractions. Pearson's correlation coefficients were calculated to quantify the strength and direction of these relationships, providing insight into how phenolic acid concentrations change with increasing pearling time.

4.3 Results and Discussion

4.3.1 Kernel morphology

The progressive change in wheat kernel morphology with increasing pearling times (50, 100, 150, 250, 350, 450 seconds) highlighted the gradual removal of the outer bran layers and exposure of the starchy endosperm (**Figure 4.2**). At shorter pearling times (50 and 100 seconds), the kernel retained much of its original structure, with significant portions of the bran still intact. However, as pearling progressed (150 seconds and beyond), the outer layers, including the bran and germ, were progressively removed, resulting in a more spherical shape due to the prominence of the softer endosperm. By 450 seconds, most of the bran and germ were removed, leaving a kernel composed almost entirely of endosperm. This morphological shift had important implications for phenolic extraction, as phenolic compounds are primarily concentrated in the bran and outer layers of the kernel (Zhu et al., 2022).

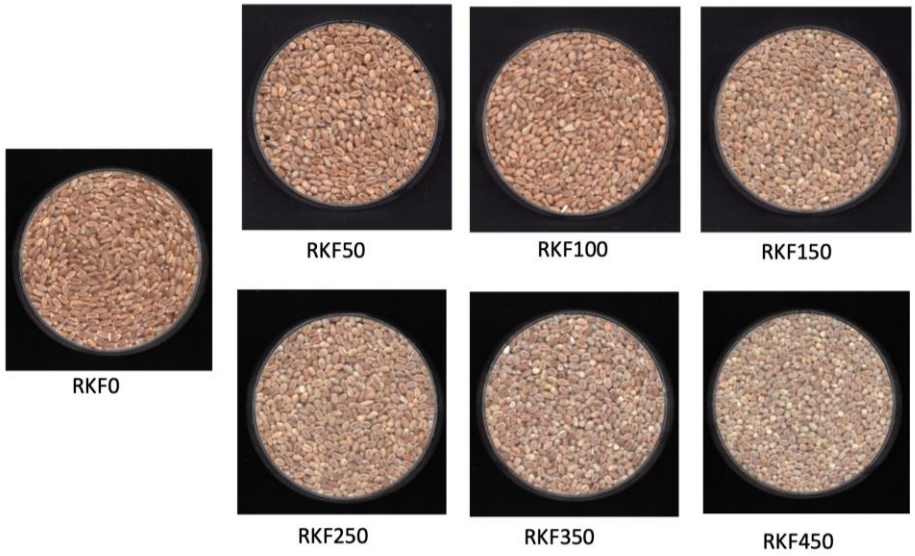


Figure 4.2: Visual representation of wheat kernels at different pearling times

(RKF0: Raw whole wheat kernel; RKF50: Kernels pearled for 50 seconds; RKF100: Kernels pearled for 100 seconds; RKF150: Kernels pearled for 150 seconds; RKF250: Kernels pearled for 250 seconds; RKF350: Kernels pearled for 350 seconds; RKF450: Kernels pearled for 450 seconds).

Images of the bran fractions removed during pearling are displayed in **Figure 4.3**. At first glance it was observed that the paler color was obtained as the pearling process was progressing. The first bran fraction obtained from pearling the wheat kernel for 50 s was the brownest compared to the rest of the fractions. In opposition, the bran from the 450 s pearling process had the lightest color.

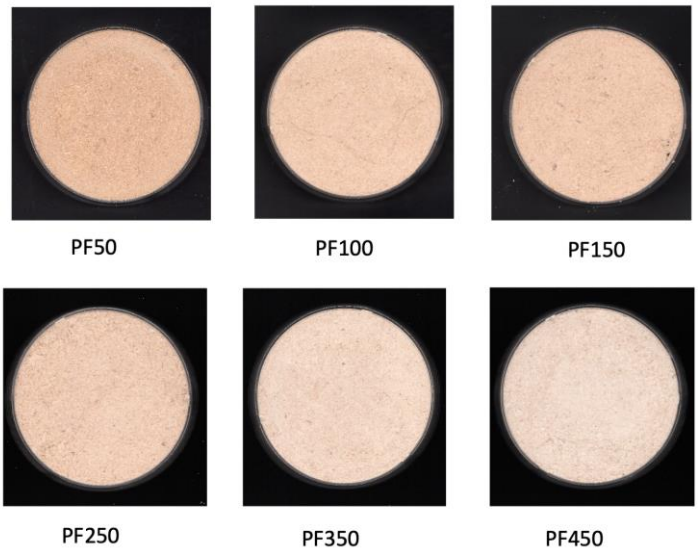


Figure 4.3: Visual representation of pearled fractions at different pearling times

(PF50: Pearled fraction obtained from pearling for 50 seconds; PF100: Pearled fraction obtained from pearling for 100 seconds; PF150: Pearled fraction obtained from pearling for 150 seconds; PF250: Pearled fraction obtained from pearling for 250 seconds; PF350: Pearled fraction obtained from pearling for 350 seconds; PF450: Pearled fraction obtained from pearling for 450 seconds).

4.3.2 Total phenolic content

4.3.2.1 TPC of refined kernel and flour

In this study, TPC analysis confirmed that as pearling progressed, from the outer layers to the inner endosperm, the TPC consistently decreased in both refined kernel fraction (RKF) as shown in **Figure 4.4** and refined flour fractions (RFF) as shown in **Figure 4.5**. A notable exception was

observed with the RKF50 and RFF50 fractions, corresponding to pearling at 50 seconds, where a higher TPC was recorded compared to RKF0 and RFF0. This trend suggests that the removal of 5% of the outermost layer may eliminate foreign matter and pesticides, potentially contributing to the higher phenolic content in RKF50 and RFF50.

These findings align with previous research. For instance, Barron *et al*. (2007) demonstrated that the aleurone layer, located just beneath the outer bran, is a rich source of phenolic compounds. Shorter pearling times, which preserve this layer, result in higher concentrations of bioactive phenolic compounds. This is consistent with the current results, where fractions with shorter pearling times (such as RKF50 and RFF50) retained more of the aleurone layer, leading to higher TPC.

Additionally, Beta *et al*. (2005) reported that phenolic content decreases as pearling progresses through the aleurone layer, with the highest concentrations observed in the initial 10% of pearling fractions. This study's TPC results from RKF50 and RFF50 corroborate these findings, suggesting that the early stages of pearling preserve more phenolic compounds.

Furthermore, the variation in TPC between RKF and RFF across all pearling times was statistically significant ($P< 0.05$), with RFF consistently showing higher TPC than RKF. The higher TPC in RFF compared to RKF may also be explained by the better solvent penetration and surface area in flour samples, enhancing the extraction of phenolic compounds. Additionally, differences in genetic factors and growing conditions, as noted by Menga *et al*. (2010), may have contributed to the variation in TPC observed among the three samples of refined kernel and flour fractions.

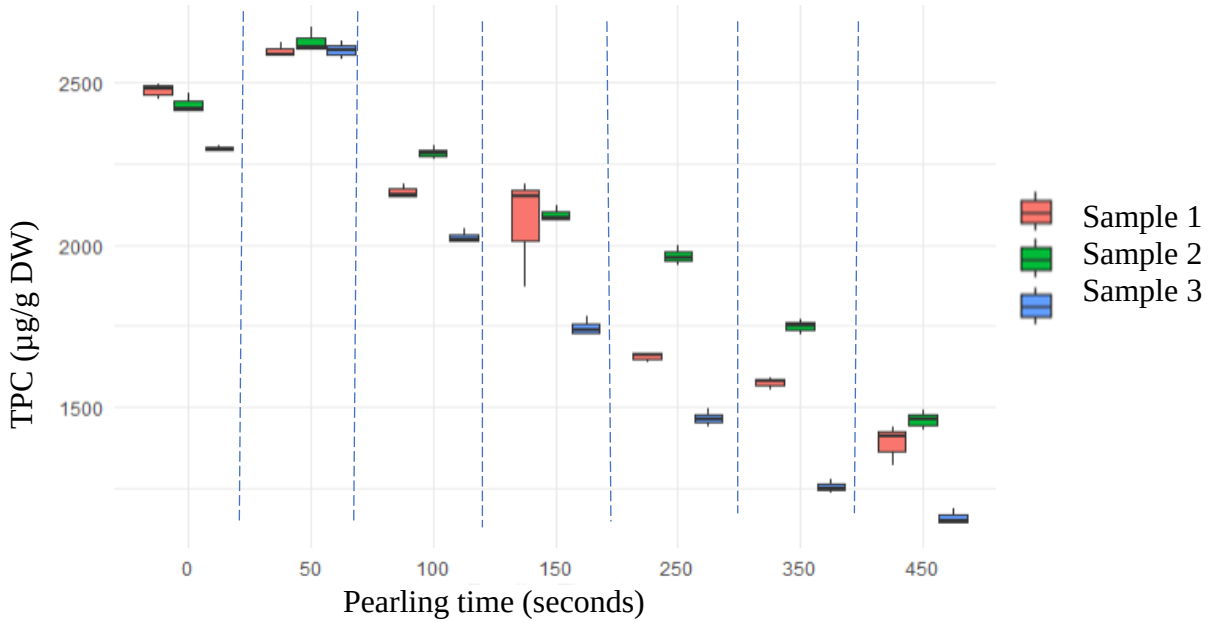


Figure 4.4: Total Phenolic Content (TPC) in refined kernel fractions (RKF) across pearling times (50, 100s, 150, 250, 350, 450 seconds)

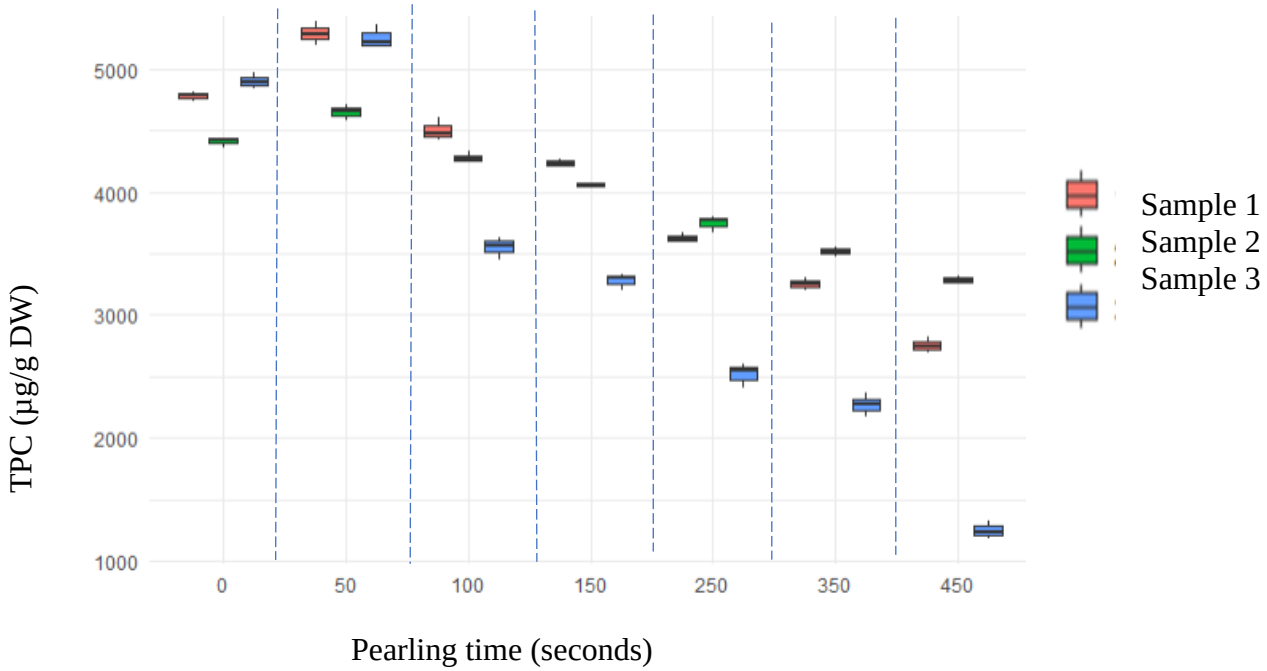


Figure 4.5: Total Phenolic Content (TPC) in refined flour fractions (RFF) across pearling times (50, 100, 150, 250, 350, 450 seconds)

4.3.2.2 TPC of pearled fractions

The TPC of the pearled fractions (PF) was also significantly impacted by pearling time shown in **Figure 4.6**. While TPC generally decreased as pearling progressed, PF100, representing the 10% pearling fraction, exhibited higher TPC than PF50 (5% pearling fraction). This suggests that the 10% pearling fraction still retained much of the aleurone layer, which is known to be rich in phenolic compounds (Barron et al., 2007). The study's MANOVA results showed that pearling time had a significant effect on TPC ($p < 0.05$), with the highest TPC observed in PF100.

This finding is consistent with Giordano *et al.* (2019) who reported that the 5-10% common wheat pearling fraction contained higher amounts of soluble phenolic acids (184 µg/g) compared to the 0-5% fraction (174 µg/g), indicating that the outermost layers (5-10%) are particularly rich in phenolics. Similarly, Liyana-Pathirana & Shahidi (2007) demonstrated that shorter pearling times result in higher antioxidant activity, primarily due to greater retention of phenolic compounds in the outer wheat layers, including the bran and aleurone.

As pearling time increased, the subsequent pearled fractions (e.g., PF150, PF250, PF350, and PF450) exhibited a progressive reduction in TPC, in line with the increasing removal of phenolic-rich outer layers. This trend aligns with findings from Giordano *et al.* (2017), who reported that the bran fraction contains significantly higher levels of phenolic acids and antioxidant activity compared to later pearled fractions. The current results further support that pearling removes the outermost layers where phenolics are concentrated, particularly in the early stages (5-10%).

The findings of Zhu *et al*. (2022) has highlighted the concentration of phenolic acids in the outermost layers of wheat kernels, which was confirmed in their study involving six varieties of bread wheat. In the same study, seven consecutive pearling fractions (P1 through P7), representing different percentages of the wheat kernel, were analyzed, with P1 (0-7%) and P2 (8-13%)

containing the highest concentrations of phenolic compounds. Their results demonstrated a significant spatial variance in phenolic content across the wheat kernel, with the outer layers (P1 and P2) being especially rich in bioactive compounds such as phenolic acids. These findings are reflected in this study's PF50 and PF100 results, which also show higher TPC in the early pearling fractions. This further supports the notion that shorter pearling times, which remove primarily the outer bran layers, yield higher concentrations of phenolic compounds compared to the inner endosperm.

Interestingly, the results from durum wheat show a contrasting trend. Giordano *et al.* (2019) observed that the 15-20% pearling fraction in durum wheat exhibited the highest phenolic content 349 µg/g DW, followed by earlier and later fractions.

This suggests that phenolic distribution may vary between wheat species, with common wheat showing higher phenolic content in outer fractions, while durum wheat may have a different phenolic distribution across its kernel layers. The results of this study reaffirm that phenolic content in common wheat decreases with more extensive pearling, as more of the phenolic-rich outer layers are removed.

The uneven distribution of phenolic compounds from the outer layer to the inner portion of wheat kernels explains the observed trends in TPC. The aleurone and bran layers are known to be rich in phenolic compounds, and as pearling time progresses, the removal of these layers leads to a significant decrease in TPC. The findings from PF100, which exhibited the highest TPC among the pearled fractions, align with studies like Beta *et al*. (2005) and Giordano *et al.* (2019), which identified the 5-10% pearling fraction as the richest in phenolic compounds.

The overall trend of decreasing TPC with longer pearling times is consistent with Parker *et al*. (2005), who linked the biosynthesis of phenolic compounds to arabinoxylan synthesis in the wheat grain's outer layers. As pearling removes these layers, the biosynthesis and retention of phenolics are reduced, leading to lower TPC in more heavily pearled fractions.

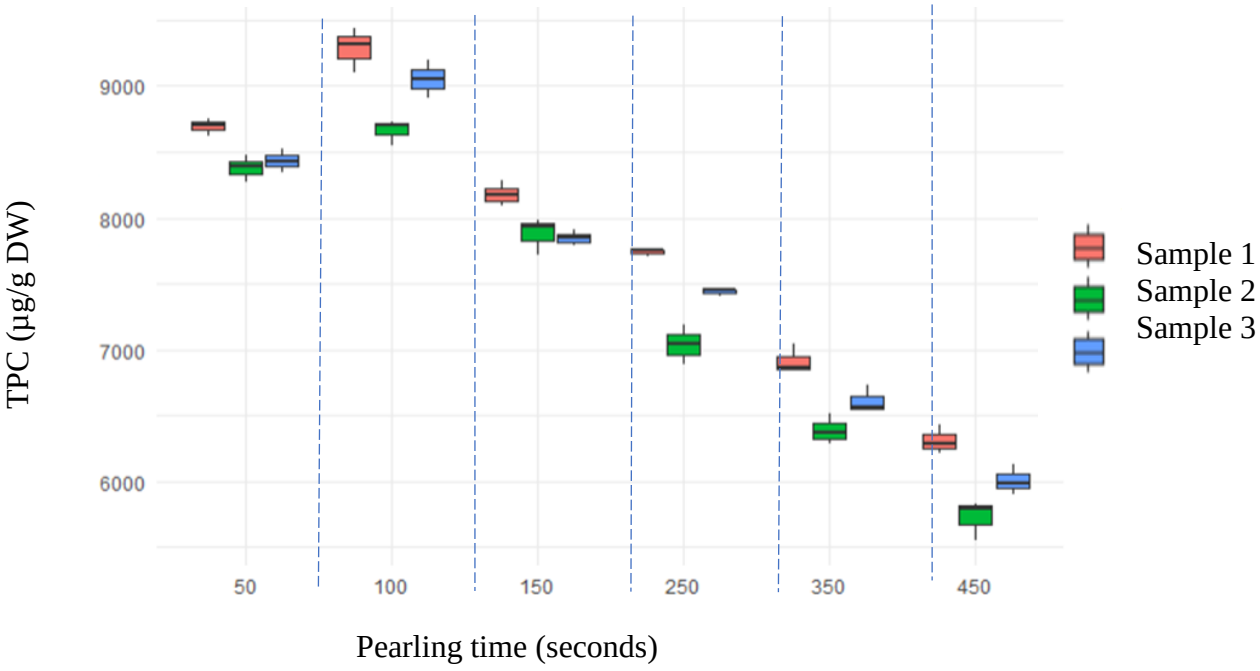


Figure 4.6: Total Phenolic Content (TPC) in pearled fractions (PF) across pearling times (50, 100, 150, 250, 350, 450 seconds)

4.3.3 Phenolic acid profiles

4.3.3.1 Phenolic acid profiles of refined kernels and flours

The phenolic acid profiles of refined kernels (**Figure 4.7**) and their derived flour fractions (**Figure 4.8**) were significantly influenced by pearling time. As pearling time increased, more of the outer layers of the wheat kernel were removed, leading to a progressive reduction in phenolic acid content. This reduction was particularly evident in later pearling times, where the fractions were dominated by the endosperm, which contains lower concentrations of phenolic acids such as ferulic, sinapic, and *p*-coumaric acids. These phenolic acids are primarily concentrated in the aleurone layer and outer pericarp, and their concentration decreased sharply with longer pearling times.

Studies by Tian *et al*. (2024) and Zhu *et al*. (2022) corroborated these findings, showing that shorter pearling times retained more of the bran, which resulted in higher concentrations of phenolic acids. The current study's MANOVA results further supported the impact of pearling time on phenolic acid concentrations extracted from both RKF and RFF. In RFF, Pillai's trace (0.98875) and an approximate F-value of 407.44 (p < 2.2e-16) indicated a significant effect of pearling time on phenolic acid content. This effect was even more pronounced in RKF, where Pillai's trace (0.99468) and an F-value of 866.97 (p < 2.2e-16) suggested that phenolic acid profiles were more sensitive to pearling time when the extraction was performed on RKF compared to that made on RFF.

The variation in the impact of pearling time on extracted phenolic acids between RKF and RFF can be attributed to the distribution of phenolic compounds within the wheat grain. Kernels, which contain higher concentrations of phenolic acids in their outer layers, experienced more substantial reductions in phenolic content as pearling removed these layers. In contrast, flour, primarily

derived from the inner endosperm, showed less reduction in phenolic content due to the lower initial concentration of phenolic acids in the endosperm.

Post-hoc Tukey's HSD tests revealed significant differences in individual phenolic acids across pearling times. In RKF, there was a sharp decline in phenolic acids such as gallic and hydroxybenzoic acids as pearling time increased, with the most significant reductions observed between RKF100 and RKF450. RFF exhibited similar trends, but the reductions were less pronounced, consistent with the lower phenolic content in the endosperm.

Correlation analysis revealed strong negative relationships between pearling time and phenolic acid concentrations in RKF, with the weakest correlation observed for caffeic acid (-0.8538) and the strongest for salicylic acid (-0.9745). These findings emphasize that longer pearling times significantly reduce phenolic content, while shorter pearling durations better preserve these compounds. This effect is particularly pronounced in RKF, where the outer layers, rich in bioactive phenolic acids, remain more intact during the earlier pearling stages.

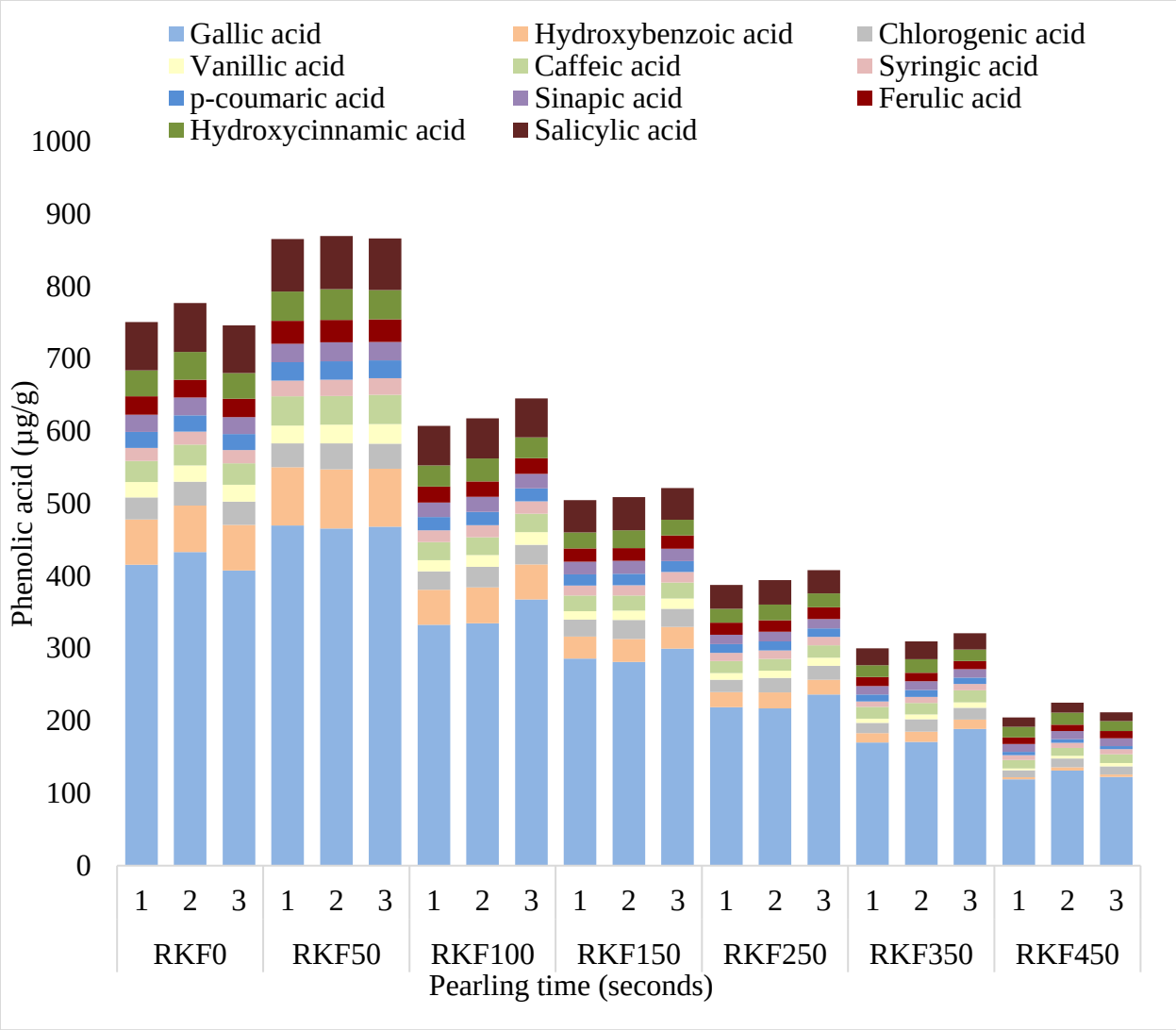


Figure 4.7: Phenolic acid profile of refined kernel fractions (RKF) across pearling times (50, 100, 150, 250, 350, 450 seconds)

Refined kernel fractions: RKF0: Raw whole wheat kernel sample; RKF50: Kernels pearled for 50 seconds; RKF100: Kernels pearled for 100 seconds; RKF150: Kernels pearled for 150 seconds; RKF250: Kernels pearled for 250 seconds; RKF350: Kernels pearled for 350 seconds; RKF450: Kernels pearled for 450 seconds.

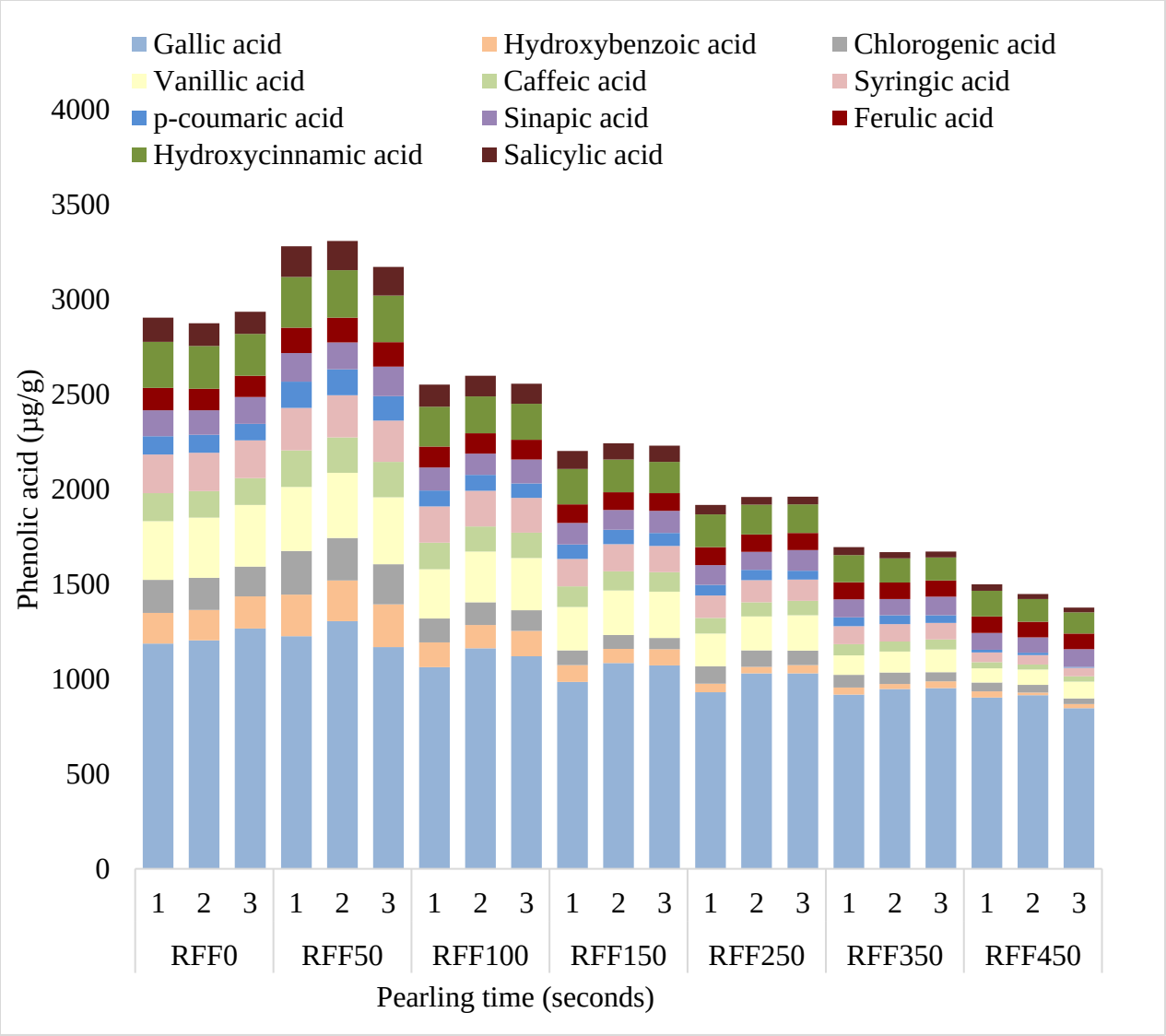


Figure 4.8: Phenolic acid profile of refined flour fractions (RFF) across pearling times (50, 100, 150, 250, 350, 450 seconds)

Refined flour samples: RFF0: Raw whole wheat flour sample; RFF50: Flour obtained from kernels pearled for 50 seconds; RFF100: Flour obtained from kernels pearled for 100 seconds; RFF150: Flour obtained from kernels pearled for 150 seconds; RFF250: Flour obtained from kernels pearled for 250 seconds; RFF350: Flour obtained from kernels pearled for 350 seconds; RFF450: Flour obtained from kernels pearled for 450 seconds.

4.3.3.2 Phenolic acid profiles of pearled fractions

The phenolic acid profiles of the PF were also significantly impacted by pearling time is shown in **Figure 4.9**. The study's MANOVA results demonstrated a highly significant effect of pearling time on the overall phenolic acid content in bran and other outer layers, which were progressively removed as pearling continued. The pearled fraction showed higher concentrations of all 11 phenolic acids quantified at PF100 compared to PF50, as shown in **Figure 4.9,** reflecting the abundance of phenolic compounds in the aleurone layer of the wheat kernel.

As pearling time increased, the outer fractions became depleted of their phenolic content. The reductions in individual phenolic acids, particularly those concentrated in the outer layers, were more drastic in the early (PF50 and PF100) and leveled off in later pearling times (PF350 and PF450), where the remaining material was predominantly endosperm. These results align with previous research, which also found that pearling primarily removes the outer layers of the wheat kernel, where most phenolic compounds are located. For example Barron *et al*. (2007), demonstrated that phenolic acids, such as ferulic, sinapic, and *p*-coumaric acids, are predominantly located in the outer layers of the wheat kernel namely, the aleurone, intermediate, and outer pericarp layers. Their study showed that these layers contain significantly higher concentrations of phenolic acids compared to the whole wheat kernel, with ferulic acid being the most abundant. The aleurone layer, intermediate layer, and outer pericarp contain 6.5%, 3.8%, and 4.0% (w/w) of phenolic acids, respectively, levels markedly higher than those found in other kernel tissues. Moreover, Barron *et al.* (2007) reported that the scutellum exhibited phenolic acid concentrations of 3.9 μg mg^{-1}, much higher than the 0.4 - 0.7 μg mg^{-1} observed in the embryonic axis. These findings highlight the significant impact of pearling, particularly shorter pearling times, which

preserve these outer layers and retain higher phenolic acid concentrations in the bran, while the inner endosperm, from which flour is derived, experiences a lesser reduction in phenolic content.

Tian *et al*. (2024) also reported that pearled flour of wheat retains substantial amounts of both soluble and insoluble-bound ferulic acid, further confirming the concentration of phenolic acids in the wheat outer kernel layers. In their study, pearled flour retained between 85.5% and 98.9% of soluble ferulic acid and 67.3% to 83.3% of insoluble-bound ferulic acid compared to whole wheat flour. Additionally, their analysis of specific phenolic acids in whole wheat flour and pearled flour from different pearling times (P1 and P2, corresponding to 140s and 45s) showed notable changes. For instance, the concentration of 4-hydroxybenzoic acid decreased slightly from 16.27 μg/g in whole wheat to 15.18 μg/g and 15.94 μg/g in P1 and P2 flours, respectively. Similarly, vanillic acid levels declined from 18.90 μg/g in whole wheat to 15.65 μg/g in P1 and 17.46 μg/g in P2. Syringic acid concentrations were 16.13 μg/g in whole wheat, dropping to 13.41 μg/g in P1 and 14.68 μg/g in P2. In the case of *p*-coumaric acid, levels fell from 29.13 μg/g in whole wheat to 22.06 μg/g in P1 and 26.13 μg/g in P2, while ferulic acid was highest in whole wheat flour at 686.88 μg/g, decreasing to 475.71 μg/g in P1 and 563.67 μg/g in P2. Interestingly, sinapic acid levels increased from 96.89 μg/g in whole wheat to 133.74 μg/g in P1 but decreased to 106.54 μg/g in P2. These results underscore the significant retention of phenolic acids in the bran fractions, supporting the conclusion that pearling primarily affects the outer kernel layers.

Giordano *et al*. (2017) also highlighted the importance of the bran fraction in phenolic extraction, demonstrating that the bran contained significantly higher levels of free phenolic acids and total antioxidant activity 10 times higher than refined flour and 3 times higher than whole grain flour. Interestingly, their study also found that red wheat types exhibited higher free phenolic acid

concentrations in the 5-10% pearling fraction than in the 0-5% fraction, corroborating the findings of other studies that the initial pearling fractions contain the most phenolic-rich material.

Post-hoc Tukey's HSD tests confirmed significant differences in phenolic acid concentrations across the different pearling times. The outer pearled fractions (PF50 and PF100) exhibited the highest concentrations of phenolic acids, which are abundant in the bran and outer aleurone layers. In contrast, the inner pearled fractions (PF350 and PF450) contained significantly lower phenolic acid concentrations, reflecting the loss of phenolic-rich layers.

Correlation analysis revealed a strong negative relationship between pearling time and phenolic acid content across all measured phenolic acids in the PF. Syringic acid showed the weakest correlation at -0.8765. These findings emphasize that the removal of outer layers during pearling significantly reduces phenolic content, particularly in the early stages. During this phase, the bran and aleurone layers, which are rich in phenolic compounds, are still present and contribute substantially to the overall phenolic content.

In this study, gallic acid was extracted in higher amounts across all pearling times, whereas salicylic acid exhibited lower extraction yields in the PF50 and PF100 fractions. Additionally, *p*-coumaric acid consistently showed the lowest extraction yields across all other pearling fractions (PF150, PF250, PF350 and PF450). The increased extraction of gallic acid may be attributed to its enhanced solubility under MAE conditions with water. Galanakis *et al*. (2013) highlighted that solubilization is a complex process governed by multiple interacting factors, necessitating a sophisticated model to determine the most suitable extraction solvent. The solubility behavior of phenolic compounds is influenced by their stereochemistry specifically, the arrangement of polar and non-polar fragments within their molecules and the intermolecular forces, such as hydrogen

bonding, that occur between the phenols and solvents. In the case of gallic acid, the presence of an aromatic ring surrounded by three hydroxyl groups and a carboxyl group made polar protic water an ideal solvent.

This observation aligns with the findings of Bucić-Kojić *et al*. (2009), who reported increased recovery of gallic acid using CE from grape seeds as the percentage of alcohol in the solvent decreased, emphasizing the role of water as a polar solvent in enhancing extraction efficiency. Conversely, the lower extraction efficiency of *p*-coumaric acid in this study could be related to its reduced solubility in water under MAE conditions. Previous studies using CE, without elevated temperatures, also reported similar trends in *p*-coumaric acid extraction. Barron *et al*. (2007) observed low *p*-coumaric acid extraction in their work, which is consistent with the findings of this study.

The findings of Jiang *et al*. (2011) provided insight into the distribution of phenolic acids in wheat bran, distinguishing between soluble and bound forms. Jiang *et al*. identified that soluble phenolic acids in wheat bran were primarily vanillic, ferulic, and syringic acids, while bound phenolic acids were mainly composed of ferulic acid, which accounted for 50.1% to 82.2% of the total identified bound phenolics. This distinction between soluble and bound phenolic acids highlighted how differences in their structural forms and localization within the wheat matrix could influence extraction yields.

Verma *et al*. (2009) analyzed the phenolic acid profiles of acid and alkali hydrolysates from the bran of six wheat cultivars using CE out of extracted the free phenolics, salicylic acid represented 1.7% to 16.7% of the total identifiable pool of phenolic acids, indicating that salicylic acid levels in bran are relatively low. Similarly, Shamanin *et al*. (2022) demonstrated that phenolic acid distribution varied significantly across wheat varieties and between bound and free forms. For

example, in purple wheat samples, gallic acid, a hydroxy benzoic acid derivative and a prominent phenolic in cereals, was found in high concentrations in the free fraction, with levels ranging from 15.41 to 95.28 μg/g of dry weight. This high concentration of free gallic acid in certain wheat varieties aligned with the findings of the present study, where gallic acid was extracted in greater amounts, likely due to its structural characteristics that favored higher solubility in the free state. The alignment between these findings underscored the importance of both phenolic acid form (soluble vs. bound) and specific wheat variety in determining extraction outcomes.

Nguyen *et al.* (2023) investigated the impact of pH on the phenolic profiles of selected mustard varieties using pressurized heating at 115°C and 10.2 psi. Their findings revealed that the pH of the treatment significantly influenced the endogenous phenolic compound profiles, with a neutral pH proving to be superior to both acidic and alkaline treatments in terms of extractability.

Additionally, Barron *et al*. (2007) demonstrated that hot alkali pretreatment at 170°C with sodium hydroxide in CE significantly enhanced phenolic acid extraction, increasing recovery by 2.25-fold in the intermediate layer and 2-fold in the outer pericarp. This improvement was attributed to the breakdown of ether-linked phenolic acids which are often associated with lignin in wheat, as also noted by Antoine *et al*. (2004). Together, these studies emphasize the critical roles of pH, temperature, and pretreatment strategies in optimizing the extraction of phenolic compounds, such as ferulic acid, highlighting the need for tailored approaches in the extraction process.

In all three samples studied, the sum of phenolic acids generally decreased with increasing pearling time, except at 100-seconds pearling. Among all three samples, significant differences in the values of individual phenolic acids could be attributed to environmental and varietal differences, as highlighted by Melios *et al*. (2024). This variability suggests that genetic and environmental

factors may play a critical role in influencing phenolic composition and extraction outcomes across different wheat varieties.

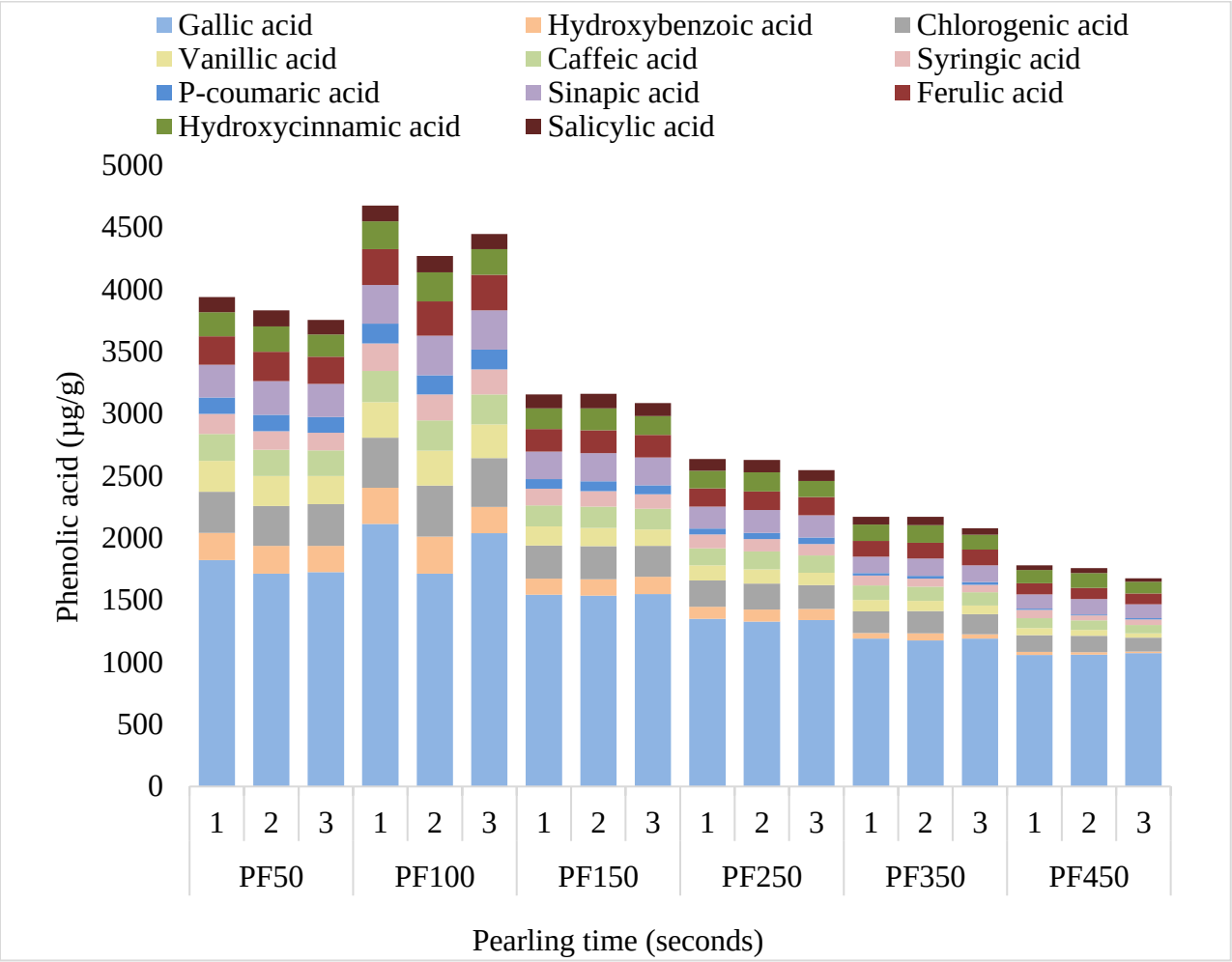


Figure 4.9: Phenolic acid profile of pearled fractions (PF) across pearling times (50, 100, 150, 250, 350, 450 seconds)
(PF50: Pearled fraction obtained from pearling for 50 seconds; PF100: Pearled fraction obtained from pearling for 100 seconds; PF150: Pearled fraction obtained from pearling for 150 seconds; PF250: Pearled fraction obtained from pearling for 250 seconds; PF350: Pearled fraction obtained from pearling for 350 seconds; PF450: Pearled fraction obtained from pearling for 450 seconds).

4.4 Conclusion

This study highlights the critical role of pearling time in influencing the retention of phenolic compounds in wheat pearled, refined kernel and flour fractions. The findings underscore that shorter pearling times effectively preserve the phenolic-rich outer layers, particularly the aleurone, which lies in 5% - 10% (w/w) of wheat kernel, is associated with higher concentrations of phenolic acids. The PF100 fraction exhibited the highest TPC among all pearled fractions, highlighting the concentration of phenolics in the outermost layers removed at early pearling stages. Analyses confirmed that pearling time strongly influenced the concentration of individual phenolic acids, with longer pearling times negatively correlated with phenolic content.

By utilizing MAE, this research demonstrates a rapid and efficient approach for optimizing phenolic extraction from wheat fractions. The insights gained here have practical implications for the food industry, especially in the development of functional foods that harness the antioxidant properties of these bioactive compounds. Future research should focus on refining extraction techniques and exploring interactions between pearling, solvents, and other bioactive compounds to further enhance the nutritional value of wheat-based products.

Author contributions

The authors contributed to the chapter "Distribution of phenolic acids throughout the wheat kernel across different pearling fractions" as follows: Kemashalini Kirusnaruban was responsible for writing the original draft, performing the investigation, conceptualizing the study, and utilizing software for data analysis. Dr. Nicola Gasparre provided training, supervised the work, contributed to the conceptualization, and was involved in software application, as well as reviewing and editing the manuscript. Dr. Ruchira Nandasiri contributed by assisting in the review and editing of the manuscript. Dr. Cristina M. Rosell provided supervision, conceptualized the study and methodologies, secured funding, and offered resources while contributing to the review and editing of the manuscript.

4.5 References

Adom, K. K., & Liu, R. H. (2002). Antioxidant Activity of Grains. *Journal of Agricultural and Food Chemistry*, *50*(21), Article 21. https://doi.org/10.1021/jf0205099

Antoine, C., Peyron, S., Lullien-Pellerin, V., Abecassis, J., & Rouau, X. (2004). Wheat bran tissue fractionation using biochemical markers. *Journal of Cereal Science*, *39*(3), Article 3. https://doi.org/10.1016/j.jcs.2004.02.001

Barron, C., Surget, A., & Rouau, X. (2007). Relative amounts of tissues in mature wheat (*Triticum aestivum* L.) grain and their carbohydrate and phenolic acid composition. *Journal of Cereal Science*, *45*(1), Article 1. https://doi.org/10.1016/j.jcs.2006.07.004

Bazzano, L. A., Song, Y., Bubes, V., Good, C. K., Manson, J. E., & Liu, S. (2005). Dietary intake of whole and refined grain breakfast cereals and weight gain in men. *Obesity Research*, *13*(11), Article 11. https://doi.org/10.1038/oby.2005.240

Beta, T., Nam, S., Dexter, J. E., & Sapirstein, H. D. (2005). Phenolic Content and Antioxidant Activity of Pearled Wheat and Roller-Milled Fractions. *Cereal Chemistry*, *82*(4), Article 4. https://doi.org/10.1094/CC-82-0390

Bucić-Kojić, A., Planinić, M., Tomas, S., Jakobek, L., & Šeruga, M. (2009). Influence of solvent and temperature on extraction of phenolic compounds from grape seed, antioxidant activity and colour of extract. *International Journal of Food Science & Technology*, *44*(12), 2394–2401. https://doi.org/10.1111/j.1365-2621.2008.01876.x

Chiremba, C., Rooney, L. W., & Beta, T. (2012). Microwave-assisted extraction of bound phenolic acids in bran and flour fractions from sorghum and maize cultivars varying in hardness.

Journal of Agricultural and Food Chemistry, *60*(18), 4735–4742. https://doi.org/10.1021/jf300279t

Galanakis, C. M., Goulas, V., Tsakona, S., Manganaris, G. A., & Gekas, V. (2013). A Knowledge Base for The Recovery of Natural Phenols with Different Solvents. *International Journal of Food Properties*, *16*(2), 382–396. https://doi.org/10.1080/10942912.2010.522750

Giordano, D., Locatelli, M., Travaglia, F., Bordiga, M., Reyneri, A., Coïsson, J. D., & Blandino, M. (2017). Bioactive compound and antioxidant activity distribution in roller-milled and pearled fractions of conventional and pigmented wheat varieties. *Food Chemistry*, *233*, 483–491. https://doi.org/10.1016/j.foodchem.2017.04.065

Giordano, D., Reyneri, A., Locatelli, M., Coïsson, J. D., & Blandino, M. (2019). Distribution of bioactive compounds in pearled fractions of tritordeum. *Food Chemistry*, *301*, 125228. https://doi.org/10.1016/j.foodchem.2019.125228

González-Thuillier, I., Salt, L., Chope, G., Penson, S., Skeggs, P., Tosi, P., Powers, S. J., Ward, J. L., Wilde, P., Shewry, P. R., & Haslam, R. P. (2015). Distribution of Lipids in the Grain of Wheat (cv. Hereward) Determined by Lipidomic Analysis of Milling and Pearling Fractions. *Journal of Agricultural and Food Chemistry*, *63*(49), 10705–10716. https://doi.org/10.1021/acs.jafc.5b05289

Hemery, Y., Rouau, X., Lullien-Pellerin, V., Barron, C., & Abecassis, J. (2007). Dry processes to develop wheat fractions and products with enhanced nutritional quality. *Journal of Cereal Science*, *46*(3), 327–347. https://doi.org/10.1016/j.jcs.2007.09.008

Jiang, H., Martin, J., Okot-Kotber, M., & Seib, P. A. (2011). Color of Whole-Wheat Foods Prepared from a Bright-White Hard Winter Wheat and the Phenolic Acids in Its Coarse

Bran. *Journal of Food Science*, *76*(6), Article 6. https://doi.org/10.1111/j.1750-3841.2011.02272.x

Liu, R. H. (2007). Whole grain phytochemicals and health. *Journal of Cereal Science*, *46*(3), Article 3. https://doi.org/10.1016/j.jcs.2007.06.010

Liyana-Pathirana, C. M., & Shahidi, F. (2006). Importance of Insoluble-Bound Phenolics to Antioxidant Properties of Wheat. *Journal of Agricultural and Food Chemistry*, *54*(4), Article 4. https://doi.org/10.1021/jf052556h

Liyana-Pathirana, C. M., & Shahidi, F. (2007). Antioxidant and free radical scavenging activities of whole wheat and milling fractions. *Food Chemistry*, *101*(3), Article 3. https://doi.org/10.1016/j.foodchem.2006.03.016

Lu, Y., & Luthria, D. (2016). Influence of gelatinization on the extraction of phenolic acids from wheat fractions. *Food Chemistry*, *194*, 1138–1142. https://doi.org/10.1016/j.foodchem.2015.08.074

Melios, S., Ninou, E., Irakli, M., Tsivelika, N., Sistanis, I., Papathanasiou, F., Didos, S., Zinoviadou, K., Karantonis, H. C., Argiriou, A., & Mylonas, I. (2024). Effect of Genotype, Environment, and Their Interaction on the Antioxidant Properties of Durum Wheat: Impact of Nitrogen Fertilization and Sowing Time. *Agriculture*, *14*(2), Article 2. https://doi.org/10.3390/agriculture14020328

Menga, V., Fares, C., Troccoli, A., Cattivelli, L., & Baiano, A. (2010). Effects of genotype, location and baking on the phenolic content and some antioxidant properties of cereal species. *International Journal of Food Science & Technology*, *45*(1), Article 1. https://doi.org/10.1111/j.1365-2621.2009.02072.x

Navarro, J. L., Biglione, C., Paesani, C., Moiraghi, M., León, A. E., & Steffolani, M. E. (2022). Effect of wheat pearling process on composition and nutritional profile of flour and its bread-making performance. *International Journal of Food Science & Technology*, *57*(1), 249–257. https://doi.org/10.1111/ijfs.15401

Nguyen, T., Nandasiri, R., Fadairo, O., & Eskin, N. A. M. (2023). Optimization of pH for extracting sinapates from mustard varieties using green technology. *Applied Food Research*, *3*(2), 100323. https://doi.org/10.1016/j.afres.2023.100323

Parker, M. L., Ng, A., & Waldron, K. W. (2005). The phenolic acid and polysaccharide composition of cell walls of bran layers of mature wheat (Triticum aestivum L. cv. Avalon) grains. *Journal of the Science of Food and Agriculture*, *85*(15), 2539–2547. https://doi.org/10.1002/jsfa.2304

Shamanin, V. P., Tekin-Cakmak, Z. H., Gordeeva, E. I., Karasu, S., Pototskaya, I., Chursin, A. S., Pozherukova, V. E., Ozulku, G., Morgounov, A. I., Sagdic, O., & Koksel, H. (2022a). Antioxidant Capacity and Profiles of Phenolic Acids in Various Genotypes of Purple Wheat. *Foods*, *11*(16), Article 16. https://doi.org/10.3390/foods11162515

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, *16*(3), 144–158. https://doi.org/10.5344/ajev.1965.16.3.144

Teslić, N., Bojanić, N., Rakić, D., Takači, A., Zeković, Z., Fišteš, A., Bodroža-Solarov, M., & Pavlić, B. (2019). Defatted wheat germ as source of polyphenols—Optimization of microwave-assisted extraction by RSM and ANN approach. *Chemical Engineering and Processing - Process Intensification*, *143*, 107634. https://doi.org/10.1016/j.cep.2019.107634

Tian, W., Zhi, L., Chen, T., Gong, X., Zhang, Y., & He, Z. (2024). Effects of pearling on proximate composition, dough rheology, bread-making quality and health-promoting components of elite wheat cultivars. *LWT*, *198*, 115962. https://doi.org/10.1016/j.lwt.2024.115962

Vaher, M., Matso, K., Levandi, T., Helmja, K., & Kaljurand, M. (2010). Phenolic compounds and the antioxidant activity of the bran, flour and whole grain of different wheat varieties. *Procedia Chemistry*, *2*(1), 76–82. https://doi.org/10.1016/j.proche.2009.12.013

Verma, B., Hucl, P., & Chibbar, R. (2009). Phenolic acid composition and antioxidant capacity of acid and alkali hydrolysed wheat bran fractions. *Food Chemistry*, *116*, 947–954. https://doi.org/10.1016/j.foodchem.2009.03.060

Wang, X., Zhang, X., Hou, H., Ma, X., Sun, S., Wang, H., & Kong, L. (2020). Metabolomics and gene expression analysis reveal the accumulation patterns of phenylpropanoids and flavonoids in different colored-grain wheats (Triticum aestivum L.). *Food Research International (Ottawa, Ont.)*, *138*(Pt A), 109711. https://doi.org/10.1016/j.foodres.2020.109711

Zhao, H., Dong, J., Lu, J., Chen, J., Li, Y., Shan, L., Lin, Y., Fan, W., & Gu, G. (2006). Effects of Extraction Solvent Mixtures on Antioxidant Activity Evaluation and Their Extraction Capacity and Selectivity for Free Phenolic Compounds in Barley (Hordeum vulgare L.). *Journal of Agricultural and Food Chemistry*, *54*(19), Article 19. https://doi.org/10.1021/jf061087w

Zhu, A., Zhou, Q., Hu, S., Wang, F., Tian, Z., Hu, X., Liu, H., Jiang, D., & Chen, W. (2022). Metabolomic analysis of the grain pearling fractions of six bread wheat varieties. *Food Chemistry*, *369*, 130881. https://doi.org/10.1016/j.foodchem.2021.130881

CHAPTER 5

5.1 Conclusions and future research directions

This study demonstrates that MAE significantly affect the extraction and concentration of phenolic compounds in wheat. MAE, when optimized for temperature, time, solvent type, and sample composition, was found to be more efficient than conventional methods. The use of water as a solvent was particularly effective in extracting phenolic compounds, such as gallic and vanillic acids, while ethanol was better suited for compounds like caffeic and *p*-coumaric acids. Furthermore, higher temperatures and prolonged extraction times generally enhanced the total phenolic content, with flour samples yielding higher TPC than kernel samples.

Pearling, which involves the progressive removal of wheat kernel layers, has a profound impact on phenolic acid distribution. Shorter pearling times retained higher concentrations of phenolic acids in the outer layers, indicating that early-stage pearling fractions are richer in bioactive compounds. Statistical analyses, including MANOVA and correlation analyses, confirmed that pearling time strongly influenced the concentration of individual phenolic acids, with longer pearling times negatively correlated with phenolic content.

The findings emphasize the importance of optimizing both extraction conditions and pearling times to maximize phenolic yield. This research provides valuable insights into developing wheat-based products with enhanced nutritional and functional properties, particularly for applications in functional foods and nutraceuticals.

Also this study findings demonstrate that microwave treatment significantly influences the color parameters of wheat kernels. The interplay of temperature, time, and solvent selection is pivotal in determining the extent of color changes. Specifically, the higher L^* and b^* values observed in

kernels treated with 80% ethanol compared to those extracted with water suggest that solvent choice is critical in optimizing color quality. Additionally, higher extraction temperatures and prolonged exposure can enhance the extraction of colored compounds but may lead to thermal degradation, resulting in undesirable color changes. These findings have practical implications for food processing applications, where careful optimization of microwave parameters could enhance the color quality of wheat-based products. Future research should delve into the specific kinetics of color transformation during MAE and investigate the interactions between proteins and pigments during thermal treatments to elucidate the mechanisms driving these changes further.

Future research should focus on further optimizing MAE parameters, such as temperature, time, and solvent composition, to maximize the yield of specific phenolic acids. This could enable more targeted extractions for producing functional foods and nutraceuticals with enhanced health benefits. Additionally, since shorter pearling times preserve higher concentrations of phenolics, future studies should investigate refining the pearling process. Understanding the dynamics of phenolic distribution across different pearling fractions may help optimize the production of wheat bran products enriched with bioactive compounds. Furthermore, additional research is needed to unravel the complex interactions between phenolic compounds and extraction conditions, aiding in the development of precise protocols for isolating and profiling these compounds across various wheat fractions. The impact of MAE on wheat quality also warrants further exploration, especially regarding how different extraction methods influence not only phenolic yields but also the sensory and quality attributes of wheat-based products. This could provide valuable insights into balancing extraction efficiency with maintaining product quality. Finally, the findings from this study could have broader applications in the development of functional foods and nutraceuticals, with future

research potentially extending these optimized extraction techniques to other grains and food systems to enhance their health-promoting properties.

5.2 Limitations

This study focused exclusively on the widely cultivated Canadian Western Red Spring (CWRS) wheat class, limiting the generalizability of the findings to other wheat classes, which may exhibit different phenolic profiles and extraction efficiencies. Additionally, the sample-to-solvent ratio for flour was initially set at 1:9; however, this caused gelatinization of the flour samples, necessitating an adjustment to a 1:99 ratio to maintain consistency and prevent interference with the extraction process. Furthermore, while ethanol and water were selected as solvents for their environmental friendliness and alignment with sustainable practices, the potential impact of other solvent systems on extraction efficiency and the resulting phenolic profile was not explored, leaving room for further investigation into optimizing solvent selection.