

Discovery of wheat bran extract's anti-aging potential against
D-galactose-induced oxidative stress in a rat aging model

by

Kaori Kobayashi

M.S., Northeast College of Health Sciences, 2020

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the
requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Food, Nutrition, Dietetics and Health
College of Health and Human Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2025

Abstract

Aging is a complex biological process associated with various health complications that require the development of effective interventions for healthy aging. Wheat bran extracts (WBE) have emerged as a promising natural alternative due to their rich composition of bioactive compounds, including phenolic compounds, dietary fiber, and arabinoxylans, which contribute to their functional properties in both food and cosmetic applications. This study also has the potential to contribute to the Sustainable Development Goals (SDGs) by reducing wheat bran as waste, which is discarded in large quantities. The present study explores the antioxidant and enzyme inhibitory activities of WBEs, emphasizing their potential for the new approach of wheat bran extracts as natural preservatives and anti-aging agents. In addition, the study seeks to bridge the gap in understanding the mechanisms by which WBEs exert their beneficial effects.

The primary objective of this research is to evaluate the potential of WBEs as functional ingredients for food and cosmetic applications, particularly their antioxidant and enzyme-inhibitory activities. The study aims to determine the effectiveness of WBE supplementation in modulating oxidative stress and aging-related biomarkers in an experimental animal model. Furthermore, research investigates how WBEs impact biological processes such as cellular aging, metabolic regulation, and tissue-specific oxidative responses.

A research study was conducted using a D-galactose-induced aging rat model to further evaluate the effects of WBE supplementation on aging-related parameters. Female Wistar rats were divided into four groups: (1) saline-injected negative control, (2) D-galactose-injected positive control, (3) D-galactose-injected with a dietary 5% wheat bran extract (WBE) supplement, and (4) D-galactose-injected with a dietary 10% WBE supplement. During a six-week period, physical parameters, antioxidant enzyme activities, and aging

markers were assessed. Measurement of body fat percentages, erythrocyte superoxide dismutase (SOD) activity, and beta-galactosidase activity in liver tissue. Furthermore, Western blot analysis was performed to assess protein quantification and expression of SOD, catalase, and telomerase reverse transcriptase to evaluate systemic oxidative stress markers.

Supplementation with WBE was found to have a significant impact on the maintenance of body fat percentages, with a mean value observed in the WBE group being approximately 30 %, compared to an average of 23 % in the control group ($P < 0.05$). The antioxidant enzyme activity exhibited a dose-dependent increase, with the erythrocyte SOD activity reaching 42,368 U/mg in the 10 % WBE group. Beta-galactosidase activity in liver tissue differed significantly among treatment groups, suggesting WBE's influence on cellular aging. In addition, biochemical assays demonstrated reductions in lipid peroxidation markers, further supporting WBE's antioxidative potential. Conversely, protein expression levels of SOD, catalase, and telomerase reverse transcriptase remained unaltered, suggesting tissue-specific responses that necessitate further investigation.

The findings suggest that WEB supplementation has the capacity to modulate aging-related parameters and oxidative stress markers, particularly in directly exposed tissues such as erythrocytes. The potential applications of WBEs in both food and cosmetics highlight their versatility as functional ingredients. Their antioxidant, antimicrobial, and enzyme-inhibitory properties render them promising candidates for natural preservatives and anti-aging formulations. While preliminary results support their role in anti-aging interventions, further comprehensive in vivo studies are required to elucidate the bioavailability and efficacy of WBE-derived bioactive compounds across various biological systems. Mechanistic studies are also required to facilitate a more complete understanding of the molecular pathways involved in WBE-induced protective effects.

Discovery of wheat bran extract's anti-aging potential against
D-galactose-induced oxidative stress in a rat aging model

by

Kaori Kobayashi

M.S., Northeast College of Health Sciences, 2020

A DISSERTATION

submitted in partial fulfillment of the
requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Food, Nutrition, Dietetics and Health
College of Health and Human Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2025

Approved by:

Major Professor
Dr. Weiqun Wang

Copyright

© Kaori Kobayashi 2025.

Abstract

Aging is a complex biological process associated with various health complications that require the development of effective interventions for healthy aging. Wheat bran extracts (WBE) have emerged as a promising natural alternative due to their rich composition of bioactive compounds, including phenolic compounds, dietary fiber, and arabinoxylans, which contribute to their functional properties in both food and cosmetic applications. This study also has the potential to contribute to the Sustainable Development Goals (SDGs) by reducing wheat bran as waste, which is discarded in large quantities. The present study explores the antioxidant and enzyme inhibitory activities of WBEs, emphasizing their potential for the new approach of wheat bran extracts as natural preservatives and anti-aging agents. In addition, the study seeks to bridge the gap in understanding the mechanisms by which WBEs exert their beneficial effects.

The primary objective of this research is to evaluate the potential of WBEs as functional ingredients for food and cosmetic applications, particularly their antioxidant and enzyme-inhibitory activities. The study aims to determine the effectiveness of WBE supplementation in modulating oxidative stress and aging-related biomarkers in an experimental animal model. Furthermore, research investigates how WBEs impact biological processes such as cellular aging, metabolic regulation, and tissue-specific oxidative responses.

A research study was conducted using a D-galactose-induced aging rat model to further evaluate the effects of WBE supplementation on aging-related parameters. Female Wistar rats were divided into four groups: (1) saline-injected negative control, (2) D-galactose-injected positive control, (3) D-galactose-injected with a dietary 5% wheat bran extract (WBE) supplement, and (4) D-galactose-injected with a dietary 10% WBE supplement. During a six-week period, physical parameters, antioxidant enzyme activities, and aging

markers were assessed. Measurement of body fat percentages, erythrocyte superoxide dismutase (SOD) activity, and beta-galactosidase activity in liver tissue. Furthermore, Western blot analysis was performed to assess protein quantification and expression of SOD, catalase, and telomerase reverse transcriptase to evaluate systemic oxidative stress markers.

Supplementation with WBE was found to have a significant impact on the maintenance of body fat percentages, with a mean value observed in the WBE group being approximately 30 %, compared to an average of 23 % in the control group ($P < 0.05$). The antioxidant enzyme activity exhibited a dose-dependent increase, with the erythrocyte SOD activity reaching 42,368 U/mg in the 10 % WBE group. Beta-galactosidase activity in liver tissue differed significantly among treatment groups, suggesting WBE's influence on cellular aging. In addition, biochemical assays demonstrated reductions in lipid peroxidation markers, further supporting WBE's antioxidative potential. Conversely, protein expression levels of SOD, catalase, and telomerase reverse transcriptase remained unaltered, suggesting tissue-specific responses that necessitate further investigation.

The findings suggest that WEB supplementation has the capacity to modulate aging-related parameters and oxidative stress markers, particularly in directly exposed tissues such as erythrocytes. The potential applications of WBEs in both food and cosmetics highlight their versatility as functional ingredients. Their antioxidant, antimicrobial, and enzyme-inhibitory properties render them promising candidates for natural preservatives and anti-aging formulations. While preliminary results support their role in anti-aging interventions, further comprehensive in vivo studies are required to elucidate the bioavailability and efficacy of WBE-derived bioactive compounds across various biological systems. Mechanistic studies are also required to facilitate a more complete understanding of the molecular pathways involved in WBE-induced protective effects.

Table of Contents

List of Figures	x
List of Tables	xi
Acknowledgements	xii
1 Literature review - functional properties and potential applications of wheat bran extracts in food and cosmetics: a review of antioxidant, enzyme-inhibitory, and anti-aging benefits	1
1.1 Introduction	2
1.2 Wheat Bran Extracts	5
1.2.1 Bioactive Compounds in Wheat Bran Extracts	5
1.2.2 Bioactive Properties in Wheat Bran Extracts	7
1.2.3 Anti-Aging Potential of Wheat Bran Extracts	11
1.2.4 Inhibitory Effect of Wheat Bran Extract on Skin Aging-Related Enzyme	13
1.2.5 Other health benefits of Wheat Bran	14
1.3 Current Findings	16
1.3.1 Animal Experiments	16
1.3.2 Superoxide Dismutase (SOD) Activity	16
1.3.3 β Galactosidase Activity	17
1.4 Application of Wheat Bran Extract	18
1.4.1 In Food Industries	18
1.4.2 In Cosmetic Industries	20
1.5 Factors That Affect the Yield of WBEs	21

1.6	Potential Risks and Mitigations	22
1.7	Challenges and Future Perspectives	23
2	Discovery of wheat bran extract’s anti-aging potential against D-galactose-induced oxidative stress in a rat aging model	25
2.1	Abstract	25
2.2	Introduction	26
2.3	Materials and methods	28
2.3.1	Materials	28
2.3.2	Analysis of SOD in blood	30
2.3.3	Analysis of Catalase, SOD2, and TERT in Skin and Liver Tissue . . .	31
2.3.4	Analysis of β -Galactosidase	32
2.3.5	Statistical Analysis	32
2.4	Results	33
2.4.1	Change of Body Weights and Food Intake	33
2.4.2	Effect of WBE supplements on Body Fat Percentage in aging rats . .	33
2.4.3	Effect of WBE supplements on SOD in aging rats	33
2.4.4	Effect of WBE supplements of β -Galactosidase in aging rats	35
2.4.5	Protein Expression in Skin and Liver	35
2.5	Discussion	37
2.6	Conclusion	40
	Bibliography	43

List of Figures

1.1	The contents of bioactive compounds, vitamins, minerals, and antioxidant activities of wheat bran extracts	7
1.2	Potential mechanisms of the antioxidant, antihypertensive, and anti-inflammatory activities of wheat bran extracts and their bioactive peptides against reactive oxygen species	9
1.3	Chemical structures of arabinoxylans. Monosubstituted β -d-xylopyranosylat O-3 with ferulic acid residue esterified at a-l-arabinofuranosyl	12
1.4	Chemical structures of (A) xylose and (B) xylo-oligosaccharides	13
1.5	Inhibitory effect of wheat bran extracts on skin aging-related enzymes	15
1.6	Anti-aging and senescence biomarker analysis in a D-galactose-induced aging rat model	17
2.1	Effect of WBE supplements on Body weight transition and food intake over the experimental period	34
2.2	Effect of WBE supplements on body fat in aging rats	35
2.3	Effect of WBE supplements on SOD in aging rats	36
2.4	Effect of WBE supplements on β -Galactosidase Activity in aging rats	37
2.5	Western blot analysis of WBE supplements on protein expressions of catalase, SOD2, and telomerase reverse transcriptase (TERT) in the skin and livers of D galactose-induced accelerated aging rats	42

List of Tables

1.1	Summary of skin-related enzymes and their role in skin aging.	15
2.1	Composition of ANG 93G and WBE diet	29
2.2	Galactose loading vs. SOD activity: erythrocytes vs. liver	41

Acknowledgments

First and foremost, I extend my deepest gratitude to my advisor, Dr. Weiqun (George) Wang, for being so patient, enthusiastic, and helpful. He has guided me a lot during my Ph.D. His knowledge, logical thinking, and helpful ideas have been very important in planning my research and have also helped me to do well at university. His support and guidance have been a great inspiration, and I am honored to be his student.

I would also like to thank my committee members: Dr. Yonghui Li, Dr. Kraig Roozeboom, and Dr. Wu Cen, for giving me so much of their time, for being so knowledgeable, and for giving me such great feedback, which has made my research so much better. I am especially thankful to Dr. Li for letting me use his lab equipment and for letting me work with his students on experiments. He is always happy to share what he knows and has some great ideas, especially about wheat bran extract, which has really helped me to understand my field.

Dr. Roozeboom gave me the chance to get some experience in his field during the summer of my first year. Seeing wheat grow and learning about fieldwork was really helpful for me. His help and advice have been really important for my research.

I am also very grateful to Dr. Wu for being so helpful. He gave me some useful statistical advice when my data did not initially show significant differences. He helped me with complex statistical analyses and supported me when I was struggling. This helped me improve my methodology and understand my results better.

I'm also thankful to my colleagues and faculty members who have shared this academic journey with me. They have helped me a lot by working with me, encouraging me, and having interesting discussions. This has made my Ph.D. experience really good. Finally, I want to say a big thank you to my family and friends for always having my back and believing in me, which helped me stay motivated.

Chapter 1

Literature review - functional properties and potential applications of wheat bran extracts in food and cosmetics: a review of antioxidant, enzyme-inhibitory, and anti-aging benefits

Kaori Kobayashi, Md Suzauddula, Ryan Bender, Cheng Li, Yonghui Li, Xiuzhi Susan Sun ,and Weiqun Wang.

This review was previously published in the journal *Foods* on February 5, 2025.

1.1 Introduction

Wheat bran contains a significant amount of bioactive compounds, particularly phenolic acids and flavonoids [1]. Wheat bran is the outermost layer of wheat grain, often considered a byproduct of the wheat milling industry. All over the world, approximately 0.12 billion tons of wheat bran is produced annually [2]. It is also an abundant and low-cost by-product of agricultural processing, which can be used as an alternative food resource [3]. The antioxidant properties of wheat bran have also been documented in the scientific literature. These properties are primarily attributed to the presence of phenolic compounds, such as ferulic acid, p-coumaric acid, and vanillic acid [4]. Previous studies have conducted various in vitro assays to evaluate the antioxidant capacity of wheat bran extracts (WBEs), including DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation decolorization, and ferric-reducing antioxidant power (FRAP) assays, resulting in significant antioxidant activity of WBEs [5]. Furthermore, research has indicated that the antioxidant capacity of WBEs may vary depending on factors such as wheat variety, growing conditions, and extraction methods [6]. Antioxidants play a crucial role in human health by neutralizing harmful free radicals and reducing oxidative stress, which is implicated in various chronic diseases and the aging process [7]. These diseases represent a range of conditions affecting different body systems, from neuro degenerative disorders like Alzheimer's and Parkinson's to metabolic conditions like type 2 diabetes, as well as various forms of tissue degeneration seen in conditions like osteoarthritis and macular degeneration. In recent years, there has been a shift toward natural antioxidants due to concerns about the safety and efficacy of synthetic alternatives. Similarly, aging-related enzyme inhibitors have also gained prominence in both health and cosmetic industries [8]. On this point, WBEs can also be considered for such uses.

Wheat bran is also rich in dietary fibers, proteins, and fats, making it a valuable component in various industries, including food, feed, medicine, and fermentation [9,10]. Rich in dietary fiber, proteins, vitamins, and minerals, wheat bran has garnered significant attention in recent years because of its potential health benefits. Moreover, wheat bran

contains various bioactive compounds, including phenolics and flavonoids, which contribute to its antioxidant properties [4]. As a result, there is growing interest in exploring the full potential of wheat bran as a functional food ingredient and a source of natural bioactive compounds for various applications. Wheat bran fiber plays a crucial role in digestive health by promoting regular bowel movements and preventing constipation [11]. The fermentation of wheat bran in the colon leads to the production of short-chain fatty acids, such as butyrate, which are beneficial for colon health [12]. The presence of butyrate-producing bacteria in the gut is enhanced by wheat bran consumption, which supports a healthy gut microbiome and may protect against colon cancer [12]. Regular consumption of whole grains, including wheat bran, is associated with a 20% to 40% reduction in the risk of cardiovascular diseases. This is due to the synergistic effects of the fiber and other constituents present in whole grains [13]. Wheat bran helps in reducing blood plasma cholesterol levels, which is a significant factor in preventing cardiovascular diseases [14].

The contamination and degradation of food products by microorganisms represent a significant challenge for the food industry, resulting in substantial detriment to economic systems and public health on a global scale [15]. Furthermore, losses incurred during storage have also been identified as a critical factor contributing to food waste [16]. Prolonging the shelf life of food products can markedly diminish waste by reducing the rates of spoilage and occurrences of stockouts, which is advantageous for both manufacturers and retailers. Additionally, extended shelf life can facilitate cost reductions in production and distribution, as it promotes more efficient inventory management and mitigates the necessity of frequent replenishment [17]. Moreover, oxidation poses a significant issue within the food industry due to its profound effects on the quality, shelf life, and marketability of food and beverage products [18]. The food industry requires various ingredients, both synthetic and natural. Notably, numerous synthetic ingredients are experiencing declining popularity owing to health-related apprehensions, diminished nutritional values, and ongoing debates regarding their safety. In contrast, natural sweeteners tend to elicit positive consumer perceptions, as they are commonly associated with healthier lifestyles and enhanced nutritional values [19]. In order to assist food industries in mitigating oxidation and microbial threats, as well

as extending shelf life, the identification of sustainable food ingredients and additives is of paramount importance. WBEs, which are abundant in natural antioxidants and bioactive compounds, emerge as a promising candidate in this endeavor. Utilizing such sustainable sources has the potential to improve product quality and stability while aligning with ecological and health-oriented objectives.

The cosmetic sector represents one of the most significant and rapidly expanding domains within the global economic framework, boasting an impressive revenue of USD 100.49 billion on an international scale [20]. The principal categories within the cosmetics market encompass skincare, haircare, makeup, perfumes, toiletries, and deodorants, as well as oral cosmetics, with skincare emerging as the predominant category in the year 2021 [21]. The incorporation of synthetic constituents in cosmetic formulations can precipitate a spectrum of adverse effects, predominantly attributable to the inclusion of allergenic and toxic agents. These adverse effects may manifest as mild dermal irritations or escalate to more grave health complications, including allergic responses and potential carcinogenic repercussions. The escalating prevalence of cosmetic usage globally has intensified apprehensions regarding these detrimental effects, thereby necessitating heightened awareness and regulatory oversight [22,23]. The literature indicates that a principal challenge confronting the cosmetic industry lies in the pursuit of safe and sustainable natural alternatives that can supplant synthetic materials utilized as ingredients in cosmetic formulations, owing to the associated environmental ramifications and the concomitant implications for human health, which are also of paramount concern to consumers. In this context, WBEs have exhibited considerable promise as a viable ingredient for the cosmetics industry due to their multifaceted functional properties. Investigations have demonstrated that WBEs can augment cosmetic formulations by imparting advantages such as skin whitening, antioxidation, wrinkle reduction, and hair care benefits [24]. WBEs are characterized by the presence of phenolic compounds and bioactive peptides that demonstrate substantial antioxidant capabilities. These compounds play a pivotal role in modulating cellular oxidative stress, a critical factor implicated in the aging process. Furthermore, the extracts have been evidenced to inhibit enzymes such as collagenase, elastase, and hyaluronidase, which are integral to the degradation of skin

architecture and elasticity. This enzymatic inhibition suggests prospective applications in cosmetic products targeted at minimizing the appearance of skin wrinkles [25]. These properties could make WBEs a promising natural ingredient for various cosmetic applications.

Despite the extensive research on WBEs, several knowledge gaps persist. First, there is limited information on its potential enzyme inhibitory effects in animal studies, particularly against skin-related enzymes, such as collagenase, elastase, and hyaluronidase. Secondly, the relationship between the antioxidant properties of WBEs and their potential enzyme-inhibitory effects has not been thoroughly explored. Understanding this relationship could provide valuable insights into the mechanisms of action of wheat bran bioactive compounds. Thirdly, while various extraction methods have been used in previous studies, there is a lack of comprehensive comparisons between traditional solvent extraction and more novel techniques like subcritical water extraction, particularly in terms of their impact on both antioxidant and enzyme-inhibitory properties. Lastly, most studies have focused on in vitro assessments, leaving a gap in our understanding of the in vivo efficacy and bioavailability of wheat bran bioactive compounds. Addressing these knowledge gaps could significantly advance our understanding of wheat bran’s potential applications in the health, cosmetics, and food industries. In previous studies, the focus has primarily been on exploring the potential health benefits of wheat bran, specifically its antioxidant properties. However, our previous research uncovered new findings about the health potential of WBEs. These discoveries provide novel insights and indicate a more promising potential for health enhancement than has been reported to date.

1.2 Wheat Bran Extracts

1.2.1 Bioactive Compounds in Wheat Bran Extracts

Wheat bran is a rich source of bioactive compounds, which include phenolic acids, bioactive peptides, and dietary fibers, all contributing to its antioxidant and health promoting properties (Figure 1). Significant amounts of phenolic acids are found in wheat

bran, including ferulic acid, gallic acid, p-hydroxybenzoic acid, syringic acid, vanillic acid, and p-coumaric acid. These compounds are known for their antioxidant properties [26,27]. A study found that a wide range of bioactive compounds exists in wheat bran, namely, saponins (1.16 ± 0.10 mg diosgenin equivalents (DE)/g), gallic acid (16.57 ± 0.16 – 18.12 ± 2.01 mg/100 g), caffeic acid (0.86 ± 0.08 – 97.61 ± 9.60 mg/100 g), p-coumaric acid (19.68 ± 0.33 – 23.40 ± 0.64 mg/100 g), ferulic acid (9.06 ± 0.15 – 9.73 ± 0.09 mg/100 g), phytic acid (2180–5220 mg/100 g), alkylresorcinols (220–400 mg/100 g), vitamin E (1.4 mg/100 g), betaine (1000–1300 mg/100 g), choline (47–100 mg/100 g), niacin (14–18 mg/100 g), pantothenic acid (2.2–3.9 mg/100 g), riboflavin (0.39–0.75 mg/100 g), biotin (0.048 mg/100 g), thiamin (0.54 mg/100 g), pyridoxine (1–1.3 mg/100 g), folate (79–200 μ g/100 g), lutein (97–140 μ g/100 g), iron (11 mg/100 g), manganese (12 mg/100 g), zinc (7.3 mg/100 g), and selenium (78 μ g/100 g) [28,29]. The existence of bioactive chemicals in wheat bran or WBEs indicates its attractive potential for industrial applications as food ingredients, nutraceuticals, and others [30]. Additionally, wheat bran contains significant amounts of flavonoids and carotenoids, which are known for their antioxidant properties. One study reported that wheat bran extracts contain flavonoids (185.96 ± 1.31 to 206.74 ± 4.80 μ g catechin equivalents (CE)/g bran), anthocyanin (36.6 ± 0.10 to 63.0 ± 0.20 μ g cyanidin 3-glucoside/g bran), beta carotene (6.11 ± 0.05 to 14.25 ± 0.12 μ g/100 g bran), zeaxanthin (16.68 ± 0.23 to 35.21 ± 0.47 μ g/100 g bran), and lutein (132.93 ± 0.82 to 174.59 ± 1.08 μ g/100 g bran) [31]. Moreover, carotenoids and unsaturated fatty acids are also found in WBEs, contributing to their nutritional value and potential health benefits, such as reducing the risk of cardiovascular diseases [32,33]. Phytosterols are natural compounds found in plants that are similar in structure to cholesterol. Phytosterols are known for their cholesterol-lowering effects and may also help prevent obesity, diabetes, and cancer. Interestingly, phytosterols, particularly oxy phytosterols, have also been identified in wheat bran [34].

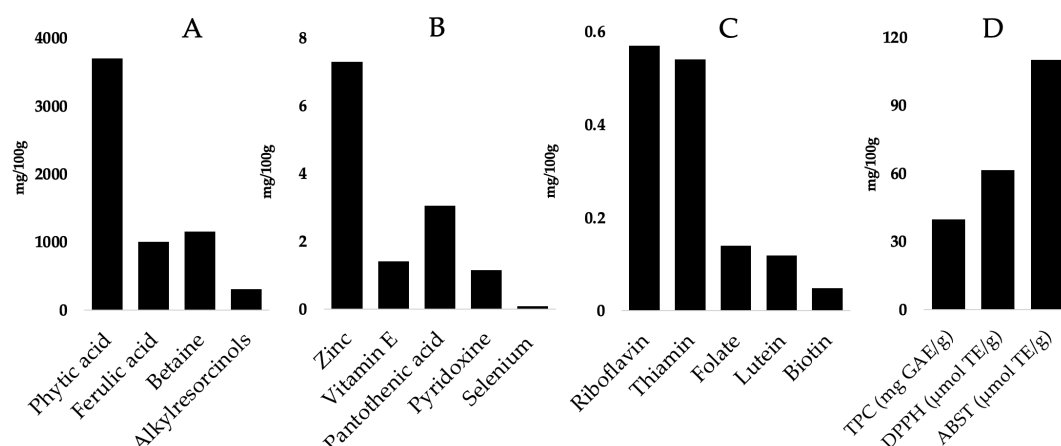


Figure 1.1: The contents of bioactive compounds, vitamins, minerals, and antioxidant activities of wheat bran extracts: (A) levels of phytochemicals; (B) mineral and vitamin contents; (C) additional vitamin levels; (D) antioxidant activities as measured by the total phenolic content (TPC) (expressed in mg gallic acid equivalents/g), DPPH radical scavenging activity (expressed in Trolox equivalence/g), and ABTS radical scavenging activity (expressed in Trolox equivalence/g). Modified from [25,29].

1.2.2 Bioactive Properties in Wheat Bran Extracts

Wheat bran is a significant source of dietary fibers, which are known for their role in cancer prevention, particularly colon and breast cancers. The fibers in wheat bran are complemented by various phytochemicals, including phytic acid, phenolic acids, lignans, and flavonoids, which also contribute to its protective effects against cancer [35,36]. A study investigated the anticancer effects of phytic acid. As a phytochemical, phytic acid demonstrates potent anti-cancer properties, particularly in colorectal cancer (CRC), through several mechanisms [37,38]. Phytic acid inhibits cell proliferation in CRC cell lines (HCT116 and HT-29) in a dose- and time-dependent manner, with IC₅₀ values of 2.96 μ M and 3.35 μ M, respectively [38]. Phytic acid also induces mitochondrial intrinsic apoptosis via the caspase-9 and caspase-3 cascade, leading to cancer cell death [38]. Additionally, it causes cell cycle arrest at the G2/M phase, preventing further division and proliferation of cancer cells. Phytic acid reduces the expression of pro-inflammatory markers such as cyclooxygenase 2 (COX-2), inducible nitric oxide synthase (iNOS), interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), and interleukin-10 (IL-10), creating a less favorable

environment for tumor growth [38]. Furthermore, phytic acid suppresses the nuclear factor kappa-light-chain- enhancer of activated B cells (NF- κ B) and β -catenin signaling pathways, decreasing the expression of downstream targets like cyclin D1 and c-Myc, which are involved in cell proliferation and survival [38]. Together, these actions underscore phytic acid's multifaceted role in cancer prevention.

The presence of ferulic acid, syringic acid, p-hydroxybenzoic acid, vanillic acid, and coumaric acid in wheat bran further enhances its antioxidant properties, which are crucial in preventing oxidative-stress-related diseases [39]. WBEs exhibit significant antioxidant activity, as evidenced by their high total phenolic and flavonoid contents [4]. A recent *in vitro* study demonstrated up to 61.36 $\mu\text{mol TE/g}$ DPPH radical scavenging activity and 110.95 $\mu\text{mol TE/g}$ ABTS+ scavenging activity (Figure 1D) in WBEs [25]. These antioxidants are effective in scavenging free radicals, such as DPPH and ABTS, and in reducing lipid peroxidation in human LDL, which is vital for cardiovascular health [39,40]. An *in vivo* study showed that ferulic acid operates through several biological mechanisms, primarily influencing hepatic functions by modulating specific signaling pathways [41]. Ferulic acid exerts protective effects on liver health by modulating hepatic functions through the inhibition of protein tyrosine phosphatase 1B (PTP1B) and the subsequent activation of adenosine monophosphate-activated protein kinase (AMPK) [41]. Ferulic acid directly binds to PTP1B, preventing its dephosphorylation of AMPK, a crucial regulator of energy homeostasis and cellular stress responses [41]. By activating AMPK, FA alleviates oxidative stress and inflammation, key contributors to liver fibrosis, and reduces the activation of hepatic stellate cells (HSCs), thereby decreasing pro-fibrotic factors. This cascade of events helps protect the liver from injury, fibrosis, and inflammation, offering a potential therapeutic approach for managing liver fibrosis and related metabolic disorders [41].

Moreover, bioactive peptides derived from WBEs exhibit a range of physiological activities, including antioxidant, antihypertensive, and anti-inflammatory effects (Figure 2). These peptides are generated through enzymatic hydrolysis and have been identified for their potential health benefits. Generally, the peptides are characterized by their specific

amino acid sequences, which contribute to their bioactivity and potential applications in nutraceuticals and functional foods. Peptides derived from wheat bran have shown significant antioxidant activities, particularly against oxidative stress in HepG2 cells.

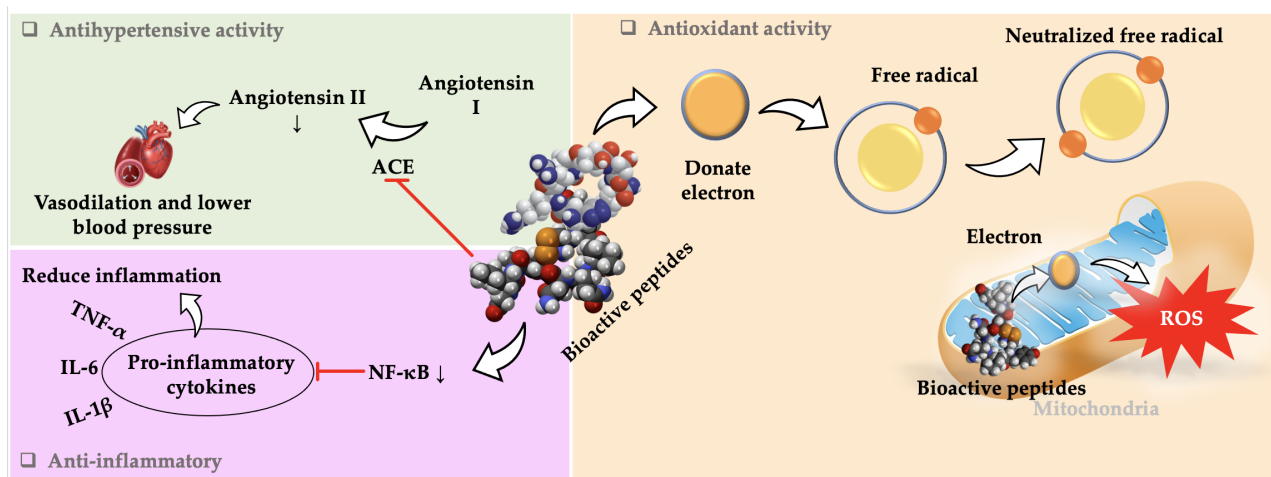


Figure 1.2: Potential mechanisms of the antioxidant, antihypertensive, and anti-inflammatory activities of wheat bran extracts and their bioactive peptides against reactive oxygen species. The top-left side of the image illustrates how hydrophobic amino acids contribute to the formation of peptides with antioxidant properties in the cytoplasm. The right side of the image depicts the role of hydrophobic amino acids in the formation of antioxidant peptides that neutralize reactive oxygen species (ROS) during cellular respiration by donating electrons. The bottom-left side shows the anti-inflammatory activity. The downward arrow (↓) indicates decreased levels of NF-κB or Angiotensin II.

The peptide sequences aspartic acid–leucine–aspartic acid–tryptophan (DLDW) and aspartic acid–leucine–glycine–leucine (DLGL), which have been identified in wheat bran extract, act as competitive inhibitors by binding to the Kelch domain of Keap1, specifically targeting the ETGE motif binding site [42]. This binding disrupts the natural interaction between Keap1 and Nrf2, reducing Nrf2 ubiquitination and preventing its degradation [42]. By stabilizing Nrf2, these peptides enhance the activation of antioxidant defense pathways, which are crucial for cellular protection [42]. DLDW and DLGL were identified for their high binding affinities, and they interfere with the Keap1–Nrf2 interaction—a critical pathway in antioxidant regulation [43]. Additionally, these peptides exhibit multifunctional bioactive properties. They act as competitive inhibitors of dipeptidyl peptidase IV (DPP-IV), an enzyme involved in glucose metabolism through the degradation of incretin

hormones [44]. Furthermore, they show moderate inhibitory activity against angiotensin-converting enzyme (ACE), a key enzyme in blood pressure regulation, highlighting their potential in cardiovascular health and antihypertensive therapies [45]. The anti-inflammatory peptides, including NL, QL, FL, HAL, AAVL, AKTVF, and TPLTR, were identified from wheat bran protein hydrolysate. These peptides exhibited significant antihypertensive effects, with the ≤ 1 kDa fraction showing better absorption and interaction with ACE and renin, leading to decreased blood pressure in spontaneously hypertensive rats [46].

In addition, a specific wheat bran extract exhibited strong anti-inflammatory properties by reducing the expression of inflammatory markers such as iNOS and COX-2 in stimulated cells [5].

The anti-inflammatory properties of wheat bran extract have been investigated through various in vitro and in vivo studies. In vitro research has demonstrated that diferulic acid, derived from ferulic acid in wheat bran extract, exhibits significant anti-inflammatory activity through multiple mechanisms. A study showed that wheat bran hydrolysates containing diferulic acid effectively reduced pro-inflammatory cytokine secretion, specifically monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor-alpha (TNF- α) in macrophages [47]. The study also revealed a synergistic effect when diferulic acids were combined with other bioactive compounds present in wheat bran, such as ferulic acid and various phenolic compounds, leading to increased anti-inflammatory effects [47]. Additional in vitro studies have demonstrated that bioactive peptides from wheat bran operate through the NF- κ B signaling pathway, a crucial mediator of inflammatory responses. These peptides effectively inhibit this pathway, resulting in reduced expression of various pro-inflammatory cytokines, including TNF- α , IL-6, and IL-1 β [48,49]. In vivo studies have provided further evidence of anti-inflammatory effects. In an animal model study, bioactive peptides demonstrated significant anti-inflammatory activity by reducing TNF- α and IL-6 concentrations in rat aqueous humor by 77.26% and 85.67%, respectively [48].

Additionally, a study indicated that the higher content of hydrophobic amino acids in

wheat bran protein implies a potential for heightened antioxidant efficacy. Hydrophobic amino acids, such as alanine, valine, leucine, proline, methionine, phenylalanine, tryptophan, and isoleucine, have non-polar side chains that tend to avoid water. This property allows them to interact more effectively with lipid membranes and other hydrophobic environments, which is crucial for antioxidant activity (Figure 2). The greater presence of these amino acids could pose great potential for interaction with free radicals, which are often found in lipid-rich environments. These compounds are known to be major contributors to the antioxidant activity of plant extracts, and their quantification provides valuable insights into the composition and potential bioactivity of the extracts [50].

1.2.3 Anti-Aging Potential of Wheat Bran Extracts

Aging is a complex biological process characterized by the gradual deterioration of physiological functions, leading to increased vulnerability to diseases and, ultimately, death. By retarding the aging process, we can avoid aging-related diseases, including cardiovascular diseases, cancer, and neuro degenerative disorders, and improve our quality of life. Antioxidants from wheat bran have potential anti-aging properties. Wheat bran contains a variety of phenolic compounds, including ferulic acid, which has shown strong antioxidant activities. These compounds can neutralize free radicals, which are implicated in cellular aging and oxidative stress-related diseases [51]. Recent studies have shown that WBEs can inhibit enzymes such as collagenase and elastase, which are involved in the breakdown of the skin’s structural proteins. This inhibition could potentially slow down skin-aging processes [4]. Chronic inflammation is associated with accelerated aging. Some components of wheat bran, particularly arabinoxylan, have shown anti-inflammatory properties, which could contribute to its anti-aging effects [52]. Arabinoxylans consist of a linear chain backbone of β d-xylopyranosyl residues linked through glycosidic linkages (Figure 3). The combination of various bioactive compounds in wheat bran may provide synergistic effects, enhancing its overall anti-aging potential beyond what individual compounds might achieve [4].

The newly discovered antioxidants in WBEs, particularly xylo-oligosaccharides (XOS) (Figure 4) and protein hydrolysates, work as anti-aging agents by targeting oxidative stress and inhibiting enzymes related to skin aging, such as collagenase, elastase, and hyaluronidase (Figures 3 and 4) [25]. Collagenase, elastase, and hyaluronidase enzymes are responsible for breaking down essential skin components, leading to wrinkles and loss of elasticity. By inhibiting these enzymes, the antioxidants from wheat bran or WBEs can maintain the skin's structure and function. In addition, the phenolic compounds present in wheat bran have strong antioxidant properties, neutralizing free radicals and reducing oxidative damage at the cellular level. This reduction in oxidative stress plays a key role in slowing down the aging process and preventing age-related diseases. Specifically, the study shows that XOS from hard wheat bran has the highest hyaluronidase inhibition activity, reaching 49.9% at a 0.5 mg/mL concentration. These properties make WBEs promising candidates for cosmetic and therapeutic applications aimed at preventing aging and maintaining skin health.

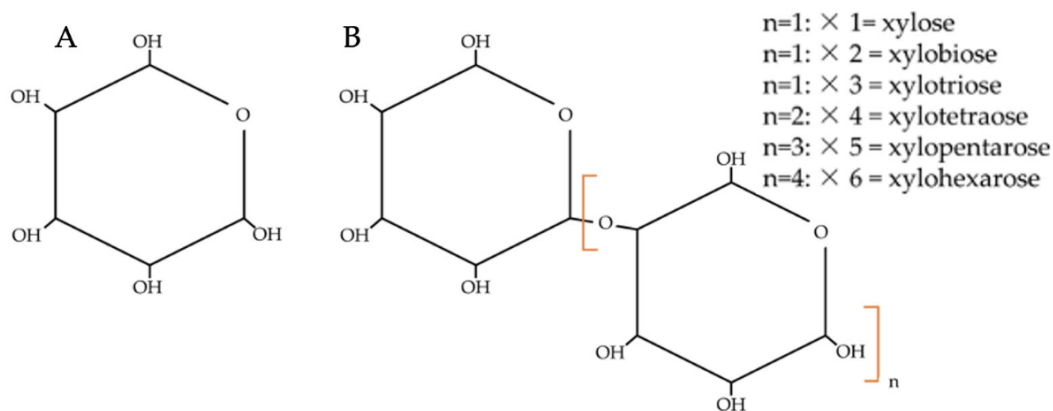


Figure 1.3: *Chemical structures of arabinoxylans. Monosubstituted β -D-xylopyranosylat O-3 with ferulic acid residue esterified at α -L-arabinofuranosyl.*

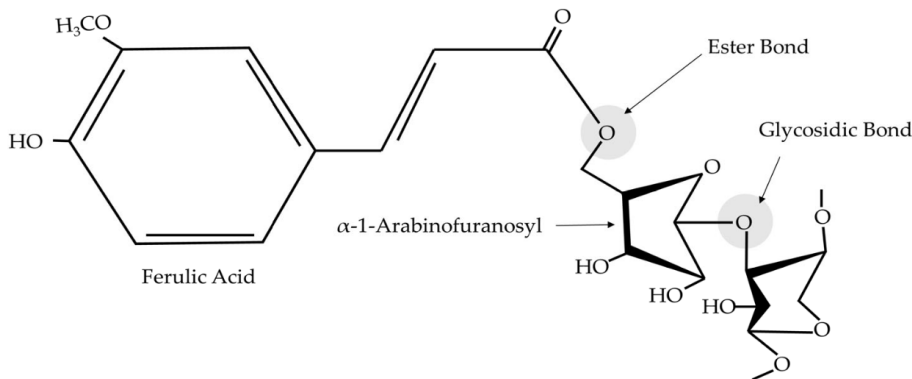


Figure 1.4: Chemical structures of (A) xylose and (B) xylo-oligosaccharides; “ n ” is a variable number of xylose units. The brackets ‘[]’ enclose a section of the molecular structure, showing that the portion repeats multiple times in the polymer chain.

1.2.4 Inhibitory Effect of Wheat Bran Extract on Skin

Aging-Related Enzyme

Research has extensively explored the antioxidant properties of wheat bran, but its potential as an inhibitor of these aging-related enzymes remains largely unexplored. As mentioned in the previous paragraphs, some enzymes, such as collagenase, elastase, and hyaluronidase, play crucial roles in the breakdown of extracellular matrix components in the skin, thus contributing to the aging process (Table 1) [53]. The degradation of collagen fibers occurs through two primary phases. In the initial phase (i.e., enzymatic degradation), facilitated by collagenases such as MMP-1, MMP-8, and MMP-13, entire fibers are broken down into diminutive fragments, resulting in the cleavage of the triple-collagen helix into two segments that subsequently undergo spontaneous denaturation to form gelatin [54]. In contrast, the other phase (non-enzymatic degradation), occurs through chemical reactions, such as glycation and oxidation, often exacerbated by environmental factors like UV exposure or metabolic conditions such as diabetes [54]. Elastases can bind to cell surface proteins, creating a microenvironment that promotes elastolysis. This interaction is mediated by exosites distinct from the active site, enhancing the enzyme’s ability to degrade elastin [55]. Hyaluronidase enzymes play a

crucial role in the breakdown of hyaluronic acid), a major component of the extracellular matrix. These enzymes facilitate the degradation of hyaluronic acid into smaller fragments, which can have various biological effects. The mechanism of hyaluronidase action involves cleaving the glycosidic bonds within hyaluronic acid, leading to the formation of low-molecular-weight hyaluronic acid fragments [56]. A current study bridges an important gap in the literature and explores the potential of WBEs in anti-aging and skin health applications [25]. The investigation examined the inhibitory impacts of protein hydrolysates and xylo-oligosaccharides derived from wheat bran on the enzymatic activities of collagenase, elastase, and hyaluronidase (Figure 5). The findings indicate that, upon optimized extraction, the wheat bran extract demonstrated significant inhibitory effects on aging-related enzymes, specifically collagenase (49.4%), elastase (22.9%), and hyaluronidase (49.9%) [25]. These enzymes are critical in the degradation of collagen, elastin fibers, and hyaluronic acid, which contributes to the development of sagging, wrinkled, and less elastic skin [13]. The results suggest that WBEs could be valuable ingredients in natural anti-aging and skin health products, offering a multifaceted approach to skin protection and rejuvenation [25]. The results of the multifaceted investigation reflect merit in the future development of novel, natural based products in both the food and cosmetic industries while also advancing our understanding of the relationships among different bioactive properties in plant extracts. Such findings can contribute to uncovering new potential applications for WBEs. Therefore, future research could focus on identifying and isolating specific compounds responsible for the observed bioactivities, as well as investigating the in vivo efficacy and safety of these extracts. Additionally, further exploration of other potential bioactivities and the development of targeted extraction methods could unlock even more applications for this abundant and sustainable resource.

1.2.5 Other health benefits of Wheat Bran

Wheat bran contains essential nutrients, including non-starch polysaccharides like arabinoxylans and β -glucans, which are beneficial for managing cardiovascular diseases,

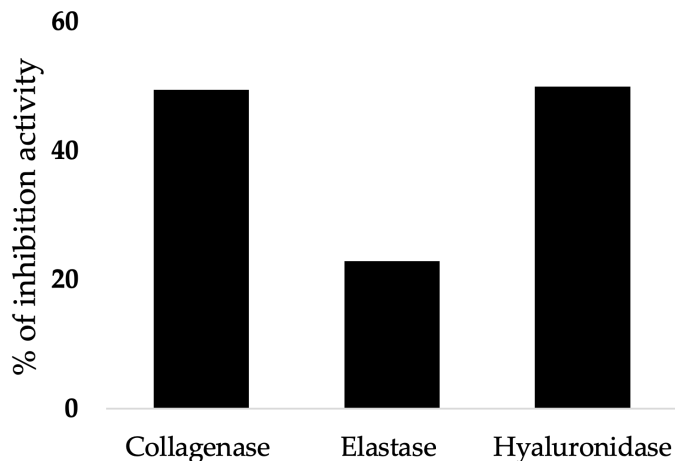


Figure 1.5: *Inhibitory effect of wheat bran extracts on skin aging-related enzymes (collagen, elastin fibers, and hyaluronic acid)* “Reprinted (adapted) with permission from [25]. Copyright (2024) American Chemical Society”

Table 1.1: *Summary of skin-related enzymes and their role in skin aging.*

Enzyme	Target	Role in Skin	Effect on Aging	Potential Benefits of Inhibition
Collagenase	Collagen	Hyaluronidase	This leads to loss of skin firmness and foundation of wrinkles.	Maintenance of skin structure and elasticity.
Elastase	Elastine	Degrades elastin fibers	results in decreased skin elasticity and sagging.	Preservation of skin elasticity and bounce.
Hyaluronidase	Hyaluronic acid	Breaks down hyaluronic acid	Reduces skin hydration and volume	Retention of skin moisture and plumpness.

cholesterol, obesity, type-2 diabetes, and cancer [14]. It is a rich source of dietary fiber, which promotes bowel health, reduces cholesterol levels, and lowers the risk of heart disease, diabetes, colon cancer, and obesity [57]. The bran’s mineral content supports metabolic functions and homeostasis, which can be enhanced through processing techniques like hydrothermal treatment and fermentation. Wheat bran is abundant in bioactive compounds such as phenolic compounds and bioactive peptides, which exhibit immunomodulating, antihypertensive, osteoprotective, and antimicrobial properties [57]. Purple wheat bran, in particular, has high levels of anthocyanins, polyphenols, and flavonoids, contributing to its superior antioxidant activity [58]. The incorporation of

wheat bran into food products, like bread, cookies, and noodles, is promising because of its health-promoting properties. However, challenges in breadmaking, such as sensory and rheological issues, can be addressed through pretreatments and enzyme additions [57]. Arabinoxylan, a primary component of wheat bran, can be extracted and used in various food applications, enhancing the nutritional profile of these products [59].

1.3 Current Findings

1.3.1 Animal Experiments

To investigate the anti-aging potential of WBEs, we conducted a pilot study using a D-galactose-induced aging model in female Wistar rats. Eight-week-old rats (average weight: $193 \text{ g} \pm 20 \text{ g}$) were obtained from Charles River (O’Fallon, Missouri) and housed in a pathogen-free facility under controlled environmental conditions (12 h light/dark cycle). After a period of acclimation, the rats were randomly assigned to one of the following four groups: Group 1—saline-injected control (i.e., negative control), fed an AIN-93G diet; Group 2—D-galactose-injected positive control, fed an AIN-93G diet; Group 3—D-galactose-injected, fed a 5% WBE-supplemented diet; and Group 4—D-galactose-injected, fed a 10% WBE-supplemented diet. Following six weeks of treatment, blood, skin, and liver samples were collected for the analysis of aging-related biomarkers.

1.3.2 Superoxide Dismutase (SOD) Activity

As shown in Figure 6A, the means of the erythrocyte SOD activity, measured using a Thermo Multiskan GO Microplate Reader, were as follows: Group 1 (saline-negative control): 3304 U/mg; Group 2 (D-galactose positive control): 2548 U/mg; Group 3 (5% WBE diet): 5842 U/mg; and Group 4 (10% WBE diet): 6565 U/mg. The D-galactose-induced reduction in SOD activity was significantly reversed in both the 5% and 10% WBE-supplemented groups ($p < 0.0001$).

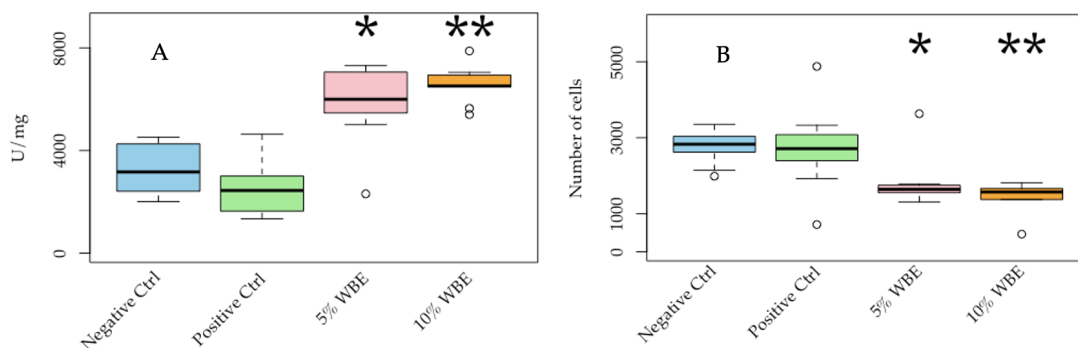


Figure 1.6: Anti-aging and senescence biomarker analysis in a D-galactose-induced aging rat model. Female Wistar rats were divided into four groups—saline-negative control, D-galactose positive control, and D-galactose positive control with 5% or 10% wheat bran extract diets—for 6 weeks. (A) SOD inhibition rate; 1 unit (U) represents the amount of SOD in a 20 μ L erythrocyte sample, which shows a 50% inhibition of WST reduction. (B) β -galactosidase activity in liver samples. * $p < 0.05$ and ** $p < 0.001$ compared with the saline-negative control. Data represent the mean $\pm$ SD ($n = 10$).

1.3.3 β Galactosidase Activity

β -Galactosidase (β -Gal), a lysosomal enzyme associated with cellular aging, predominantly in the liver, was also measured (Figure 6B). The results show that liver β -GAL activity decreased significantly in both the 5% and 10% WBE-fed groups compared to both negative and positive control, indicating a smaller proportion of senescent cells in the WBE-treated groups. The older neurons exhibited more signs of senescence, further supporting the anti-aging potential of WBEs. In addition, SOD2 and catalase activities were further measured in both skin and liver samples. However, the results do not show significant differences among the four groups. Therefore, the results are not presented. The significant increases in erythrocyte SOD and significant decreases in liver β -Gal activities in the WBE-fed groups suggest that WBEs enhance antioxidant defenses against oxidative stress, highlighting their potential role in anti-aging interventions. Further research with varying dosages, extended treatment durations, and larger sample sizes may be warranted to fully elucidate the effects of WBEs on aging and to explore their potential to promote

healthy aging.

1.4 Application of Wheat Bran Extract

1.4.1 In Food Industries

Wheat bran extract is increasingly recognized for its valuable contributions to the food industry, primarily due to its rich nutritional profile and functional properties. The extract, derived from the outer layers of wheat grain, is a source of dietary fiber, proteins, and bioactive compounds, making it a versatile ingredient in food applications. Wheat bran extract is rich in arabinoxylans, which are the primary hemicelluloses in wheat bran. These compounds are known for their health benefits and functional properties in food products, such as improving texture and stability [59]. The water-soluble extract from wheat bran contains a significant amount of carbohydrates (50.3%), proteins (24.5%), soluble fibers (6.7%), insoluble fiber (2.9%), and ash (13.0%) [60]. The study revealed that adding only 7.5% wheat bran aqueous extract (WBE) could increase bread-specific volume from 4.18 cm³/g to 4.84 cm³/g. On top of that, dough enriched with WBE demonstrated elevated peak, setback, breakdown, and final viscosities, in addition to enhanced storage and loss modulus. Analysis via scanning electron microscopy additionally indicated that the incorporation of WBE facilitated the interaction of protein and starch components within the dough [61].

A recent study isolated and purified a novel antimicrobial peptide (WBp-1) from wheat bran which exhibited considerable antibacterial potency against *Listeria monocytogenes*, with a minimum inhibitory concentration measured at 150 µg/mL [62]. Moreover, a soluble extract of wheat bran at 0.5% exhibited anti-biofilm properties, preventing and disrupting biofilm formation. Additionally, the wheat bran demonstrated an ability to modulate bacterial quorum-sensing mechanisms, evidenced by its lactonase activity that decreased acyl-homoserine lactones levels in the medium [63]. Another investigation employed both 0.4% and 0.8% methanol/water wheat bran extract (MWBE)

in the formulation of hamburgers [61]. Around a 25% suppression of Enterobacterial proliferation was observed by MWBE compared to the control sample [61]. Regarding the coloration of the hamburgers, at the end of the storage period, it was observed that the hamburgers containing MWBE exhibited a color retention of 91.93–80.77%, whereas the control sample demonstrated a color retention of 98.85–93.98%, indicating that the incorporation of these additives contributes to superior color preservation. Furthermore, the MWBE exhibited an enhanced antioxidant capacity, demonstrating an increase of up to 20% relative to the control sample [61]. Meatballs with 20% wheat bran exhibited elevated ash and protein levels, enhanced lightness and yellowness, and diminished moisture, salt, and weight loss, alongside reduced redness. Sensory analysis indicated a significant variance among meatball samples, with control samples demonstrating superior acceptability [64]. A study examined the antimicrobial and antioxidant properties of phytic acid and phytate from wheat bran [65]. Gram-positive bacteria exhibited higher sensitivity to phytic acid compared to Gram-negative bacteria. *Bacillus anthracoid* and *Staphylococcus aureus* demonstrated increased sensitivity at 5% and 6% concentrations of pure phytate [65]. The inhibition zones for *Escherichia coli* O157:H7 and *Staphylococcus aureus* were 20.33 mm and 38.67 mm, respectively, at 6% pure phytate. Furthermore, the aqueous extract of wheat bran achieved a free radical scavenging activity of 36.16% [65]. The researchers concluded that wheat-bran-extracted phytate possesses significant antimicrobial and antioxidant properties, suggesting its potential as a natural preservative. Overall, the WBEs hold significant potential for the food industry due to their rich composition of bioactive compounds and dietary fibers, as well as their ability to be transformed into functional food ingredients. These extracts can be utilized in various applications, ranging from enhancing the nutritional profile of food products to serving as substrates in biotechnological processes.

1.4.2 In Cosmetic Industries

WBEs could be a potential ingredient source in the cosmetics industry because of their functional properties, including emulsion stability, skin-whitening effects, and antimicrobial capabilities. WBEs could be utilized in some cosmetic formulations, offering natural and effective alternatives to synthetic ingredients. Wheat-bran-derived lipids have been found to enhance the stability of emulsions, and emulsions from natural sources are in high demand in cosmetic formulations [66]. The emulsions with wheat-bran-derived lipids demonstrated higher emulsion stability (49.17–52.33%) compared with the control emulsion made with soybean oil (46.00%). This indicates that the addition of wheat-bran lipids enhances the stability of an emulsion without causing particle separation. This study found that the skin-whitening effect of the emulsions increased with the concentration of wheat-bran-derived lipids [24]. These findings indicate the potential application of wheat-bran-derived lipids in the cosmetics sector. Wheat bran extract may enhance skin hydration, softness, and smoothness. These extracts are particularly beneficial in skincare formulations, as well as in shampoos and scalp treatments. Previous research indicates that the combination of wheat bran extract with carboxylic acid alkyl ester and diol enhances combability, shine, elasticity, volume, and shape retention and minimizes damage [67]. Moreover, ferulic acid encompasses a multitude of prospective therapeutic applications, functioning as a scavenger of free radicals and serving as a protective agent against ultraviolet radiation-induced skin damage. Research has determined that there exists an amount of up to 5157 ± 66 mg of alkali-extractable ferulic acid per kilogram of dry de-starched wheat bran [68]. Phycocyanobilin exhibits potential benefits for human skin, including wrinkle reduction and anti-inflammatory properties. Research indicates that phycocyanobilin extract from marine *Arthrospira maxima* possesses skin anti-wrinkling effects linked to antioxidant activity and decreased intracellular reactive oxygen species. Furthermore, phycocyanobilin extracts significantly enhance collagen synthesis while reducing matrix metalloproteinases (MMP-1) levels. Notably, the anti-wrinkling effects of phycocyanobilin extracts are markedly amplified by incorporating WBEs, achieving up to

20–30% enhancement across different concentrations, likely due to the synergistic interaction of soluble globulins and other bioactive compounds in WBEs [69]. WBEs, particularly xylo-oligosaccharides (XOS) and protein hydrolysates, have demonstrated strong antioxidant capacities. These extracts inhibit enzymes such as collagenase, elastase, and hyaluronidase, which are associated with skin aging. The inhibition of these enzymes suggests potential applications in cosmetic products aimed at reducing skin wrinkles and maintaining skin elasticity [25]. The phenolic content in WBEs, especially from hard wheat bran, has been shown to significantly inhibit hyaluronidase activity, reaching up to 49.9% inhibition at a concentration of 0.5 mg/mL. This highlights the potential of WBEs in developing functional ingredients for anti-aging purposes [25]. Wheat bran extract has also been studied for its neuro protective effects, particularly against β amyloid-induced cell death, which is a hallmark of Alzheimer’s disease. The extract was found to protect neuronal cells from apoptosis and improve memory impairment in animal models, suggesting its potential in managing neurodegenerative conditions associated with aging [70].

1.5 Factors That Affect the Yield of WBEs

The extraction of wheat bran presents several challenges and limitations, primarily due to its complex structure and the need for efficient, sustainable methods. Several extraction methods including conventional solvent extraction, ultrasound-assisted extraction, and microwave-assisted extraction have been employed to isolate useful compounds from wheat bran [57]. Wheat bran extraction methods possess effects on biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties [71]. For instance, enzymatic hydrolysis of wheat bran to obtain XOS and protein hydrolysates has shown promising antioxidant capacities [72]. Methanol extracts from colored wheat bran, such as ‘Ariheuk’, exhibit strong antioxidant activities due to a high anthocyanin content [5]. While the extraction method significantly affects the biological activities of wheat bran, the choice of method should align with the desired application,

whether it be for antioxidant, anti-inflammatory, or antimicrobial purposes. The presence of specific compounds like anthocyanins and phytic acid plays a crucial role in determining the efficacy of these extracts. In addition to the above, some researchers have also investigated the potential of WBEs in food preservation and as natural additives in functional foods [30]. For instance, arabinoxylans are the primary hemicellulose in wheat bran, known for their ability to form gels and films, which can be used in food packaging and as delivery systems for food ingredients [59]. Wheat bran contains aqueous-phase soluble proteins that can stabilize food systems, particularly in liquid and semi-solid foods. These proteins are extracted through physical and biochemical modifications to overcome barriers such as intact cell walls [73]. Supercritical CO₂ extraction of wheat bran yields extracts rich in phenolic compounds, which exhibit significant antioxidant activity. This property is crucial for preventing oxidative spoilage in foods [74]. Biochemical conversion processes, such as the Maillard reaction and enzymolysis, improve the flavor profile of wheat bran, making it a more acceptable food ingredient. This enhancement is particularly beneficial in products like steamed buns, where flavor and sensory attributes are critical [3].

1.6 Potential Risks and Mitigations

Wheat bran extracts, though widely recognized for their nutritional and bioactive properties, come with specific safety concerns that warrant consideration. Individuals with wheat allergies or celiac disease may experience adverse reactions due to the presence of wheat proteins or gluten contamination, even in trace amounts. The high phytic acid content in wheat bran can act as an antinutrient by binding essential minerals. In this chapter there are examples of various features you may want to incorporate into your document. Calcium, iron, and zinc, reduce their bioavailability and potentially lead to deficiencies with excessive intake. Additionally, over consumption of wheat bran fiber can cause digestive issues, including bloating, gas, or diarrhea, particularly in individuals unaccustomed to high-fiber diets. Furthermore, the high fiber content and bioactive compounds in wheat bran can interfere with the absorption of medications, reducing their

effectiveness. Long-term toxicological data on concentrated or bioactive-enriched wheat bran extracts remain limited, necessitating further research to ensure safety. To mitigate these risks, quality control measures, proper dosage recommendations, and consumer awareness about potential interactions and allergens are essential for the safe use of wheat bran extracts.

1.7 Challenges and Future Perspectives

There is a critical need for comprehensive in vivo studies to fully understand the efficacy and safety of WBEs. Future research should focus on bioavailability studies to determine how effectively the bioactive compounds in WBEs are absorbed, metabolized, and distributed in the body. Additionally, factors such as stability during food processing, storage, and formulation into cosmetic products must be thoroughly explored to ensure that the active compounds retain their potency. Animal studies can provide insight into the systemic effects of WBEs, including their antioxidant and anti-inflammatory effects across various organs and tissues. For cosmetic applications, dermal absorption studies and clinical trials are essential to evaluate the efficacy of WBEs in improving skin health and reducing signs of aging, particularly in real-world conditions. Furthermore, understanding the mechanisms of action at the molecular level could guide the development of more targeted applications. Long-term safety studies are crucial to assess any potential adverse effects, allergenic responses, or interactions with other substances in both food and cosmetic matrices. In addition to efficacy, these studies are necessary for securing regulatory approvals and boosting consumer confidence. Challenges also remain in scaling up extraction and production processes while ensuring cost-effectiveness and sustainability. Overcoming these barriers will unlock the full potential of WBEs as bioactive ingredients in anti-aging formulations for both food and cosmetic applications.

References

Chapter 1

1. Martín-García, B.; Gómez-Caravaca, A.M.; Marconi, E.; Verardo, V. Distribution of Free and Bound Phenolic Compounds, and Alkylresorcinols in Wheat Aleurone Enriched Fractions. *Food Res. Int.* **2021**, *140*, 109816. <https://doi.org/10.1016/j.foodres.2020.109816>.
2. Yang, Z.; Huang, T.; Guo, A.; Chen, W.; Bai, W.; Wei, L.; Tian, L. Insights into the Fermentation Patterns of Wheat Bran Cell Wall Polysaccharides Using an In-Vitro Batch Fermentation Model. *Carbohydr. Polym.* **2023**, *317*, 121100. <https://doi.org/10.1016/j.carbpol.2023.121100>.
3. Li, Q.; Sun, H.; Zhang, M.; Wu, T. Characterization of the Flavor Compounds in Wheat Bran and Biochemical Conversion for Application in Food. *J. Food Sci.* **2020**, *85*, 1427–1437. <https://doi.org/10.1111/1750-3841.14965>.
4. Mikhailova, D.V.; Shevchenko, O.G.; Golubev, D.A.; Platonova, E.Y.; Zemskaya, N.V.; Shoeva, O.Y.; Gordeeva, E.I.; Patov, S.A.; Shaposhnikov, M.V.; Khlestkina, E.K.; et al. Antioxidant Properties and Geroprotective Potential of Wheat Bran Extracts with Increased Content of Anthocyanins. *Antioxidants* **2023**, *12*, 2010. <https://doi.org/10.3390/antiox12112010>.
5. Kim, S.-G.; Park, S.-H.; Auh, J.-H. Antioxidant and Anti-Inflammatory Activities of the Methanol Extract from the Bran of the Colored Wheat, “Ariheuk”. *Appl. Biol. Chem.* **2024**, *67*, 19. <https://doi.org/10.1186/s13765-024-00872-z>.
6. Butterfield, D.A.; Swomley, A.M.; Sultana, R. Amyloid β -Peptide (1-42)-Induced Oxidative Stress in Alzheimer Disease: Importance in Disease Pathogenesis and Progression. *Antioxid. Redox Signal.* **2013**, *19*, 823–835. <https://doi.org/10.1089/ars.2012.5027>.
7. Yang, J.; Luo, J.; Tian, X.; Zhao, Y.; Li, Y.; Wu, X. Progress in Understanding Oxidative Stress, Aging, and Aging-Related Diseases. *Antioxidants* **2024**, *13*, 394. <https://doi.org/10.3390/antiox13040394>.
8. Ali, S.; Khan, S.A.; Hamayun, M.; Lee, I.-J. The Recent Advances in the Utility of Microbial Lipases: A Review. *Microorganisms* **2023**, *11*, 510. <https://doi.org/10.3390/microorganisms11020510>.

9. Fan, L.; Ma, S.; Li, L.; Huang, J. Fermentation Biotechnology Applied to Wheat Bran for the Degradation of Cell Wall Fiber and Its Potential Health Benefits: A Review. *Int. J. Biol. Macromol.* **2024**, *275*, 133529. <https://doi.org/10.1016/j.ijbiomac.2024.133529>.
10. Saini, P.; Islam, M.; Das, R.; Shekhar, S.; Sinha, A.S.K.; Prasad, K. Wheat Bran as Potential Source of Dietary Fiber: Prospects and Challenges. *J. Food Compos. Anal.* **2023**, *116*, 105030. <https://doi.org/10.1016/j.jfca.2022.105030>.
11. Jung, S.-J.; Oh, M.-R.; Park, S.-H.; Chae, S.-W. Effects of Rice-Based and Wheat-Based Diets on Bowel Movements in Young Korean Women with Functional Constipation. *Eur. J. Clin. Nutr.* **2020**, *74*, 1565–1575. <https://doi.org/10.1038/s41430-020-0636-1>.
12. Duncan, S.H.; Russell, W.R.; Quartieri, A.; Rossi, M.; Parkhill, J.; Walker, A.W.; Flint, H.J. Wheat Bran Promotes Enrichment within the Human Colonic Microbiota of Butyrate-producing Bacteria That Release Ferulic Acid. *Environ. Microbiol.* **2016**, *18*, 2214–2225. <https://doi.org/10.1111/1462-2920.13158>.
13. Baumann, L.; Bernstein, E.F.; Weiss, A.S.; Bates, D.; Humphrey, S.; Silberberg, M.; Daniels, R. Clinical Relevance of Elastin in the Structure and Function of Skin. *Aesthetic Surg. J. Open Forum* **2021**, *3*, ojab019. <https://doi.org/10.1093/asjof/ojab019>.
14. Sztupecki, W.; Rhazi, L.; Depeint, F.; Aussenac, T. Functional and Nutritional Characteristics of Natural or Modified Wheat Bran Non-Starch Polysaccharides: A Literature Review. *Foods* **2023**, *12*, 2693. <https://doi.org/10.3390/foods12142693>.
15. Barbosa, J.R.; Da Silva, S.B.; Da Silva Martins, L.H.; Bezerra, F.W.F.; Freitas, L.C.; Ferreira, M.C.R.; De Carvalho Junior, R.N. Microbial Degradation of Food Products. In *Recent Advances in Microbial Degradation*; Inamuddin, Ahamed, M.I., Prasad, R., Eds.; Environmental and Microbial Biotechnology; Springer Singapore: Singapore, 2021; pp. 155–172, ISBN 978-981-16-0517-8.
16. Cozzolino, D.; Alagappan, S.; Hoffman, L.C. The Missing Link Between Shelf Life and Food Waste: Are Infrared Sensing Technologies Underutilised? *Trends Food Sci. Technol.* **2024**, *148*, 104494. <https://doi.org/10.1016/j.tifs.2024.104494>.
17. Shibu, C.; Misra, P. Food preservation and storage. In *Futuristic Trends in Agriculture Engineering & Food Sciences Volume 3 Book 9*; Varma, V.S., Rathod, K., Nayak, Y., Kulkarni, S.S., Yadav, R., Dhanapal, A., Bhattacharjee, B., Shukla, S., Alamu, W., Singh, A., et al., Eds.; Iterative International Publishers, Selfypage Developers Pvt Ltd.: Karnataka, India, 2024; pp. 119–135, ISBN 978- 93-5747-615-7.

18. Decker, E.A.; Elias, R.J.; McClements, D.J. *Oxidation in Foods and Beverages and Antioxidant Applications*; Woodhead Publishing Series in Food Science, Technology and Nutrition; Woodhead publ: Oxford, UK, 2010; ISBN 978-1-84569-648-1.
19. Castro-Muñoz, R.; Correa-Delgado, M.; Córdova-Almeida, R.; Lara-Nava, D.; Chávez-Muñoz, M.; Velásquez-Chávez, V.F.; Hernández-Torres, C.E.; Gontarek-Castro, E.; Ahmad, M.Z. Natural Sweeteners: Sources, Extraction and Current Uses in Foods and Food Industries. *Food Chem.* **2022**, *370*, 130991. <https://doi.org/10.1016/j.foodchem.2021.130991>.
20. Cosmetics Market Size, Share And Growth Report, 2030. Available online: <https://www.grandviewresearch.com/industry-analysis/cosmetics-market> (accessed on 30 October 2024).
21. Mondello, A.; Salomone, R.; Mondello, G. Exploring Circular Economy in the Cosmetic Industry: Insights from a Literature Review. *Environ. Impact Assess. Rev.* **2024**, *105*, 107443. <https://doi.org/10.1016/j.eiar.2024.107443>.
22. Samanta, S. Impacts of Chemical Beauty: Impacts of Chemical Beauty. *Indian J. Physiol. Allied Sci.* **2022**, *73*, 1–6. <https://doi.org/10.55184/ijpas.v73i1.24>.
23. Anitha, M.G.; Hiremath, P.; Divya, K.; Abhiram, P. A Survey Study on Adverse Effects of Synthetic Cosmetics. *Int. J. Community Med. Public Health* **2023**, *10*, 4147–4152. <https://doi.org/10.18203/2394-6040.ijcmph20233442>.
24. Han, N.; Kim, H.-J.; Kim, K.-H.; Park, J.-H.; Oh, Y.-G.; Kim, H.-S.; Lee, J.-Y.; Lee, Y.-Y.; Kim, M.; Kang, M.-S. Application of Emulsion Containing Wheat-Bran-Derived Lipids as a Functional Cosmetic Raw Material. *Korean J. Food Sci. Technol.* **2023**, *55*, 278–281. <https://doi.org/10.9721/KJFST.2023.55.3.278>.
25. Li, C.; Wu, W.; Tilley, M.; Chen, R.; Sun, X.S.; Wang, W.; Li, Y. In Vitro Antioxidant Properties of Wheat Bran Extracts and Their Inhibitory Effects on Collagenase, Elastase, and Hyaluronidase. *ACS Food Sci. Technol.* **2024**, *4*, 1960–1966. <https://doi.org/10.1021/acsfoodscitech.4c00310>.
26. Saroj, R.; Kaur, S.; Malik, M.A.; Puranik, V.; Kaur, D. Thermal Processing of Wheat Bran: Effect on the Bioactive Compounds and Dietary Fiber. *Bioact. Carbohydr. Diet. Fibre* **2024**, *32*, 100433. <https://doi.org/10.1016/j.bcdf.2024.100433>.
27. Mencin, M. Biotehnološki Procesi Kot Sredstvo Za Povečanje Dostopnosti in Antioksidativne Aktivnosti Fenolnih Spojin Iz Zrn Krušne Pšenice in Pire. *Acta Agric. Slov.* **2024**, *120*, 1–10. <https://doi.org/10.14720/aas.2024.120.2.17019>.

28. López-Perea, P.; Guzmán-Ortiz, F.A.; Román-Gutiérrez, A.D.; Castro-Rosas, J.; Gómez-Aldapa, C.A.; Rodríguez-Marín, M.L.; Falfán-Cortés, R.N.; González-Olivares, L.G.; Torruco-Uco, J.G. Bioactive Compounds and Antioxidant Activity of Wheat Bran and Barley Husk in the Extracts with Different Polarity. *Int. J. Food Prop.* **2019**, *22*, 646–658. <https://doi.org/10.1080/10942912.2019.1600543>.
29. Anson, N.M.; Hemery, Y.M.; Bast, A.; Haenen, G.R.M.M. Optimizing the Bioactive Potential of Wheat Bran by Processing. *Food Funct.* **2012**, *3*, 362. <https://doi.org/10.1039/c2fo10241b>.
30. Rebolleda, S.; González-San José, M.L.; Sanz, M.T.; Beltrán, S.; Solaesa, Á.G. Bioactive Compounds of a Wheat Bran Oily Extract Obtained with Supercritical Carbon Dioxide. *Foods* **2020**, *9*, 625. <https://doi.org/10.3390/foods9050625>.
31. Brewer, L.R.; Kubola, J.; Siriamornpun, S.; Herald, T.J.; Shi, Y.-C. Wheat Bran Particle Size Influence on Phytochemical Extractability and Antioxidant Properties. *Food Chem.* **2014**, *152*, 483–490. <https://doi.org/10.1016/j.foodchem.2013.11.128>.
32. Talawar, S.T.; Chetana, R.; Roopa, B.S.; Suresh Kumar, G. Effect of Wheat Bran Oil Concentrates on Quality and Nutrition of WBO Dark Compound Chocolates. *LWT* **2021**, *142*, 111005. <https://doi.org/10.1016/j.lwt.2021.111005>.
33. Zhu, R.; Xu, H.; Cai, H.; Wang, S.; Mao, J.; Zhang, J.; Xiong, X.; Wang, X.; Zhou, W.; Guo, L. Effects of Cereal Bran Consumption on Cardiometabolic Risk Factors: A Systematic Review and Meta-Analysis. *Nutr. Metab. Cardiovasc. Dis.* **2023**, *33*, 1849–1865. <https://doi.org/10.1016/j.numecd.2023.04.020>.
34. Zhu, Y.; Soroka, D.; Sang, S. Oxyphytosterols as Active Ingredients in Wheat Bran Suppress Human Colon Cancer Cell Growth: Identification, Chemical Synthesis, and Biological Evaluation. *J. Agric. Food Chem.* **2015**, *63*, 2264–2276. <https://doi.org/10.1021/jf506361r>.
35. Ma, L.; Wang, H.; Qiu, Y.; Bai, Z.; Yang, Z.; Li, E.; Ma, X.; Xiao, D. Alternative Uses of Fermented Wheat Bran: A Mini Review. *Fermentation* **2024**, *10*, 611. <https://doi.org/10.3390/fermentation10120611>.
36. Yuan, M.; Zhang, G.; Bai, W.; Han, X.; Li, C.; Bian, S. The Role of Bioactive Compounds in Natural Products Extracted from Plants in Cancer Treatment and Their Mechanisms Related to Anticancer Effects. *Oxid. Med. Cell. Longev.* **2022**, *2022*, 1429869. <https://doi.org/10.1155/2022/1429869>.

37. Liu, L.; Winter, K.M.; Stevenson, L.; Morris, C.; Leach, D.N. Wheat Bran Lipophilic Compounds with In Vitro Anticancer Effects. *Food Chem.* **2012**, *130*, 156–164. <https://doi.org/10.1016/j.foodchem.2011.07.023>.
38. Feng, J.; Liu, Y.; Zhang, C.; Ji, M.; Li, C. Phytic Acid Regulates Proliferation of Colorectal Cancer Cells by Downregulating NF- κ B and β -Catenin Signalling. *Eur. J. Inflamm.* **2023**, *21*, 1721727X231182622. <https://doi.org/10.1177/1721727X231182622>.
39. Kiokias, S.; Proestos, C.; Oreopoulou, V. Effect of Natural Food Antioxidants against LDL and DNA Oxidative Changes. *Antioxidants* **2018**, *7*, 133. <https://doi.org/10.3390/antiox7100133>.
40. Sun, Y.; Liu, C.; Bao, X.; Wang, C.; Yang, L.; Bian, R.; Zhang, Y.; Wang, J. Wheat Bran Protein Improves Lipid Metabolism by Downregulating the Amount of HMG-CoA Reductase in the Liver of High-Fat Diet-Fed Rats. *J. Food Biochem.* **2023**, *2023*, 1–10. <https://doi.org/10.1155/2023/1792652>.
41. Wu, J.; Xue, X.; Fan, G.; Gu, Y.; Zhou, F.; Zheng, Q.; Liu, R.; Li, Y.; Ma, B.; Li, S.; et al. Ferulic Acid Ameliorates Hepatic Inflammation and Fibrotic Liver Injury by Inhibiting PTP1B Activity and Subsequent Promoting AMPK Phosphorylation. *Front. Pharmacol.* **2021**, *12*, 754976. <https://doi.org/10.3389/fphar.2021.754976>.
42. Zhuang, M.; Li, J.; Wang, A.; Li, G.; Ke, S.; Wang, X.; Ning, M.; Sheng, Z.; Wang, B.; Zhou, Z. Structurally Manipulated Antioxidant Peptides Derived from Wheat Bran: Preparation and Identification. *Food Chem.* **2024**, *442*, 138465. <https://doi.org/10.1016/j.foodchem.2024.138465>.
43. Zhuang, C.; Miao, Z.; Sheng, C.; Zhang, W. Updated Research and Applications of Small Molecule Inhibitors of Keap1-Nrf2 Protein-Protein Interaction: A Review. *Curr. Med. Chem.* **2014**, *21*, 1861–1870. <https://doi.org/10.2174/0929867321666140217104648>.
44. de Valk, H.W. DPP-4 Inhibitors and Combined Treatment in Type 2 Diabetes: Re-Evaluation of Clinical Success and Safety. *Rev. Diabet. Stud. RDS* **2007**, *4*, 126–133. <https://doi.org/10.1900/RDS.2007.4.126>.
45. Ramlal, A.; Nautiyal, A.; Baweja, P.; Kumar, V.; Mehta, S.; Mahto, R.K.; Tripathi, S.; Shanmugam, A.; Pujari Mallikarjuna, B.; Raman, P.; et al. Angiotensin-Converting Enzyme Inhibitory Peptides and Isoflavonoids from Soybean [Glycine Max (L.) Merr.]. *Front. Nutr.* **2022**, *9*, 1068388. <https://doi.org/10.3389/fnut.2022.1068388>.

46. Zou, Z.; Wang, M.; Wang, Z.; Aluko, R.E.; He, R. Antihypertensive and Antioxidant Activities of Enzymatic Wheat Bran Protein Hydrolysates. *J. Food Biochem.* **2020**, *44*, e13090. <https://doi.org/10.1111/jfbc.13090>.
47. Bautista-Expósito, S.; Tomé-Sánchez, I.; Martín-Diana, A.B.; Frias, J.; Peñas, E.; Rico, D.; Casas, M.J.G.; Martínez-Villaluenga, C. Enzyme Selection and Hydrolysis under Optimal Conditions Improved Phenolic Acid Solubility, and Antioxidant and Anti-Inflammatory Activities of Wheat Bran. *Antioxidants* **2020**, *9*, 984. <https://doi.org/10.3390/antiox9100984>.
48. Liu, H.; Zhang, L.; Yu, J.; Shao, S. Advances in the Application and Mechanism of Bioactive Peptides in the Treatment of Inflammation. *Front. Immunol.* **2024**, *15*, 1413179. <https://doi.org/10.3389/fimmu.2024.1413179>.
49. Zhu, W.; Ren, L.; Zhang, L.; Qiao, Q.; Farooq, M.Z.; Xu, Q. The Potential of Food Protein-Derived Bioactive Peptides against Chronic Intestinal Inflammation. *Mediat. Inflamm.* **2020**, *2020*, 6817156. <https://doi.org/10.1155/2020/6817156>.
50. Milner, S.E.; Brunton, N.P.; Jones, P.W.; O'Brien, N.M.; Collins, S.G.; Maguire, A.R. Bioactivities of Glycoalkaloids and Their Aglycones from Solanum Species. *J. Agric. Food Chem.* **2011**, *59*, 3454–3484. <https://doi.org/10.1021/jf200439q>.
51. Rudrapal, M.; Khairnar, S.J.; Khan, J.; Dukhyil, A.B.; Ansari, M.A.; Alomary, M.N.; Alshabrm, F.M.; Palai, S.; Deb, P.K.; Devi, R. Dietary Polyphenols and Their Role in Oxidative Stress-Induced Human Diseases: Insights Into Protective Effects, Antioxidant Potentials and Mechanism(s) of Action. *Front. Pharmacol.* **2022**, *13*, 806470. <https://doi.org/10.3389/fphar.2022.806470>.
52. Chudan, S.; Ishibashi, R.; Nishikawa, M.; Tabuchi, Y.; Nagai, Y.; Ikushiro, S.; Furusawa, Y. Effect of Wheat-Derived Arabinoxylan on the Gut Microbiota Composition and Colonic Regulatory T Cells. *Molecules* **2023**, *28*, 3079. <https://doi.org/10.3390/molecules28073079>.
53. Ndlovu, G.; Fouche, G.; Tselanyane, M.; Cordier, W.; Steenkamp, V. In Vitro Determination of the Anti-Aging Potential of Four Southern African Medicinal Plants. *BMC Complement. Altern. Med.* **2013**, *13*, 304. <https://doi.org/10.1186/1472-6882-13-304>.
54. Artacho-Cordón, F.; Ríos-Arrabal, S.; Lara, P.C.; Artacho-Cordón, A.; Calvente, I.; Núñez, M.I. Matrix Metalloproteinases: Potential Therapy to Prevent the Development of Second Malignancies after Breast Radiotherapy. *Surg. Oncol.* **2012**, *21*, e143–e151. <https://doi.org/10.1016/j.suronc.2012.06.001>.

55. Hornebeck, W.; Emonard, H. The Cell-Elastin-Elastase(s) Interacting Triade Directs Elastolysis. *Front. Biosci.-Landmark* **2011**, *16*, 707–722. <https://doi.org/10.2741/3714>.
56. Buhren, B.A.; Schrumpf, H.; Hoff, N.-P.; Bölke, E.; Hilton, S.; Gerber, P.A. Hyaluronidase: From Clinical Applications to Molecular and Cellular Mechanisms. *Eur. J. Med. Res.* **2016**, *21*, 5. <https://doi.org/10.1186/s40001-016-0201-5>.
57. Li, C.; Stump, M.; Wu, W.; Li, Y. Exploring the Chemical Composition, Antioxidant Potential, and Bread Quality Effects of the Nutritional Powerhouse: Wheat Bran—A Mini-Review. *J. Agric. Food Res.* **2023**, *14*, 100898. <https://doi.org/10.1016/j.jafr.2023.100898>.
58. Saini, P.; Kumar, N.; Kumar, S.; Panghal, A.; Attkan, A.K. Analysis of Engineering Properties, Milling Characteristics, Antioxidant Potential, and Nutritional Benefits of Purple Wheat and Its Bran. *Food Bioeng.* **2023**, *2*, 406–419. <https://doi.org/10.1002/fbe2.12073>.
59. Chen, Z.; Mense, A.L.; Brewer, L.R.; Shi, Y. Wheat Bran Arabinoxylans: Chemical Structure, Extraction, Properties, Health Benefits, and Uses in Foods. *Compr. Rev. Food Sci. Food Saf.* **2024**, *23*, e13366. <https://doi.org/10.1111/1541-4337.13366>.
60. Li, C.; Chen, G.; Tilley, M.; Chen, R.; Perez-Fajardo, M.; Wu, X.; Li, Y. Enhancing Gluten Network Formation and Bread-Making Performance of Wheat Flour Using Wheat Bran Aqueous Extract. *Foods* **2024**, *13*, 1479. <https://doi.org/10.3390/foods13101479>.
61. Šulniūtė, V.; Jaime, I.; Rovira, J.; Venskutonis, P.R. Rye and Wheat Bran Extracts Isolated with Pressurized Solvents Increase Oxidative Stability and Antioxidant Potential of Beef Meat Hamburgers. *J. Food Sci.* **2016**, *81*, H519–H527. <https://doi.org/10.1111/1750-3841.13197>.
62. Zou, Z.; Purnawan, M.A.; Wang, Y.; Ismail, B.B.; Zhang, X.; Yang, Z.; Guo, M. A Novel Antimicrobial Peptide WBp-1 from Wheat Bran: Purification, Characterization and Antibacterial Potential against *Listeria Monocytogenes*. *Food Chem.* **2025**, *463*, 141261. <https://doi.org/10.1016/j.foodchem.2024.141261>.
63. González-Ortiz, G.; Quarles Van Ufford, H.C.; Halkes, S.B.A.; Cerdà-Cuellar, M.; Beukelman, C.J.; Pieters, R.J.; Liskamp, R.M.J.; Pérez, J.F.; Martín-Orue, S.M. New Properties of Wheat Bran: Anti-biofilm Activity and Interference with Bacteria Quorum- sensing Systems. *Environ. Microbiol.* **2014**, *16*, 1346–1353. <https://doi.org/10.1111/1462-2920.12441>.

64. Simona Chiș, M.; Corina Fărcaș, A. Cereal By-Products Valorization in Bakery, Pastry, and Gastronomy Products Manufacturing. In *Exploring the World of Cereal Crops [Working Title]*; IntechOpen: London, UK, 2024.
65. Sorour, M.A.M.; Abul-Hamd, E.M.; Elsayed, A.A.; Asmaa, G. Antimicrobial and Antioxidant Activities of Phytic Acid Extracted from Wheat Bran. *J. Sohag Agriscience* **2022**, *7*, 231–238.
66. Manful, M.E.; Ahmed, L.; Barry-Ryan, C. Cosmetic Formulations from Natural Sources: Safety Considerations and Legislative Frameworks in the European Union. *Cosmetics* **2024**, *11*, 72. <https://doi.org/10.3390/cosmetics11030072>.
67. Nakano, A.; Lourenco, C. A wheat bran extract in a hair care blend. U.S. Patent Application 16/303,864, 22 June 2023; published as 20230190633.
68. Pazo-Cepeda, M.V.; Aspromonte, S.G.; Alonso, E. Extraction of Ferulic Acid and Feruloylated Arabinoxyl-Oligosaccharides from Wheat Bran Using Pressurized Hot Water. *Food Biosci.* **2021**, *44*, 101374. <https://doi.org/10.1016/j.fbio.2021.101374>.
69. Koh, E.-J.; Kim, T.; Ryu, Y.-K.; Lee, W.-K.; Sunwoo, I.-Y.; Ro, H.S.; Jeon, G.; Kim, G.R.; Lee, H.Y.; Choi, W.-Y. Enhancement of Skin Anti-Wrinkling Effects of Arthrospira Maxima Phycocyanobilin by Combining with Wheat Bran Extract. *Appl. Sci.* **2024**, *14*, 10216. <https://doi.org/10.3390/app142210216>.
70. Lee, C.; Park, G.-H.; Lee, J.-W.; Jang, J.-H. Protective Effect of Wheat Bran Extract against β -Amyloid-Induced Cell Death and Memory Impairment. *Korea J. Herbol.* **2015**, *30*, 67–75.
71. Shang, X.-L.; Liu, C.-Y.; Dong, H.-Y.; Peng, H.-H.; Zhu, Z.-Y. Extraction, Purification, Structural Characterization, and Antioxidant Activity of Polysaccharides from Wheat Bran. *J. Mol. Struct.* **2021**, *1233*, 130096. <https://doi.org/10.1016/j.molstruc.2021.130096>.
72. Valls, C.; Pastor, F.I.J.; Vidal, T.; Roncero, M.B.; Díaz, P.; Martínez, J.; Valenzuela, S.V. Antioxidant Activity of Xylooligosaccharides Produced from Glucuronoxylan by Xyn10A and Xyn30D Xylanases and Eucalyptus Autohydrolysates. *Carbohydr. Polym.* **2018**, *194*, 43–50. <https://doi.org/10.1016/j.carbpol.2018.04.028>.
73. Janssen, F.; Courtin, C.M.; Wouters, A.G.B. Aqueous Phase Extractable Protein of Wheat Bran and Germ for the Production of Liquid and Semi-Solid Foods. *Crit. Rev. Food Sci. Nutr.* **2023**, *64*, 9585–9603. <https://doi.org/10.1080/10408398.2023.2214615>.
74. Argun, M.S. Functional Properties of Extracts from Wheat (*Triticum Aestivum* L.) Coarse Bran and Shorts (Razmol) Obtained

Chapter 2

Discovery of wheat bran extract's anti-aging potential against D-galactose-induced oxidative stress in a rat aging model

Kaori Kobayashi, Keshari Sudasinghe, Ryan Bender, Md Suzauddula, Cheng Li, Cen Wu, Xiuzhi Susan Sun, Yonghui Li, Weiqun Wang

This manuscript is in preparation for a journal submission.

2.1 Abstract

Wheat bran has been recognized for its anti-aging effects, primarily attributed to its antioxidant properties. By using an innovative extraction approach, our previous study identified novel enriched antioxidants in wheat bran, including xylo-oligosaccharides and protein hydrolysates. However, the potential anti-aging effects of these newly identified

wheat bran extracts remain unclear. This study aimed to evaluate the anti-aging potential of these extracts in a D-galactose-induced rat aging model. Wistar rats were randomly assigned to one of four groups: (1) saline-injected negative control, (2) D-galactose-injected positive control, (3) D-galactose-injected with a dietary 5% wheat bran extract (WBE) supplement, and (4) D-galactose-injected with a dietary 10% WBE supplement. After six weeks of treatment, no significant differences were observed in average body weight across the groups, although D-galactose led to a significant reduction in food intake and body fat, as measured by rodent densitometry. Additionally, D-galactose induced systemic oxidative stress by decreasing erythrocyte superoxide dismutase (SOD) activity. However, dietary supplementation with 5-10% WBE effectively reversed D-galactose-induced reduction, significantly enhancing erythrocyte SOD activity in a dose-dependent manner. Interestingly, while D-galactose did not significantly increase senescence-associated β -galactosidase activity in the liver, both 5% and 10% WBE supplementations significantly reduced its baseline levels. However, protein expression levels of the antioxidative biomarker SOD2 in liver tissues and catalase in both skin and liver tissues, as well as anti-aging biomarker telomerase reverse transcriptase in liver tissues, did not show significant differences among groups. These findings suggest that our novel wheat bran extracts exhibit potential anti-aging properties by mitigating D-galactose-induced oxidative stress in circulation, though their effects at the tissue level remain inconclusive. Further research is warranted to elucidate the underlying molecular mechanisms and optimize study duration for potential interventional clinical trials.

2.2 Introduction

Aging is a complex biological process characterized by a gradual decline in physiological functions, increased vulnerability to diseases, and reduced cellular repair mechanisms [1,2]. This process is closely linked with oxidative stress, mitochondrial dysfunction, and telomere attrition, all of which contribute to cellular and tissue degeneration [3–5]. The rapidly aging global population underscores the necessity of

innovative research into the biological and environmental factors influencing aging, as well as strategies to mitigate aging-related disorders and promote healthy aging [6]. Biomarkers such as antioxidant superoxide dismutase (SOD) and catalase as well as anti-aging senescence-associated beta-galactosidase (SA- β -gal) and telomerase reverse transcriptase (TERT) have been identified as critical indicators of cellular aging and potential dietary interventional targets in gerontology research [7–9].

Among these, SOD is a key mitochondrial enzyme responsible for neutralizing superoxide radicals, thereby reducing oxidative stress, a major driver of aging [10]. Decreased activity of this enzyme has been observed to correlate with increased cellular damage and the presence of age-related pathologies, as evidenced by studies on SOD knockout models (mice with a targeted mutation in the superoxide dismutase 2 gene), which exhibit accelerated aging phenotypes [11]. Catalase, another essential antioxidant enzyme, plays a central role in oxidative stress regulation by catalyzing the decomposition of hydrogen peroxide into water and oxygen [12]. Functioning alongside SOD, catalase contributes to the detoxification of reactive oxygen species (ROS), thereby maintaining redox homeostasis. Given its significance in cellular defense mechanisms, a reduction in catalase activity has been associated with increased oxidative damage and aging-related disorders [13].

Similarly, SA- β -gal activity serves as a widely used biomarker of cellular senescence, with significant age-related increases reported in human tissues. Research has revealed a significant age-related increase in the prevalence of cells exhibiting elevated SA- β -gal activity, ranging from $30 \pm 3\%$ in blood samples from individuals in their 20s to $64 \pm 4\%$ in those from individuals in their 60s, with some individuals demonstrating over 85% of CD8+ T cells characterized by heightened SA- β -gal activity [14]. Furthermore, TERT plays a fundamental role in maintaining telomere length and genomic stability, with its decline contributing to chromosomal instability and cellular senescence [15,16]. A decline in TERT expression with age is directly associated with telomere shortening, chromosomal instability, and cellular senescence, highlighting its importance in aging research [17].

The focus on dietary compounds, particularly those derived from plant sources, is

driven by their potential to mitigate oxidative stress and age-related cellular damage. Antioxidants present in whole grains effectively neutralize reactive oxygen species, positioning them as promising alternatives to synthetic interventions with expectations of their safety. Wheat bran, a by-product of wheat milling, has been identified as a notable source of bioactive antioxidant compounds [18,19]. These compounds exhibit potent antioxidant, anti-inflammatory, and anti-aging properties, thus rendering wheat bran extracts (WBE) an attractive candidate for research related to the aging process [20].

By using an innovative extraction approach, our recent studies have identified novel enriched antioxidants in wheat bran, including xylo-oligosaccharides and protein hydrolysates, which exhibit strong free radical scavenging and enzyme-inhibitory properties [19]. Investigations *in vitro* have shown that these newly discovered compounds effectively inhibit collagenase, elastase, and hyaluronidase, suggesting their potential in both food and cosmetic applications [19]. However, despite the known nutritional and bioactive potential of wheat bran, its direct impact on aging biomarkers, such as SOD activity, SA- β -gal levels, and TERT expression, remains largely unexplored. Most existing research has focused on the general health benefits of wheat bran rather than its specific effects on molecular markers of aging or accelerated aging models [21]. The present study aims to bridge this gap by evaluating the anti-aging potential of novel enriched antioxidants in wheat bran using a D-galactose-induced aging model. Ultimately, these findings may contribute to the development of wheat bran-based nutraceuticals as accessible and sustainable interventions for promoting healthy aging.

2.3 Materials and methods

2.3.1 Materials

Hard red winter wheat bran (protein content: 16.8%) and soft red winter wheat bran (protein content: 13.3%) were sourced from Hal Ross Flour Mill (Manhattan, Kansas) [22]. The wheat bran underwent grinding and a series of processing steps to extract

xylo-oligosaccharides and protein hydrolysates, novel superantioxidants identified in our recent studies [19]. These compounds exhibit strong free radical scavenging and enzyme-inhibitory properties, contributing to their potential anti-aging benefits [19]. These extracts were subsequently incorporated into experimental diets to evaluate their efficacy in mitigating oxidative stress and promoting healthy aging. These wheat bran extract (WBE) diets, along with the basal AIN-93G diet, were formulated and pelleted by Envigo Teklad (Madison, WI). The WBE diets were designed to match the basal AIN-93G diet in terms of fat, protein, fiber, essential micronutrient levels, and total caloric content, with carbohydrate composition modified to include either 5% or 10% WBE (Table 2-1).

Table 2.1: *Composition of ANG 93G and WBE diet*

Diets	AIN-93G	5% WBE	10% WBE
Energy composition (%)			
Carbohydrate	60.1	59.2	58.8
Protein	17.7	17.5	17.4
Fat	7.2	7.1	7.0
Ingredients (g/kg)			
Casein	200	187	176
L-Cystine	3	3	3
Corn Starch	397.486	397.486	360.986
Maltodextrin	132	132	132
Sucrose	100	100	100
Soybean Oil	70	69	68.5
Cellulose	50	35	12
Mineral Mix S10022G	35	35	35
Vitamin Mix V10037	10	10	10
Choline Bitartrate	2.5	2.5	2.5
TBHQ, antioxidant	0.014	0.014	0.014
Wheat Bran Extracts		50	100

Forty female Wistar rats (8 weeks old, weighing 193 ± 20 g) were purchased from Charles River Laboratories (O’Fallon, Missouri) and housed in a pathogen-free facility under controlled environmental conditions. The animals were maintained on a standard 12:12-hour light-dark cycle and provided with a standard AIN-93G pellet diet and water ad libitum during a one-week acclimatization period. Following acclimatization, the rats were randomly assigned to four experimental groups (n = 10 per group):

1. Negative control group: Standard AIN-93G diet with saline daily i.p. injection.
2. Positive control group: Standard AIN-93G diet with D-galactose daily i.p. injection (200 mg/kg) to induce accelerated aging.
3. 5% WBE group: 5% WBE-supplemented AIN-93G diet with D-galactose daily i.p. injection (200 mg/kg).
4. 10% WBE group: 10% WBE-supplemented AIN-93G diet with D-galactose daily i.p. injection (200 mg/kg).

Each group followed their respective dietary and injection regimens for six weeks. Weekly body weight and food intake were monitored. At the end of the study, body fat percentage was assessed using a PIXImus Densitometer (GE, Chicago, IL) (Figure 2-2A). The rats were then sacrificed, and biological samples, including blood, liver, and skin tissues, were collected. Blood samples were processed and stored separately for erythrocyte, serum, and plasma analyses. Liver and skin tissues were stored at -20°C for subsequent biochemical and molecular assessments.

2.3.2 Analysis of SOD in blood

Superoxide dismutase (SOD) is a key antioxidant enzyme that plays a vital role in protecting cells from oxidative stress [23]. To quantify SOD activity in erythrocytes, a photometric analysis was conducted using the SOD Assay Kit - WST (Dojindo Molecular Technologies, Inc., Rockville, MD). The assay was performed following the manufacturer's protocol. First, all necessary reagents, including the WST-1 substrate, enzyme standards, and buffers, were prepared. Erythrocyte samples were then collected, processed, and diluted as per the assay requirements. The prepared samples and enzyme standards were added to a reaction mixture containing the WST-1 substrate in a 96-well microplate. Absorbance was measured at 450 nm before and after the reaction to monitor changes in optical density. The difference in absorbance values was used to calculate SOD activity, which was determined by comparing sample readings to a standard curve generated using known concentrations of SOD enzyme. To ensure accuracy and reproducibility, all

experiments were conducted in triplicate, and results were expressed as mean values with standard deviations.

2.3.3 Analysis of Catalase, SOD2, and TERT in Skin and Liver Tissue

Protein expression levels of catalase, SOD2, and TERT in posterior skin and liver tissues were analyzed via western blotting. Total protein was extracted from tissues using RIPA Lysis Buffer (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's instructions. Protein concentrations were quantified using the Qubit 4 Fluorometer with the Protein Broad Range Assay (Thermo Fisher Scientific Inc, Waltham, MA). For sample preparation, 85 mg of back skin tissue and 20–40 mg of randomly collected liver tissue were incubated with RIPA buffer (200 μ L for skin and 400 μ L for liver) containing a protease inhibitor on ice for 20 minutes. The tissues were then homogenized using a Dounce pestle, with skin homogenized for >100 seconds (5–8 passes) and liver for 90 seconds (4 passes). Following homogenization, the samples were incubated on a tube rack for 20 minutes. Skin homogenates were centrifuged at 13,000 RPM for 3 minutes, while liver homogenates were centrifuged at 14,000 RPM for 10 minutes at 4°C. Supernatants were collected, and the lipid layer in liver samples was removed and discarded. Protein concentrations in the lysates were equalized before western blotting, and homogenized samples were stored at -80°C until analysis. Western blotting was performed using the Jess Protein Simple automated western blotting system (Bio-Techne, Minneapolis, MN). Target proteins included catalase (Abcam, EPR20198, Rabbit), SOD2 (Novus, AF3419, Goat), and TERT (Thermo Fisher Scientific Inc, Waltham, MA). Protein expression data were quantified using the area under the curve (AUC) values generated by Compass software (Compass Engineering, Cincinnati, OH).

2.3.4 Analysis of β -Galactosidase

The senescence-associated β galactosidase (SA- β -gal) assay was conducted using the Beta Galactosidase Assay Kit according to the manufacturer's instructions (Abcam, Waltham, MA). Briefly, frozen liver sections were prepared, washed, and stained with β -galactosidase staining solution and incubated at 37°C in a dry incubator. Fluorescence (Ex/Em = 480/530 nm) was measured using the Multiskan™ GO microplate spectrophotometer (Thermo Fisher Scientific, MA). Fluorescence values were applied to a standard curve to derive absorbance measurements. Percent inhibition is determined by the following formula.

$$\text{Inhibition rate (\%)} = \frac{(S1 - S3) - (SS - S2)}{(S1 - S3)} \times 100 \quad (1)$$

Where:

- $S1$: the slope of blank 1
- $S2$: the slope of blank 2
- $S3$: the slope of blank 3
- SS : the slope of the sample

2.3.5 Statistical Analysis

Data were expressed as means $\pm$ SEM. Statistical analyses were conducted using GraphPad Prism 8 software (GraphPad Software, Inc., California) and R. Comparisons between two groups were made using unpaired Student's t-tests, while differences among more than two groups were analyzed using one-way ANOVA or the Kruskal-Wallis test. A P-value<0.05 was considered statistically significant.

2.4 Results

2.4.1 Change of Body Weights and Food Intake

Throughout the experimental period, the aging rats (positive control) and WBE treatment groups received daily D-galactose injections (200 mg/kg). Weekly weight measurements showed an average weight gain of approximately 12 g per week across all groups. However, the weight gain was not consistent throughout the experimental period, with the 5% WBE and positive control groups exhibiting fluctuations in weight gain. (Figures 2-1A and 2-1B). However, food intake varied significantly among groups. The galactose-positive control group consumed significantly less food than the negative control group ($P < 0.05$). In contrast, the 5% and 10% WBE supplementation groups exhibited food intake levels comparable to the negative control group, with the 10% WBE group consuming significantly more than the positive control group ($P < 0.01$) (Figure 2-1C and 2-1D).

2.4.2 Effect of WBE supplements on Body Fat Percentage in aging rats

The negative control group exhibited an average body fat percentage of 27%. Both the 5% and 10% WBE supplementation groups maintained similar body fat percentages of approximately 30%, indicating consistent results across these groups. In contrast, the galactose-positive control group displayed a significantly lower body fat percentage (23%) ($P < 0.05$), suggesting that D-galactose administration altered body composition (Figure 2-2B).

2.4.3 Effect of WBE supplements on SOD in aging rats

The activity of SOD in erythrocytes and liver tissue was assessed to determine the antioxidant effects of WBE supplementation. The galactose-positive control group

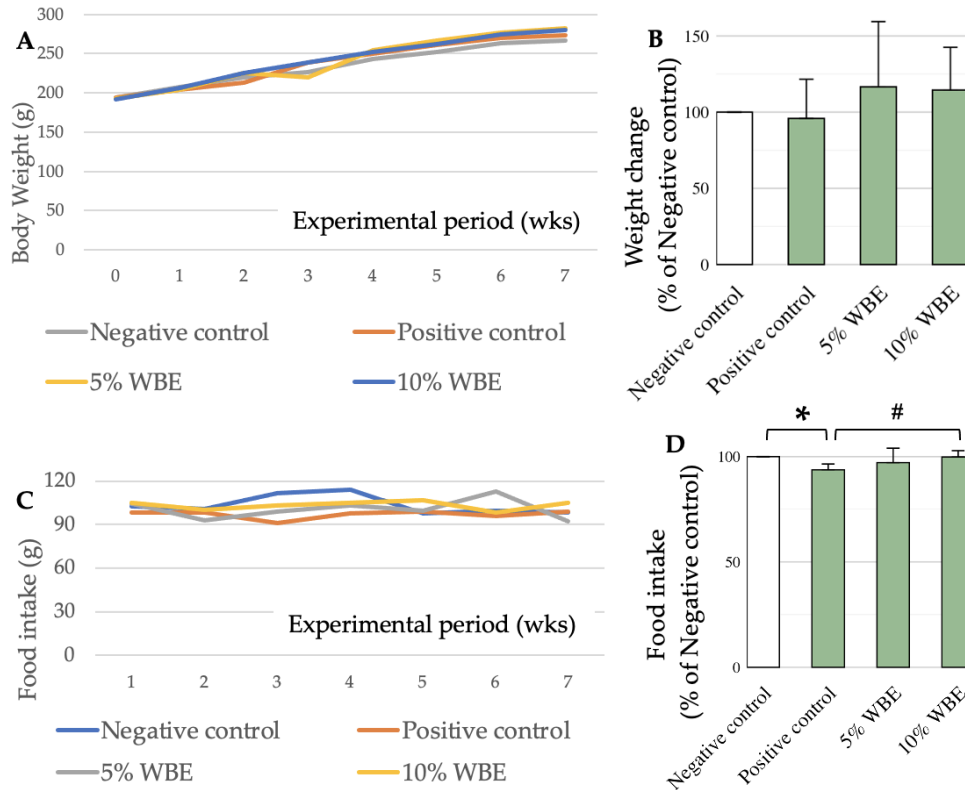


Figure 2.1: Body weight transition and food intake over the experimental period. **A.** Results of body weight in aging rats during experiment **B.** The rate of weight change in each group. Each rat's weight change rate was calculated by dividing the final week's weight by the initial week's weight, then multiplying by 100. **C.** The transition in mean diet intake over the experimental period. **D.** The mean diet intake over the experimental period. The saline-injected negative control group was set at 100% and compared with each rat in all groups. Data represent mean $\pm$ SD ($n=10$). Negative control, Saline-injected negative control group; Positive control, D-galactose-injected positive control group; 5% WBE, D-galactose-injected with 5% Wheat bran extract diets; 10% WBE, D-galactose-injected with 10% Wheat bran extract diets.

exhibited significantly lower SOD activity in erythrocytes compared to the negative control group, indicating oxidative stress induction. WBE supplementation resulted in a dose-dependent increase in erythrocytes SOD activity, with 5% WBE elevating activity to 32,749 U/m and 10% WBE increasing it to 42,368 U/m ($P<0.05$). However, liver tissue analysis revealed no significant differences in SOD activity among the groups. The disparity between erythrocytes and the liver suggests that WBE primarily exerts its antioxidative effects in circulating blood cells rather than hepatic mitochondria. As

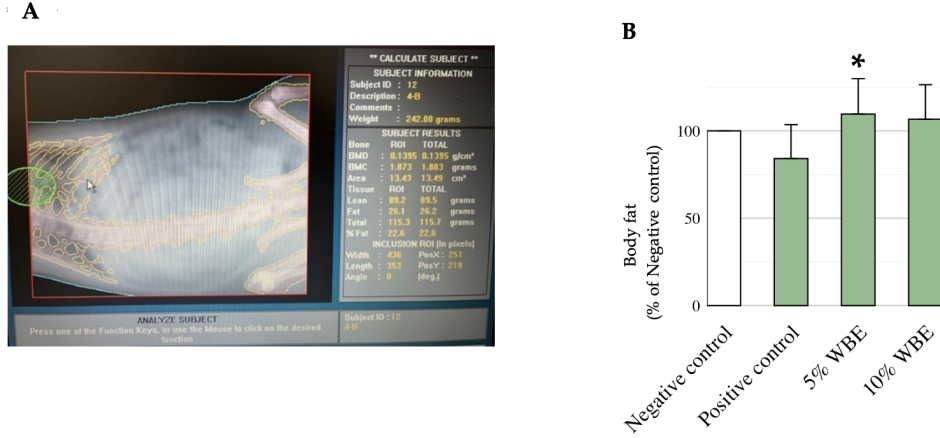


Figure 2.2: Effect of WBE treatment on body fat percentage in D-galactose-induced accelerated aging rats. **A.** The body fat percentage was captured in the 7th week of sacrifice using a Lunar PIXI Densitometer. **B.** Mean body fat percentage across all groups at the end of the experiment. The saline-injected negative control group was set at 100% and compared with each rat in all groups. * $P < 0.05$ as compared with the galactose-injected positive group. Data represent mean $\pm$ SD ($n=10$). Negative control, Saline-injected negative control group; Positive control, D-galactose-injected positive control group; 5% WBE, D-galactose-injected with 5% Wheat bran extract diets; 10% WBE, D-galactose-injected with 10% Wheat bran extract diets.

summarized in Table 2, differences in oxidative stress sources, SOD isoforms, and metabolic adaptation mechanisms may explain the tissue-specific variation in response.

2.4.4 Effect of WBE supplements of β -Galactosidase in aging rats

Senescence-associated β -galactosidase (SA- β -gal) activity was significantly elevated in the galactose-positive control group, confirming increased cellular senescence. Notably, while D-galactose did not significantly increase SA- β -gal activity in the liver, WBE supplementation still led to a dose-dependent reduction in baseline SA- β -gal levels, suggesting a protective effect against aging-related cellular deterioration (Figure 2-4).

2.4.5 Protein Expression in Skin and Liver

Western blot analysis was performed to assess the protein expression levels of key aging biomarkers (SOD2, catalase, and telomerase reverse transcriptase [TERT]) in skin

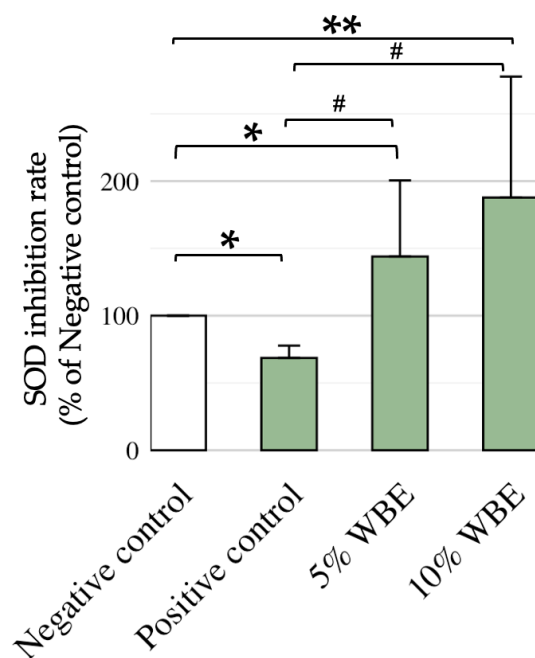


Figure 2.3: Effect of WBE treatment on SOD in D-galactose-induced accelerated aging rats. A. Superoxide dismutase (SOD) activities (U/ mg protein) in erythrocytes of galactose-induced aging female Wistar rats treated with WBE-supplemented diets at concentrations of 5% or 10%. SOD values were determined as described in the Materials and Methods section. The saline-injected negative control group was set at 100% and compared with each rat in all groups. * $P < 0.05$ as compared with the saline-negative control group. ** $P < 0.05$ as compared with the saline-negative control group. # $P < 0.05$ as compared with the saline-negative control group. Negative control, Saline-injected negative control group; Positive control, D-galactose-injected positive control group; 5% WBE, D-galactose-injected with Wheat bran extract diets; 10% WBE, D-galactose-injected with 10% Wheat bran extract diets.

and liver tissues (Figure 2-5). No significant differences were observed between groups, indicating that WBE's effects may be limited to enzymatic activity rather than transcriptional or translational regulation of these proteins. This suggests that while WBE influences oxidative stress markers in circulation, its impact at the tissue level may be more nuanced and influenced by additional regulatory mechanisms.

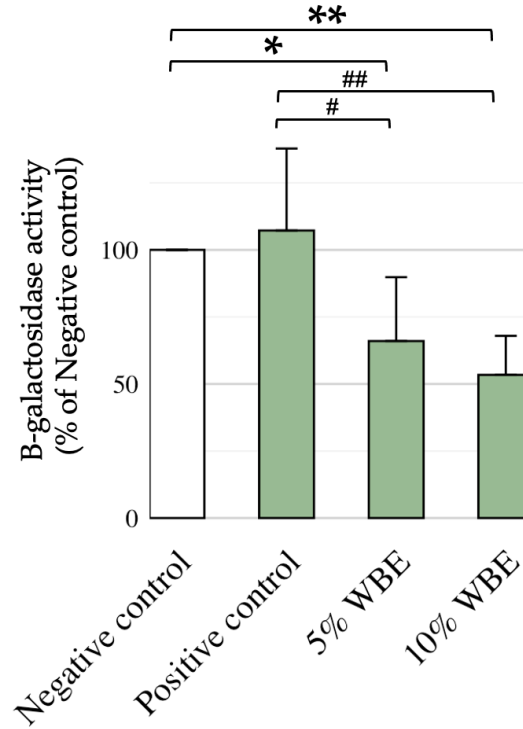


Figure 2.4: β -Galactosidase Activity in erythrocyte. β -Galactosidase (β -gal) activities in the liver of galactose-induced aging female Wistar rats treated with WBE-supplemented diets at concentrations of 5% or 10%. β -gal values were determined as described in the Materials and Methods section. The saline-injected negative control group was set at 100% and compared with each rat in all groups. * $P < 0.001$ as compared with the saline-negative control group. ** $P < 0.001$ as compared with the saline-negative control group. $P < 0.001$ as compared with the galactose-positive control group. # $P < 0.0001$ as compared with the galactose-positive control group. Data represent mean $\pm$ SD ($n = 10$). Negative control, Saline-injected negative control group; Positive control, D-galactose-injected positive control group; 5% WBE, D-galactose-injected with 5% Wheat bran extract diets; 10% WBE, D-galactose-injected with 10% Wheat bran extract diets.

2.5 Discussion

This study investigated the potential of novel enriched antioxidants in wheat bran to counteract the effects of aging. Utilizing a D-galactose-induced aging model, the study focused on recently identified antioxidants in wheat bran, including xylo-oligosaccharides and protein hydrolysates, with an emphasis on oxidative stress and cellular aging biomarkers. The results demonstrated that dietary supplementation with WBE led to a significant enhancement in erythrocyte superoxide dismutase (SOD) activity and a

decrease in baseline levels of senescence-associated β -galactosidase (SA- β -gal), indicating notable systemic antioxidative and anti-senescence effects. However, no significant changes were observed in hepatic protein expression levels of SOD2, catalase, or telomerase reverse transcriptase (TERT), indicating that WBE's antioxidative effects are more evident in circulating compartments than in solid tissues.

A key finding of this study was the significant restoration of erythrocyte SOD activity following WBE supplementation. D-galactose administration is known to induce oxidative stress by generating reactive oxygen species (ROS) and disrupting redox balance, leading to a decline in SOD activity [25]. The observed dose-dependent increase in erythrocyte SOD activity with 5% and 10% WBE supplementation suggests that WBE effectively scavenges ROS and restores antioxidant defenses in circulation. This finding is consistent with previous studies that identified bioactive components in wheat bran, including phenolic acids and flavonoids, as potent antioxidants capable of neutralizing oxidative stress [21]. Furthermore, the reduction in SA- β -gal activity baseline in WBE-fed groups suggests that WBE may exert a protective effect against cellular senescence, independent of D-galactose-induced stress. This finding is particularly noteworthy, as SA- β -gal activity is a widely recognized marker of cellular aging [26]. The ability of WBE to lower SA- β -gal levels may indicate an underlying rejuvenative or senescence-preventive mechanism, possibly mediated by enhanced mitochondrial function and reduced oxidative damage [27].

Despite the pronounced systemic antioxidative effects, no significant changes were observed in the expression of SOD2, catalase, or TERT protein in liver and skin tissue. This discrepancy may be explained by several factors. Firstly, erythrocytes act as primary oxidative stress responders in circulation [28], while tissue-level responses may require a longer exposure to WBE supplementation for measurable effects [29] (see Table 2). Secondly, the liver possesses robust endogenous antioxidant mechanisms that may overshadow the contributions of WBE [30] (see Table 2-2). Moreover, oxidative damage induced by D-galactose may exert a more acute impact on circulating erythrocytes than on specific tissue compartments, thereby making systemic biomarkers, such as erythrocyte SOD activity, more responsive indicators of the antioxidative efficacy of WBE [31,32]. The

six-week duration of the study may have been insufficient to capture long-term tissue-level adaptations [33,34]. Aging is a progressive process, during which subtle changes in protein expression may require extended intervention periods to become apparent. It is recommended that future studies consider lengthier study durations in order to assess whether sustained WBE supplementation leads to significant molecular changes in aging tissues.

Our findings regarding body composition revealed interesting metabolic effects of both D-galactose administration and WBE supplementation. Prior research has established that healthy adult female Wistar rats typically exhibit body fat percentages ranging from 20% to 30% at 15 weeks of age, depending on their diet and activity levels [24]. Interestingly, within our four experimental groups, the negative control group showed significantly lower body fat compared to other groups (Figure 2B). This observation aligns with the known metabolic effects of D-galactose, which is metabolized in the liver and other tissues via the Leloir pathway [35]. This pathway involves three enzymes: galactokinase (GALK), galactose-1-phosphate uridylyl transferase (GALT), and uridine diphosphate (UDP) galactose-4-epimerase (GALE), ultimately converting galactose to glucose for oxidation in glycolysis or storage as glycogen [36]. D-galactose administration induces several significant metabolic alterations that disrupt normal physiological functions [25]. When D-galactose intake exceeds the body’s processing capacity, it can lead to metabolic disturbances, including disruption of glucose metabolism through competitive inhibition of glucose transporters [37]. This competition influences fat storage through metabolic programming effects observed in various studies [37,38]. D-galactose competes with glucose for transport mechanisms [38], leading to reduced glucose availability, which subsequently affects fat accumulation, resulting in the observed reduction in body fat percentage in the D-galactose-injected positive control group. Furthermore, research suggests that galactose consumption disrupts glucose homeostasis by interfering with glucose transporters such as GLUT1 and GLUT4, contributing to insulin resistance and metabolic dysfunction [38]. These disruptions can contribute to metabolic diseases such as diabetes and obesity, as well as age-related neurodegenerative diseases like Alzheimer’s disease [38]. The effects of

galactose on insulin signaling pathways are well-documented, with evidence indicating that galactose exposure induces insulin resistance and alters cellular signaling, driving metabolic disturbances [39].

Beyond its antioxidative and anti-aging benefits, this study found no adverse effects associated with WBE supplementation. Throughout the study, no significant changes were observed in body weight and liver enzyme levels, suggesting that WBE is well tolerated and does not exert toxic effects at the administered doses. These findings indicate that WBE could be considered a safe dietary supplement for long-term consumption. However, further studies in diverse models, including human trials, are needed to confirm its safety profile and establish optimal dosages.

Further research is warranted to elucidate the precise molecular mechanisms underlying WBE’s anti-aging effects. Specifically, investigating mitochondrial function, inflammatory pathways, and DNA damage repair mechanisms may provide deeper insights into WBE’s role in aging modulation. Additionally, expanding the study to include different tissue types, such as kidney and brain, may reveal whether WBE’s effects extend beyond systemic circulation.

Optimizing the dosage and bioavailability of WBE is another critical area for exploration since this study demonstrated dose-dependent benefits but did not determine the optimal concentration for maximal anti-aging effects without remaining essential adverse metabolic impacts remaining essential.

2.6 Conclusion

In summary, this study highlights the potential of WBE as a dietary intervention for mitigating oxidative stress and cellular aging. WBE supplementation significantly enhanced systemic antioxidant defenses and reduced senescence markers, though its effects at the tissue level remain inconclusive. These findings support the development of wheat bran-based nutraceuticals for promoting healthy aging and emphasize the need for further investigation into long-term molecular and physiological impacts.

Table 2.2: *Galactose loading vs. SOD activity: erythrocytes vs. liver*

Characteristics	Erythrocyte	Liver
ROS generation source	Systemic oxidative stress due to galactose loading [45]	ROS (predominantly of mitochondrial origin) resulting from galactose metabolism [46].
Type of SOD	Mainly Cu/Zn-SOD (cytosolic type) [47].	Mn-SOD (mitochondrial type) and Cu/Zn-SOD [48].
Antioxidant effects	The direct action of antioxidants is easily observed [28].	Antioxidant effects may be gradual or sub-marked [29].
The time scale of impact	Short-term changes in oxidative stress are likely to be reflected [49].	Long-term metabolic compensatory effects may weaken the impact [50].
Changes in SOD activity	Often significantly increased or stabilized by antioxidants [51].	Baseline SOD activity is high, and changes may be small [30].
Effects of damage	Oxidative stress-induced membrane damage and shortened erythrocyte lifespan are reduced [49].	Limited effect of antioxidants if oxidative stress is too high [52].

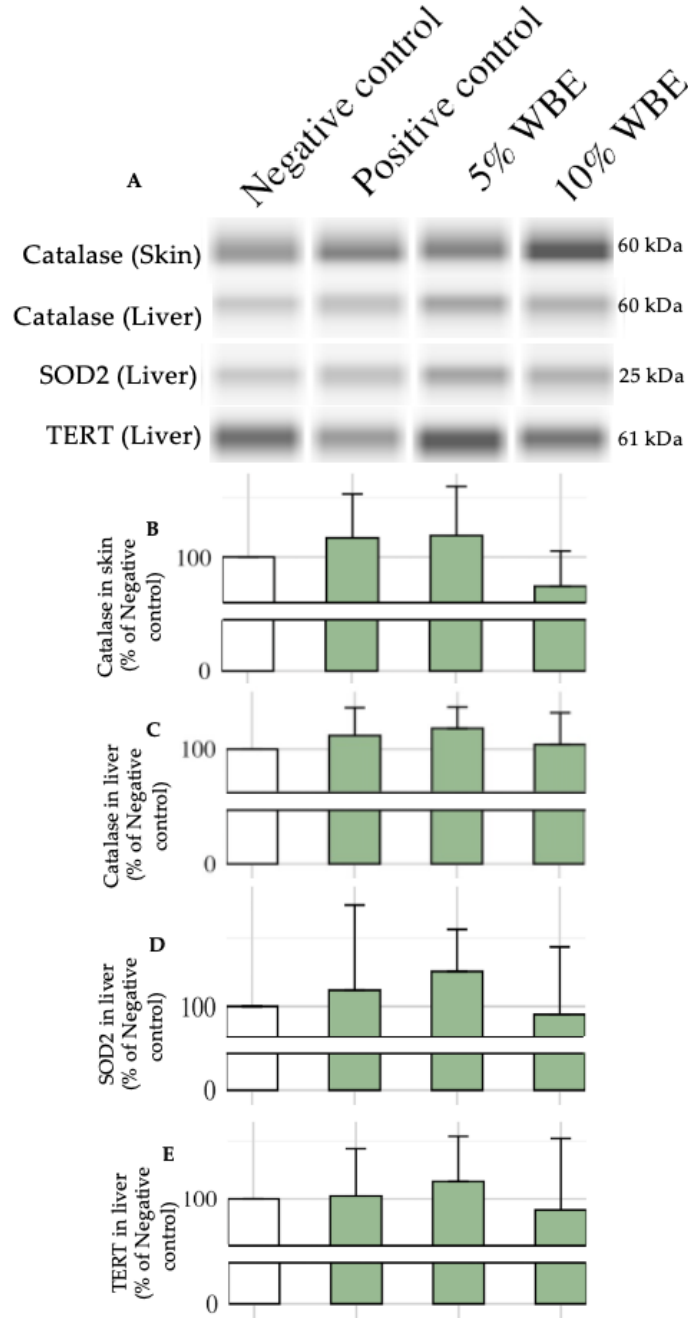


Figure 2.5: Western blot analysis of WBE treatment on protein expressions of catalase, SOD2, and TERT in the skin and livers of D galactose-induced accelerated aging rats. **A.** The protein expression images of catalase (skin and liver), SOD2 (liver), and TERT (liver) in each group by western blot. Gray values of: **B.** Catalase in skin, **C.** Catalase in liver, **D.** SOD2 in liver, and **E.** TERT in liver. Bar graphs were constructed according to the gray values of the protein bands. Data represent mean $\pm$ SD (n=6). Negative control, Saline-injected negative control group; Positive control, D-galactose-injected positive control group; 5% WBE, D-galactose-injected with 5% Wheat bran extract diets; 10% WBE, D-galactose-injected with 10% Wheat bran extract diets.

Bibliography

References

Chapter 2

1. A Comprehensive Review of Wheat Phytochemicals: From Farm to Fork and beyond - Tian - 2022 - Comprehensive Reviews in Food Science and Food Safety - Wiley Online Library Available online: <https://ift.onlinelibrary.wiley.com/doi/10.1111/1541-4337.12960> (accessed on 10 March 2025).
2. Guo, J.; Huang, X.; Dou, L.; Yan, M.; Shen, T.; Tang, W.; Li, J. Aging and Aging-Related Diseases: From Molecular Mechanisms to Interventions and Treatments. *Signal Transduct. Target. Ther.* **2022**, *7*, 1–40, doi:10.1038/s41392-022-01251-0.
3. Höhn, A.; Weber, D.; Jung, T.; Ott, C.; Hugo, M.; Kochlik, B.; Kehm, R.; König, J.; Grune, T.; Castro, J.P. Happily (n)Ever after: Aging in the Context of Oxidative Stress, Proteostasis Loss and Cellular Senescence. *Redox Biol.* **2017**, *11*, 482–501, doi:10.1016/j.redox.2016.12.001.
4. Somasundaram, I.; Jain, S.M.; Blot-Chabaud, M.; Pathak, S.; Banerjee, A.; Rawat, S.; Sharma, N.R.; Duttaroy, A.K. Mitochondrial Dysfunction and Its Association with Age-Related Disorders. *Front. Physiol.* **2024**, *15*, 1384966, doi:10.3389/fphys.2024.1384966.
5. Rossiello, F.; Jurk, D.; Passos, J.F.; d'Adda Di Fagagna, F. Telomere Dysfunction in Ageing and Age-Related Diseases. *Nat. Cell Biol.* **2022**, *24*, 135–147, doi:10.1038/s41556-022-00842-x.
6. Austad, S.N.; Wood, M.A.; Villeda, S.A.; Voss, J.L.; Sahay, A.; Albert, M. Innovative Approaches in Cognitive Aging. *Neurobiol. Aging* **2019**, *83*, 150–154, doi:10.1016/j.neurobiolaging.2019.04.013.
7. Dutta, C. BIOMARKERS OF AGING. *Innov. Aging* **2024**, *8*, 301, doi:10.1093/geroni/igae098.0982.
8. Tao, W.; Yu, Z.; Han, J.-D.J. Single-Cell Senescence Identification Reveals Senescence Heterogeneity, Trajectory, and Modulators. *Cell Metab.* **2024**, *36*, 1126–1143.e5, doi:10.1016/j.cmet.2024.03.009.
9. Arbatskiy, M.S.; Balandin, D.E. Universal Markers of Cellular and Replicative Senescence. *Adv. Gerontol.* **2024**, *14*, 127–134, doi:10.1134/S2079057025600028.

10. Palma, F.R.; He, C.; Danes, J.M.; Paviani, V.; Coelho, D.R.; Gantner, B.N.; Bonini, M.G. Mitochondrial Superoxide Dismutase: What the Established, the Intriguing, and the Novel Reveal About a Key Cellular Redox Switch. *Antioxid. Redox Signal.* **2020**, *32*, 701–714, doi:10.1089/ars.2019.7962.
11. Zhang, Y.; Unnikrishnan, A.; Deepa, S.S.; Liu, Y.; Li, Y.; Ikeno, Y.; Sosnowska, D.; Van Remmen, H.; Richardson, A. A New Role for Oxidative Stress in Aging: The Accelerated Aging Phenotype in Sod1[−] Mice Is Correlated to Increased Cellular Senescence. *Redox Biol.* **2017**, *11*, 30–37, doi:10.1016/j.redox.2016.10.014.
12. Rasheed, Z. Therapeutic Potentials of Catalase: Mechanisms, Applications, and Future Perspectives. *Int. J. Health Sci.* **2024**, *18*, 1–6.
13. Anwar, S.; Alrumaihi, F.; Sarwar, T.; Babiker, A.Y.; Khan, A.A.; Prabhu, S.V.; Rahmani, A.H. Exploring Therapeutic Potential of Catalase: Strategies in Disease Prevention and Management. *Biomolecules* **2024**, *14*, 697, doi:10.3390/biom14060697.
14. Martínez-Zamudio, R.I.; Dewald, H.K.; Vasilopoulos, T.; Gittens-Williams, L.; Fitzgerald-Bocarsly, P.; Herbig, U. Senescence-Associated β -Galactosidase Reveals the Abundance of Senescent CD8⁺ T Cells in Aging Humans. *Aging Cell* **2021**, *20*, e13344, doi:10.1111/accel.13344.
15. Yuan, X.; Larsson, C.; Xu, D. Mechanisms Underlying the Activation of TERT Transcription and Telomerase Activity in Human Cancer: Old Actors and New Players. *Oncogene* **2019**, *38*, 6172–6183, doi:10.1038/s41388-019-0872-9.
16. Lee, J.H.; Hong, J.; Zhang, Z.; De La Peña Avalos, B.; Proietti, C.J.; Deamicis, A.R.; Guzmán G., P.; Lam, H.-M.; Garcia, J.; Roudier, M.P.; et al. Regulation of Telomere Homeostasis and Genomic Stability in Cancer by N⁶-Adenosine Methylation (m⁶A). *Sci. Adv.* **2021**, *7*, eabg7073, doi:10.1126/sciadv.abg7073.
17. Harley, J.; Santosa, M.M.; Ng, C.Y.; Grinchuk, O.V.; Hor, J.-H.; Liang, Y.; Lim, V.J.; Tee, W.W.; Ong, D.S.T.; Ng, S.-Y. Telomere Shortening Induces Aging-Associated Phenotypes in hiPSC-Derived Neurons and Astrocytes. *Biogerontology* **2024**, *25*, 341–360, doi:10.1007/s10522-023-10076-5.
18. Mikhailova, D.V.; Shevchenko, O.G.; Golubev, D.A.; Platonova, E.Y.; Zemskaya, N.V.; Shoeva, O.Y.; Gordeeva, E.I.; Patov, S.A.; Shaposhnikov, M.V.; Khlestkina, E.K.; et al. Antioxidant Properties and Geroprotective Potential of Wheat Bran Extracts with Increased Content of Anthocyanins. *Antioxid. Basel Switz.* **2023**, *12*, 2010, doi:10.3390/antiox12112010.

19. Li, C.; Wu, W.; Tilley, M.; Chen, R.; Sun, X.S.; Wang, W.; Li, Y. In Vitro Antioxidant Properties of Wheat Bran Extracts and Their Inhibitory Effects on Collagenase, Elastase, and Hyaluronidase. *ACS Food Sci. Technol.* **2024**, *4*, 1960–1966, doi:10.1021/acsfoodscitech.4c00310.
20. Brindzová, L.; Zalibera, M.; Jakubík, T.; Mikulášová, M.; Takácsová, M.; Mošovská, S.; Rapta, P. Antimutagenic and Radical Scavenging Activity of Wheat Bran. *Cereal Res. Commun.* **2009**, *37*, 45–55, doi:10.1556/CRC.37.2009.1.6.
21. Kobayashi, K.; Suzaudulla, M.; Bender, R.; Li, C.; Li, Y.; Sun, X.S.; Wang, W. Functional Properties and Potential Applications of Wheat Bran Extracts in Food and Cosmetics: A Review of Antioxidant, Enzyme-Inhibitory, and Anti-Aging Benefits. *Foods* **2025**, *14*, 515, doi:10.3390/foods14030515.
22. Mikhailova, D.V.; Shevchenko, O.G.; Golubev, D.A.; Platonova, E.Y.; Zemskaya, N.V.; Shoeva, O.Y.; Gordeeva, E.I.; Patov, S.A.; Shaposhnikov, M.V.; Khlestkina, E.K.; et al. Antioxidant Properties and Geroprotective Potential of Wheat Bran Extracts with Increased Content of Anthocyanins. *Antioxidants* **2023**, *12*, 2010, doi:10.3390/antiox12112010.
23. heng, M.; Liu, Y.; Zhang, G.; Yang, Z.; Xu, W.; Chen, Q. The Applications and Mechanisms of Superoxide Dismutase in Medicine, Food, and Cosmetics. *Antioxid. Basel Switz.* **2023**, *12*, 1675, doi:10.3390/antiox12091675.
24. George, B.G.; Richard, M.J.; Alfred, T.B. Body Weight, Hematological and Biochemical Parameters of Wistar Albino Rats Fed with Chia Seed Concentrate. *Eur. J. Med. Plants* **2023**, *34*, 10–19, doi:10.9734/ejmp/2023/v34i1111167.
25. Han, H.; Liu, Z.; Yin, J.; Gao, J.; He, L.; Wang, C.; Hou, R.; He, X.; Wang, G.; Li, T.; et al. D-Galactose Induces Chronic Oxidative Stress and Alters Gut Microbiota in Weaned Piglets. *Front. Physiol.* **2021**, *12*, doi:10.3389/fphys.2021.634283.
26. Valieva, Y.; Ivanova, E.; Fayzullin, A.; Kurkov, A.; Igrunkova, A. Senescence-Associated β -Galactosidase Detection in Pathology. *Diagnostics* **2022**, *12*, 2309, doi:10.3390/diagnostics12102309.
27. Wang, J.; Wu, Y.; Chen, Z.; Chen, Y.; Lin, Q.; Liang, Y. Exogenous Bioactive Peptides Have a Potential Therapeutic Role in Delaying Aging in Rodent Models. *Int. J. Mol. Sci.* **2022**, *23*, 1421, doi:10.3390/ijms23031421.

28. Oxidants and Antioxidants in the Redox Biochemistry of Human Red Blood Cells | ACS Omega Available online: <https://pubs.acs.org/doi/10.1021/acsomega.2c06768> (accessed on 6 February 2025).
29. Alpinetin Exhibits Antioxidant and Anti-Inflammatory Effects in C57BL/6 Mice with Alcoholic Liver Disease Induced by the Lieber–DeCarli Ethanol Liquid Diet Available online: <https://www.mdpi.com/1422-0067/26/1/86> (accessed on 6 February 2025).
30. Trist, D.B.G.; Hilton, D.J.B.; Hare, P.D.J.; Crouch, P.P.J.; Double, P.K.L. Superoxide Dismutase 1 in Health and Disease: How a Frontline Antioxidant Becomes Neurotoxic. *Angew. Chem. Int. Ed Engl.* **2020**, *60*, 9215, doi:10.1002/anie.202000451.
31. Remigante, A.; Morabito, R.; Spinelli, S.; Trichilo, V.; Loddo, S.; Sarikas, A.; Dossena, S.; Marino, A. D-Galactose Decreases Anion Exchange Capability through Band 3 Protein in Human Erythrocytes. *Antioxidants* **2020**, *9*, 689, doi:10.3390/antiox9080689.
32. Spinelli, S.; Remigante, A.G.D.; Dossena, S.; Marino, A.; Morabito, R. Hemoglobin Glycation and Oxidative Stress Induce Functional Modification of Band 3 Protein in D-Galactose-Treated Erythrocytes: Double Effect of Quercetin. *FASEB J.* **2022**, *36*, doi:10.1096/fasebj.2022.36.S1.R4411.
33. Ross, K.S.; Smith, C. D-Galactose: A Model of Accelerated Ageing Sufficiently Sensitive to Reflect Preventative Efficacy of an Antioxidant Treatment. *Biogerontology* **2020**, *21*, 745–761, doi:10.1007/s10522-020-09891-x.
34. 1H NMR-Based Fecal Metabolomics Reveals Changes in Gastrointestinal Function of Aging Rats Induced by d-Galactose Available online: https://www.researchgate.net/publication/343914722_1H_NMR-Based_Fecal_Metabolomics_Reveals_Changes_in_Gastrointestinal_Function_of_Aging_Rats_Induced_by_d-Galactose (accessed on 18 March 2025).
35. Replacing Part of Glucose with Galactose in the Postweaning Diet Protects Female But Not Male Mice from High-Fat Diet–Induced Adiposity in Later Life - ScienceDirect Available online: <https://www.sciencedirect.com/science/article/pii/S0022316622166563?via%3Dihub> (accessed on 10 January 2025).
36. Conte, F.; van Buuringen, N.; Voermans, N.C.; Lefeber, D.J. Galactose in Human Metabolism, Glycosylation and Congenital Metabolic Diseases: Time for a Closer

Look. *Biochim. Biophys. Acta BBA - Gen. Subj.* **2021**, 1865, 129898, doi:10.1016/j.bbagen.2021.129898.

37. Gjedde, A. Blood-Brain Transfer of Galactose in Experimental Galactosemia, with Special Reference to the Competitive Interaction between Galactose and Glucose. *J. Neurochem.* **1984**, 43, 1654–1662, doi:10.1111/j.1471-4159.1984.tb06092.x.
38. Chadt, A.; Al-Hasani, H. Glucose Transporters in Adipose Tissue, Liver, and Skeletal Muscle in Metabolic Health and Disease. *Pflugers Arch.* **2020**, 472, 1273–1298, doi:10.1007/s00424-020-02417-x.
39. Na, Z.; Jiang, H.; Meng, Y.; Song, J.; Feng, D.; Fang, Y.; Shi, B.; Li, D. Association of Galactose and Insulin Resistance in Polycystic Ovary Syndrome: A Case-Control Study. *EClinicalMedicine* **2022**, 47, 101379, doi:10.1016/j.eclinm.2022.101379.
40. Succio, M.; Sacchetti, R.; Rossi, A.; Parenti, G.; Ruoppolo, M. Galactosemia: Biochemistry, Molecular Genetics, Newborn Screening, and Treatment. *Biomolecules* **2022**, 12, 968, doi:10.3390/biom12070968.
41. Alam, Y.H.; Kim, R.; Jang, C. Metabolism and Health Impacts of Dietary Sugars. *J. Lipid Atheroscler.* **2022**, 11, 20–38, doi:10.12997/jla.2022.11.1.20.
42. Sun, P.; Bouwman, L.M.S.; De Deugd, J.; Van Der Stelt, I.; Oosting, A.; Keijer, J.; Van Schothorst, E.M. Galactose in the Post-Weaning Diet Programs Improved Circulating Adiponectin Concentrations and Skeletal Muscle Insulin Signaling. *Int. J. Mol. Sci.* **2022**, 23, 10207, doi:10.3390/ijms231810207.
43. Wang, T.; Wang, J.; Hu, X.; Huang, X.-J.; Chen, G.-X. Current Understanding of Glucose Transporter 4 Expression and Functional Mechanisms. *World J. Biol. Chem.* **2020**, 11, 76–98, doi:10.4331/wjbc.v11.i3.76.
44. Guo, B.; Guo, Q.; Wang, Z.; Shao, J.-B.; Liu, K.; Du, Z.-D.; Gong, S.-S. D-Galactose-Induced Oxidative Stress and Mitochondrial Dysfunction in the Cochlear Basilar Membrane: An in Vitro Aging Model. *Biogerontology* **2020**, 21, 311–323, doi:10.1007/s10522-020-09859-x.
45. Bettiol, A.; Galora, S.; Argento, F.R.; Fini, E.; Emmi, G.; Mattioli, I.; Bagni, G.; Fiorillo, C.; Becatti, M. Erythrocyte Oxidative Stress and Thrombosis. *Expert Rev. Mol. Med.* **2022**, 24, e31, doi:10.1017/erm.2022.25.
46. Allameh, A.; Niayesh-Mehr, R.; Aliarab, A.; Sebastiani, G.; Pantopoulos, K. Oxidative Stress in Liver Pathophysiology and Disease. *Antioxidants* **2023**, 12, 1653, doi:10.3390/antiox12091653.

47. Copper Concentration in Erythrocytes, Platelets, Plasma, Serum and Urine: Influence of Physical Training - PMC Available online:
<https://pmc.ncbi.nlm.nih.gov/articles/PMC8025359/> (accessed on 6 February 2025).
48. Kitada, M.; Xu, J.; Ogura, Y.; Monno, I.; Koya, D. Manganese Superoxide Dismutase Dysfunction and the Pathogenesis of Kidney Disease. *Front. Physiol.* **2020**, *11*, doi:10.3389/fphys.2020.00755.
49. Obeagu, E.I.; Igwe, M.C.; Obeagu, G.U. Oxidative Stress's Impact on Red Blood Cells: Unveiling Implications for Health and Disease. *Medicine (Baltimore)* **2024**, *103*, e37360, doi:10.1097/MD.00000000000037360.
50. Li, S.; Tan, H.-Y.; Wang, N.; Zhang, Z.-J.; Lao, L.; Wong, C.-W.; Feng, Y. The Role of Oxidative Stress and Antioxidants in Liver Diseases. *Int. J. Mol. Sci.* **2015**, *16*, 26087–26124, doi:10.3390/ijms161125942.
51. Ściskalska, M.; Ołdakowska, M.; Marek, G.; Milnerowicz, H. Changes in the Activity and Concentration of Superoxide Dismutase Isoenzymes (Cu/Zn SOD, MnSOD) in the Blood of Healthy Subjects and Patients with Acute Pancreatitis. *Antioxidants* **2020**, *9*, 948, doi:10.3390/antiox9100948.
52. Vascotto, C.; Tiribelli, C. Oxidative Stress, Antioxidant Defenses, and the Liver. In *Studies on Hepatic Disorders*; Albano, E., Parola, M., Eds.; Springer International Publishing: Cham, 2015; pp. 41–64 ISBN 978-3-319-15539-5.