

Impact of advanced oxidative process (AOP) decontamination on the techno-functional properties of flaxseed during storage

By

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A thesis submitted to the Faculty of Graduate and Postdoctoral Studies of

The University of Manitoba

In partial fulfilment of the requirements of the degree of

MASTER OF SCIENCE

Department of Food and Human Nutritional Sciences

University of Manitoba

Winnipeg

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Acknowledgments

I would like to express my sincere gratitude to my supervisor, Dr. Filiz Koksel, for giving me the opportunity to pursue my Master's degree under her supervision. I am deeply thankful for her continuous support, encouragement, and mentorship throughout my graduate studies. From welcoming me into her research group, to guiding me through every stage of this project, her expertise, thoughtful advice, and dedication have been invaluable to my academic and professional development. I am sincerely grateful to my thesis advisory committee members, Dr. Jitendra Paliwal and Dr. Nandika Bandara, for their insightful comments, constructive suggestions, and valuable feedback, which greatly improved this thesis. I also thank Dr. Mudassir Chaudhry for his guidance, encouragement, and support throughout this research.

I would like to thank my colleagues and friends in the FoodPro Lab; Dr. Aayushi Kadam, Amanjeet Singh, Ravinder Singh, Peifan Du, Susane Trevisan, and Irene Rangira, for their friendship, encouragement, technical assistance, and the many enjoyable moments we shared. I also appreciate John Bachu and Finley Makila at the Richardson Centre for Food Technology and Research for their technical assistance with the nitrogen analyzer.

I gratefully acknowledge the financial support provided by the Sustainable Canadian Agricultural Partnership (Sustainable CAP), the Manitoba Crop Alliance, and the Natural Sciences and Engineering Research Council of Canada (NSERC). I also sincerely appreciate the financial assistance provided by the University of Manitoba through the Graduate Entrance Scholarship and the Research Completion Scholarship.

Finally, I extend my heartfelt thanks to my friends in Winnipeg for their friendship and encouragement throughout my time here. I am especially grateful to my parents, my sister, and my close relatives for their unconditional love, unwavering support, and constant encouragement. Their belief in me has been my greatest source of strength, and this achievement would not have been possible without them.

Preface

This thesis is presented in manuscript style: Chapter 1 introduces the research, and Chapter 2 presents a comprehensive literature review that establishes scientific background and rationale for the study. The remaining chapters present the research manuscript, including the materials and methods, results and discussion, conclusions, and future recommendations.

This thesis is an original work by the author, Guwanth Wanigasuriya, completed under the supervision of Dr. Filiz Koksel in the Department of Food and Human Nutritional Sciences at the University of Manitoba.

The research presented in this thesis was conducted in partial fulfillment of the requirements for the Master of Science degree. The author was responsible for sample preparation, laboratory analyses, data collection, statistical analyses, interpretation of the results, and preparation of the thesis manuscript. Dr. Filiz Koksel provided guidance on research design, data interpretation, and manuscript preparation throughout the study. Dr. Filiz Koksel served as the corresponding author and reviewed the manuscript. All authors reviewed and approved the final version of the manuscript.

To the best of the author's knowledge, this thesis has not been previously published in its entirety, nor has it been submitted to any other institution in fulfillment of the requirements for any other degree. Portions of this thesis may be submitted for publication in peer-reviewed journals, with appropriate acknowledgment of all contributors.

Abstract

Flaxseed, an important oilseed crop with various food and beverage applications, has gained attention for its potential health benefits, attributed to its composition rich in omega-3 fatty acids, lignans, and dietary fibre. However, the increasing use of raw flaxseed, particularly in applications such as salad and yogurt toppings, smoothies, trail mixes, etc., raises concerns about microbial contamination acquired during production, harvesting, and storage. Advanced oxidative process (AOP) offers a promising non-thermal decontamination approach with minimal impact on the quality of agricultural commodities while improving microbial safety. The objective of the study was to quantify the changes in techno-functional properties of flaxseed before and after AOP decontamination as well as during storage. Flaxseed was treated using an AOP system (Clean Flow Healthcare mini) combining ozone (5-9 ppm), UV-C light (8-12 mW/cm²), and 3% hydrogen peroxide (H₂O₂). Ozone (O₃) and UV-C were applied for 30 or 40 s of exposure time, while H₂O₂ was delivered at low (20 mL/min) or high (30 mL/min) flow rates, producing four treatments (T1-30, T1-40, T2-30, and T2-40). Untreated flaxseed served as the control. The treated and control samples were stored at 20 °C and 75% relative humidity for four months and analyzed for water holding capacity (WHC), oil holding capacity (OHC), emulsion capacity (EC), emulsion stability (ES), least gelation concentration (LGC), and protein solubility (PS). WHC was significantly ($p < 0.05$) affected by treatment, storage time, and their interaction, but remained within 3.32-3.71 g water/g dry flaxseed. OHC was significantly ($p < 0.05$) affected by treatment and storage time, with no interaction, and ranged from 1.86 to 1.95 g oil/g dry flaxseed. EC ranged from 41.3 to 44.5%, with significant ($p < 0.05$) treatment and treatment $\times$ storage effects. ES remained approximately 49.7-52.0%, with slightly lower values observed for T2-30, and T2-40. Also, LGC was significantly ($p < 0.05$) affected by treatment and storage time at all pH levels, while PS was affected by storage time, especially at pH 7.0. Overall, AOP caused statistically significant ($p < 0.05$) but minor changes in flaxseed techno-functionality during storage, supporting its potential as a non-thermal decontamination strategy.

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List of Abbreviations

db.	Dry basis
ALA	Alpha-linolenic acid
CFU	Colony-forming units
H ₂ O ₂	Hydrogen peroxide
O ₃	Ozone
AOP	Advanced oxidative process
UV	Ultraviolet
ROS	Reactive oxygen species
FTIR	Fourier-transform infrared spectroscopy
WHC	Water holding capacity
OHC	Oil holding capacity
EC	Emulsion capacity
ES	Emulsion stability
LGC	Least gelation concentration
PS	Protein solubility
FPI	Flaxseed protein isolate
FG	Flaxseed gum
ASAE	American Society of Agricultural Engineers
AOCS	American Oil Chemists' Society
AACC	American Association of Cereal Chemists
AAPH	2,2'-azobis (2-amidinopropane) dihydrochloride

Chapter 1. Introduction

Flaxseed (*Linum usitatissimum* L.) has been cultivated for thousands of years and has served multiple purposes ranging from food and medicine to linen fibre, paint, and flooring materials. As Vaisey-Genser & Morris (2003) noted, its longstanding role in human civilization reflects its versatility and nutritional value. While the term *linseed* is commonly used in Europe, Hall et al. (2006) indicated that *flaxseed* is the preferred term in North America, especially in contexts related to human consumption.

In recent decades, flaxseed has emerged as an important dietary component, largely because growing scientific evidence supports its health-promoting properties. According to Hall et al. (2006), flaxseed contains approximately 38-45% oil, with nearly half of its fatty acid profile consisting of α -linolenic acid (ALA), an essential omega-3 fatty acid. Moreover, the seed is a substantial source of plant lignans, reaching levels up to 13 mg/g, and contains roughly 28% dietary fibre and 20% protein. Tarique et al. (2022) emphasized that the combined presence of healthy lipids, proteins, fibres, and phytochemicals contributes to flaxseed's diverse functional benefits, which include improved cardiovascular health, antioxidant activity, anti-inflammatory effects, and efficiency and health of the digestive system. These characteristics have enabled flaxseed to be positioned within the broader category of functional foods, or as Nowak & Jeziorek (2023) described, "superfoods" that naturally contain bioactive compounds with potential physiological benefits. The nutritional composition of flaxseed further emphasizes its growing relevance in food and nutraceutical markets and continues to drive its inclusion in a broad range of products, such as bakery items, beverages, cereals, smoothies, and dietary supplements, particularly in response to rising demand for plant-based and minimally processed foods.

Flaxseed holds economic significance, particularly in Canada. Pradhan et al. (2010) identified flaxseed as Canada's third-largest oilseed crop after canola and soybean, noting its annual export value of approximately CAD \$150-180 million. Canada contributes nearly 40% of global flaxseed production, thereby playing a major role in shaping international flax markets. Saskatchewan Flax Development Commission (2024) further highlighted that other key producing regions include China, the United States,

and India, while the European Union's primary producers are Germany, France, and the United Kingdom. Large-scale cultivation, mechanised harvesting, and extensive global trade illustrate flaxseed's economic and agricultural relevance. The global flaxseed market was valued at approximately USD 800 million in 2023 and is projected to reach USD 1.38 billion by 2028, with an estimated compound annual growth rate of ~11.6% during the forecast period (Tyagi, 2024).

Despite its nutritional advantages, microbial contamination can compromise the safety and quality of flaxseed. Like most field crops, flaxseed is exposed to numerous sources of contamination throughout its production chain, including soil, air, dust, manure, water, handling equipment, shipping containers, and processing surfaces. Willis et al. (2009) and Vegi et al. (2016) documented that flaxseed, similar to other oilseeds, can harbour spoilage microorganisms and pathogenic bacteria. Many of these microorganisms can survive in low-moisture environments, which Beuchat et al. (2013) and Van der Linden et al. (2013) described as particularly favourable to the persistence of pathogens such as *Salmonella* spp., *Escherichia coli*, *Bacillus cereus*, *Clostridium* spp., and *Staphylococcus aureus*. Several outbreaks linked to oilseed-derived foods have been attributed to *Salmonella*, the most frequently encountered pathogen in flaxseed processing chains. Tamber et al. (2016) reported *Salmonella* contamination in flaxseed-containing products, raising concerns given that flaxseed is often consumed without heat treatment. Fungal microflora present an additional challenge, as molds and yeasts, including *Fusarium*, *Penicillium*, *Aspergillus*, and *Alternaria* species, can contaminate seeds during growth, storage, or distribution. Rollemberg et al. (2022) noted that xerotolerant fungi and mycotoxin-producing species may proliferate under fluctuating storage conditions, increasing the risk of spoilage, off-flavours, and potentially hazardous toxin production. Shah et al. (2018) added that microbial growth may accelerate lipid oxidation, particularly critical for flaxseed due to its high content of unsaturated fatty acids. Survey studies reveal that the microbial load present on flaxseed can be substantial (Silva et al., 2022; Vegi et al., 2016). Vegi et al. (2016) reported total aerobic bacterial counts as high as 5.7 log CFU/g, along with significant populations of yeasts and molds. Because flaxseed is often consumed raw or incorporated into ready-to-eat products, these microbial levels pose direct risks to

consumer safety, prompting producers and exporters to seek effective methods to reduce microbial loads while preserving seed quality.

A range of chemical decontamination methods has been applied to seeds and grains, though many present limitations for flaxseed. Chlorine-based sanitizers, such as sodium hypochlorite, are widely used because of their cost-effectiveness and high antimicrobial efficacy. Gilbert et al. (2023) demonstrated that such treatments can achieve microbial reductions exceeding 3 log CFU. Hylton et al. (2019) reported that peracetic acid, obtained by mixing acetic acid (vinegar) and hydrogen peroxide, was an effective sanitizing treatment for flaxseed, reducing *Salmonella* levels to below the limit of detection by more than 5 log CFU/g. However, McDonald & Komulainen (2005) and Ding et al. (2013) cautioned that chemical sanitizers may form potentially toxic or chlorinated byproducts and pose occupational hazards.

Physical treatments, including hot water, dry heat, irradiation, and high-pressure processing, have also been investigated, as reported by Ding et al. (2013), Bari et al. (2009) and Hu et al. (2004). Previous findings (Shah et al., 2018; Shah et al., 2017) indicate that pasteurization, along with storage, significantly reduces microbial loads in both whole and milled flaxseed. Bliznyuk et al. (2023) reported that electron beam irradiation reduced both fungal presence and colony size in flaxseed by 15% and 30%, respectively. Yet these methods often require prolonged exposure or elevated temperatures, which may compromise seed viability (Bliznyuk et al., 2023) nutritional integrity, and sensory attributes. Koch & Roberts (2014) noted that thermal processes can degrade heat-sensitive nutrients. Similar effects on the protein profile of flaxseed were reported by Waszkowiak et al. (2018), while Jiang et al. (2006) showed that high temperatures can alter structural and visual properties of seeds.

Given these limitations, interest is growing in alternative decontamination technologies that are both effective and environmentally sustainable. Advanced Oxidative Processes (AOPs) have emerged as a promising category of treatments capable of generating hydroxyl radicals ($\bullet\text{OH}$) with strong oxidizing power. Fan & Song (2020) explained that AOPs can be produced by combining ultraviolet (UV-C) light, ozone (O_3), hydrogen peroxide (H_2O_2), pulsed light, or cold plasma. With an oxidation potential of 2.80 V,

hydroxyl radicals can rapidly attack microbial lipids, proteins, and nucleic acids, thereby efficiently inactivating microbes at low temperatures (Fan & Song, 2020).

Although AOPs are widely used in water treatment, their use in food systems is relatively recent. Studies involving fresh produce and certain grain commodities have reported microbial reductions ranging from 1 to 5 log CFU depending on treatment parameters and commodity characteristics (Abdi et al., 2024; W. Guan et al., 2013; Ho et al., 2020; Murray et al., 2018). Hadjok et al. (2008) observed that synergistic effects between UV-C light and chemical oxidants can enhance microbial inactivation, though such effects are not consistently observed across different products. Importantly, few studies have explored AOPs in low-moisture, lipid-rich systems such as flaxseed, where matrix composition may influence oxidative reactions and treatment outcomes and affect flaxseed's techno-functionality.

Alongside microbial inactivation, AOPs may alter the physicochemical and nutritional properties of food materials. Flaxseed is especially vulnerable to oxidative degradation due to its high content of polyunsaturated fatty acids. Choe & Min (2005) explained that lipid oxidation can produce off-flavours, reduce shelf life, and negatively impact nutritional value. Recent research by Findlay et al. (2023) demonstrated that AOP-treated flaxseed exhibited increased fatty acid levels, indicating lipid hydrolysis and oxidation, as well as subtle structural changes (the presence and nature of C-H bonds) detectable through FTIR analysis. Similarly, Bechlin et al. (2019) found that O₃ exposure accelerated free fatty acid formation in flaxseed. These observations suggest that while AOPs may offer efficient decontamination, treatment parameters must be optimized to minimize undesirable oxidative effects.

Flaxseed's techno-functional properties, including water holding capacity (WHC), oil holding capacity (OHC), emulsion capacity (EC) and stability (ES), protein solubility (PS), and least gelation concentration (LGC), are important properties in the development and formulation of food products. These properties are influenced by the structural composition of proteins, mucilage polysaccharides, lipids, and other flaxseed constituents (Hall et al., 2006; Oomah & Mazza, 1997; Tiwari & Singh, 2012). Oxidative processes may alter these components, potentially affecting hydration behaviour, solubility, emulsification,

and overall techno-functional performance. Given the increasing use of flaxseed as a functional ingredient in a variety of foods, understanding how AOP treatments influence these techno-functional properties is essential. Therefore, comprehensive research is needed to evaluate the effects of AOP decontamination on flaxseed. Such research must address several critical questions, including how much AOPs reduce microbial loads on flaxseed. It must also examine how these treatments influence oxidative degradation and physicochemical attributes. It should also determine whether techno-functional properties such as hydration, solubility, emulsification, and gelation change. It should also evaluate the compositional and structural changes that occur after exposure to AOPs.

The overall objective of this MSc thesis work was to: (1) quantify the changes in techno-functional properties of flaxseed before and after AOP decontamination as well as during storage. The techno-functional properties evaluated included WHC, OHC, EC, ES, LGC, and PS. It was hypothesized that the used H_2O_2 concentrations and exposure times, in combination with O_3 and UV-C radiation will not substantially affect its techno-functional properties before and during storage.

Addressing these research gaps is particularly important, given the growing emphasis on sustainable processing technologies and the need to balance microbial safety with preservation of nutritional and techno-functional properties. A deeper understanding of how AOPs affect flaxseed quality will provide valuable guidance to processors, researchers, and regulators considering the adoption of these technologies. Insights into microbial reduction and techno-functional performance will support the development of optimized AOP protocols that enhance safety while maintaining or improving the compositional and functional integrity of flaxseed. Ultimately, this research will contribute to advancing safe, sustainable, and high-quality flaxseed products within the global food system.

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Bridge to Chapter 2

Herein, we review the literature relevant to advanced oxidative decontamination (AOP) of flaxseed. It covers flaxseed composition, microbial contamination, seed decontamination methods, AOP principles and applications, and the effects of AOP on food quality, with particular emphasis on techno-functional properties. The chapter also discusses the major techno-functional properties of flaxseed and the factors influencing them. Overall, the review provides the scientific foundation for this study, identifies existing knowledge gaps, and establishes the rationale for the research presented in this thesis.

Chapter 2. Literature Review

2.1 Flaxseed and its composition

Flaxseed (*Linum usitatissimum* L.) is a small oilseed with distinct physical features that influence its processing and functional applications. Flaxseed has gained significant attention in recent years due to its exceptional nutritional profile and health-promoting properties. This seed of the flax plant, native to the Mediterranean and West Asia, contains a remarkable concentration of bioactive compounds that contribute to its classification as a ‘superfood’ (Nowak & Jeziorek, 2023; Tarique et al., 2022).

2.1.1 Physical characteristics

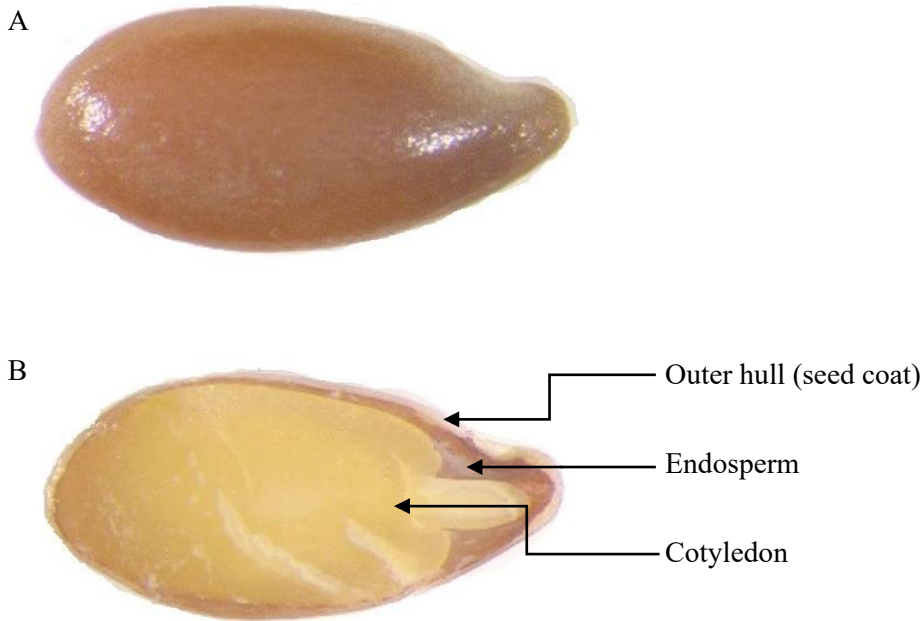


Figure 1. Photomicrographs of a brown flaxseed. A: Outer appearance, B: Longitudinally bisected.

[Adapted from Findlay et al. (2023)]

Flaxseed typically has an oblong and flattened shape, measuring about 4-6 mm in length and 2-3 mm in width. It is comprised of an embryo that has two cotyledons surrounding a thin endosperm; a yellow to dark brown seed coat (hull) with a smooth, hard, and glossy surface (Cullis, 2007; Shim et al., 2014; Troshchynska et al., 2022). The hull of the seed is rich in carbohydrates (Oomah & Mazza, 1997) and has a hard texture due to its higher fibre content. The hull comprises both insoluble fibres, such as cellulose, hemicellulose, and lignin, and soluble fibres, mainly gum polysaccharides. The hull is the source of mucilage, a viscous mixture of water-soluble hydrocolloids, primarily polysaccharides, released when the seed is wetted, causing it to become sticky (Haseeb et al., 2024; Zhang et al., 2023).

Flaxseed is commonly found in two colour varieties: brown and yellow flaxseed (also known as golden flaxseed). Both colour types are similar in nutritional value, though minor differences in pigment and antioxidant content may exist (Abtahi et al., 2022). The seeds feature a unique texture, with a crunchy outer layer and a soft, chewy interior. Their flavour is mild yet distinctive, characterized by a nutty taste (Coşkuner & Karababa, 2007).

In the food industry, the physical properties of flaxseed, such as density and porosity, are crucial for efficient processing, storage, and quality control (Eissa, 2011; Zhang et al., 2023). According to Coşkuner & Karababa (2007) flaxseeds have a linear decrease in bulk density from an average value of 726.6 to 555.6 kg m⁻³ when the moisture level increased from 6.09% to 16.81% (db.). In contrast, the true density and porosity of flaxseeds linearly increased from 1000 to 1111 kgm⁻³ and from 27.34% to 57.44% by increasing seed moisture content from 6.09% to 16.81% on a dry basis.

2.1.2 Chemical composition

The chemical composition of flaxseed, whether in its raw or processed forms, can be affected by factors such as genetics, growing environment, and seed processing. In addition, analysis methods can also affect composition (Bernacchia, 2014; Yang et al., 2021). Various reports describe the chemical

composition of processed forms of flaxseed, such as whole-roasted, defatted, or ground flour. Table 1 presents some of this information.

Table 1. Chemical compositions of different flaxseed forms.

Components	Cultivar	Moisture (db.)	Lipids	Protein	Carbohydrates	Crude fibre	Ash	Total dietary fibre	Insoluble fibre	Soluble fibre	Omega-3 fatty acid (%)	References
Whole Flaxseed (%)	-	6.5	37.1	20.3	28.9	-	-	4.8	-	-	-	Bhatti & Cherdkiatgumchai (1990); S. Hussain et al. (2006); Singh et al. (2011)
	-	7.1-8.3	31.9-37.8	26.9-31.6	-	-	-	36.7-46.8	30	10	-	
	-	7	41	20	-	-	-	28	-	-	-	
	-	6	30-45	20-25	30-35	-	-	10	-	-	-	
	-	-	40-45	24-26	25-27	-	-	-	-	-	-	
	Neelam	6.99 (0.24)	42.41 (1.01)	21.76 (0.58)	26.11 (0.80)	5.71 (0.08)	4.00 (0.25)	-	-	-	54.66 (0.22)	Singh et al. (2012)
	Brown Canadian flax	7.7	41	20	-	-	3.4	28	12.8-17.1	4.3 - 8.6	-	Bernacchia (2014)
Full-fat flour (%)	Chandni	4.53 (0.12)	38.76 (1.28)	21.23 (0.53)	-	-	3.47 (0.13)	8.02 (0.27)	-	-	-	Hussain et al. (2008)
	-	4.50	37	22.4	-	-	-	8.2	-	-	-	Bhatti & Cherdkiatgumchai (1990); S. Hussain et al. (2006); Singh et al. (2011)

2.1.3 Functional components

Flaxseed is a source of proteins, lipids, carbohydrates, and bioactive ingredients. Flaxseed is also a rich source of plant lignans (up to 13 mg/g of flaxseed). The demand for alpha-linolenic Acid (ALA) and lignans as food ingredients has opened up new opportunities for flaxseed to be used in food. However, there has been no similar level of interest observed for other flaxseed components, such as protein and dietary fibre, which account for 20% and 28% of the flaxseed, respectively (Hall et al., 2006).

a) Proteins

Flaxseed protein content ranges from 20 to 30%, and most of it consists of two storage proteins: globulins and albumins. Approximately 80% of the total proteins are globulins (linin and conlinin), which are 11-12S type proteins that are salt-soluble and have higher molecular weights. Albumins are 1.6-2S type proteins that are not salt-soluble and have low molecular weights (Bernacchia, 2014; Hall et al., 2006). Non-protein nitrogen in the seed contributes to 21.7% of the total nitrogen (Care, 1954). In the past five years, Canadian flaxseed has been found to have a crude protein content of approximately 23%, indicating that the protein content of the meal after oil extraction is 43-46% (Duguid, 2010). The protein content of flaxseed that has been hulled or defatted can vary considerably, depending on the cultivar, growing location, and seed processing. It has been reported that the hull fraction contains relatively less protein; therefore, dehulling increases the protein content of flaxseed from 19.2% to 21.8% (Oomah & Mazza, 1997). Flaxseed is regarded as a highly desirable protein source because it contains substantial amounts of sulfur-containing amino acids like cysteine and methionine, which most pulses are limiting in (Tiwari & Singh, 2012), essential amino acids such as tyrosine, threonine, and lysine, as well as branched-chain amino acids including leucine, isoleucine, and valine (Mueed et al., 2022).

b) Lipids

Flaxseed is an oilseed crop with an oil content of roughly 38–45%, which depends on various factors, e.g., cultivar. According to Singh et al. (2011), the nutritional value of flaxseed is primarily influenced by its lipid component, because it has a favorable ratio of polyunsaturated, monounsaturated, and saturated fatty acids. The cotyledons contain the majority of the lipid (~75%), with only ~22% and ~3% allocated to the seed coat and embryo, respectively (Bekhit et al., 2018). The lipid composition of flaxseed oil is low in saturated lipids (9% of total fatty acids), with moderate levels of monounsaturated lipids (18%) and high levels of polyunsaturated lipids (73%) (Cunnane et al., 1993). In oil, ALA, a polyunsaturated lipid, constitutes 52% of the fatty acids. ALA is an omega-3 fatty acid with numerous health benefits.

c) Carbohydrates

Flaxseed is low in digestible carbohydrates (sugars and starch), and the majority of its carbohydrates are resistant to digestive enzymes. Flaxseed is a source of both soluble and insoluble dietary fibre, containing a ratio of 20:80 to 40:60 of soluble to insoluble fibre. Cellulose and lignin are the major insoluble fibre fractions, while mucilage gums are the soluble fibre fractions (Oomah & Mazza, 1997; Shahidi & Naczki, 2003). Xylose is the main component in the neutral fraction of flaxseed polysaccharides, while rhamnose is the main component in the acidic fraction, followed by galactose (Hall et al., 2006). Flaxseed mucilage is a great source of dietary fibre and because of its highly viscous nature, it could also be utilized as a food gum (Diederichsen et al., 2006; Duguid, 2010).

d) Phenolic compounds

Flaxseed contains phenolic compounds, which are classified as bioactive compounds including lignans, flavonoids, and phenolic acids. (Bernacchia, 2014). The amount of phenolic compounds in flaxseed is influenced by cultivar and environmental factors (Bekhit et al., 2018). These compounds are often associated with various health benefits, particularly because of their antioxidant activity. Polyphenols,

including phenolic acids and flavonoids, have therapeutic properties that can reduce inflammation, reduce thrombosis, prevent allergies, and provide many other health-related benefits (Bekhit et al., 2018).

Flaxseeds are the most abundant source of lignan precursors in the human diet. The majority of them are located in the seed's outer coat (Bekhit et al., 2018). According to Johnsson et al. (2000), flaxseed dietary lignans have great potential as an ingredient in functional foods. Many studies have recently focused on these lignans, which are powerful antioxidant substances found in flaxseed and also other plants.

2.2 Contaminations in flaxseed

Flaxseed is susceptible to several types of contamination, including chemical, physical, genetic, and microbial. These contaminants can occur during different phases of crop production, such as pre-harvest and post-harvest, including storage and processing. These contaminants can pose health risks and compromise the seed's functional and nutritional integrity.

2.2.1 Chemical contaminations

Chemical contamination of flaxseed can be caused by pesticide, herbicide and fungicide residues, heavy metals, or naturally occurring cyanogenic glycosides.

Flaxseed crops may come in contact with various agrochemicals, such as herbicides, fungicides, and insecticides, whose accumulation in flaxseed can lead to chemical contamination. While flaxseed is considered a low-input crop, pesticide use or drift from neighboring fields can result in residue accumulation (Cui et al., 2022). Nonpolar pesticides can be particularly harmful in flaxseed and other fatty foods due to their greater solubility in lipids (Koesukwiwat et al., 2010).

Heavy metals build up in plant tissue as plants absorb higher amounts of heavy metals from soil that has been contaminated. Environmental factors such as soil composition and industrial pollution may

lead to the accumulation of heavy metals, including cadmium, lead, and arsenic, in flaxseed (Dhall et al., 2022; Zainab et al., 2020). According to Saleem et al. (2020), for over 40 years, flaxseed has been used in research to investigate the removal of toxic substances, specifically heavy metals, when grown in soils contaminated with heavy metals. Therefore, it is evident that flaxseed can be contaminated by heavy metals through bio-accumulation (Angelova et al., 2004).

Although flaxseed has numerous health and culinary benefits, it may be contaminated with toxic compounds at high levels, which could affect nutrient bioavailability and impair health. Flaxseed contains components that are also considered antinutrients, including cyanogenic compounds, particularly cyanogenic glycosides. These toxic compounds can degrade into toxic hydrogen cyanide (HCN) upon ingestion. While typical dietary intake of flaxseed poses minimal risk, excessive consumption of raw or unprocessed flaxseed can exceed safe thresholds for cyanogenic glycosides (Bekhit et al., 2018; Kobaisy et al., 1996; Schrenk et al., 2019).

2.2.2 Physical contaminations

Physical contaminants, though less discussed in scientific literature, can include extraneous matter such as plant debris, stones, metal fragments, and insect parts (Dzuovor et al., 2018). These contaminants are usually caused by poor post-harvest handling and inadequate cleaning or sorting systems (Osei-Kwarteng et al., 2024).

2.2.3 Genetic contaminations

Conventional flaxseeds can be contaminated with genetically engineered flaxseeds through various mechanisms, such as seed contamination and cross-pollination in the field. In the past decade, several countries and trade unions have implemented regulatory systems to control the production and consumption

of genetically modified (GM) agricultural crops (Jackson & Anderson, 2005). With regulatory measures in the EU, Japan, and Brazil, the presence of GM flaxseed in Canadian flaxseed shipments was detected, and as a result, the volume of Canadian flaxseed shipped to Europe during the 2009-2011 period significantly dropped (Jhala et al., 2011; Singh et al., 2022). After 2011, Canadian flaxseed shipments to the EU market remained below previous levels (Government of Canada, 2024).

2.3 Microbial contamination in flaxseed

Microbial contamination is the most important concern for flaxseed, particularly when used in raw or minimally processed food products (Findlay et al., 2023). Flaxseed, like other oilseeds, can harbor a wide range of microorganisms, including bacteria, yeasts, and molds. These contaminants can be introduced during harvesting, for example through soil contact, handling, or storage, particularly in humid or unclean conditions (Christensen & Meronuck, 1986; Sparks, 2021). Although flaxseed is a low-moisture oilseed, it can harbor both microbial pathogens and spoilage microorganisms. In its raw form, flaxseed may contain enteric and environmental microbes that may pose food safety and quality risks (FSAI, 2022; M. K. Shah & Bergholz, 2016). Raw flaxseed is frequently found to have high microbial loads, as evidenced by a survey that found uncleaned seeds with 5.7 log CFU/g total aerobic bacteria, 3.6 log CFU/g yeasts, and 3.0 log CFU/g molds (Vegi et al., 2016). These microorganisms can persist because of poor cleaning and drying. Flaxseed products have been found to show spikes in *Salmonella* and *Escherichia coli*, while even non-pathogenic coliforms tend to increase in contaminated batches. Flaxseed's low water activity ($a_w \approx 0.3-0.5$ when dry) typically inhibits microbial growth, but vegetative microbes can grow if moisture increases, including through high humidity or rehydration (M. K. Shah & Bergholz, 2016; Tamber et al., 2016b).

- **Pathogenic bacteria:** *Salmonella* is the most common pathogen in recalls for low-moisture foods such as flaxseed. Flaxseed-based products have been consistently linked to *Salmonella* outbreaks, resulting in recalls of milled flaxseed due to *Salmonella* Typhimurium. Shiga -toxin-producing *E. coli* is also of concern; although less common, a few outbreaks have been linked

to low-moisture foods. Even though other enteric bacteria (like *Campylobacter* and *Listeria*) do not usually grow on dry flaxseed, they can still cause contamination in mixed-ingredient products (FSAI, 2022; M. K. Shah & Bergholz, 2016).

- **Spoilage bacteria:** Oilseeds often contain *Bacillus* species, including *B. cereus*, and other spore-forming bacteria whose spores can survive drying and heat. If flaxseed absorbs moisture (e.g., during processing), *B. cereus* can germinate, grow, and even produce toxins. These bacteria are largely dormant when stored at low water activity, but improper humidity control can lead to bacterial aerobic spoilage (Shah & Bergholz, 2016).
- **Yeasts and molds:** Low water activity in dry flaxseed prevents the growth of most yeasts and molds. However, mold growth and spoilage can occur even with minor moisture uptake from humid air or partial hydration, leading to visible pellicles and musty odors. Mold growth can also lead to rancidity by increasing lipid oxidation (Rollemberg et al., 2022; Sperber, 2009). According to a study conducted by Rollemberg et al. (2022), potential mycotoxin producers, such as *Aspergillus* spp. and other xerotolerant molds (*A. cibarius*, *A. appendiculatus*, and *A. amstelodami*), have been discovered on flaxseed.
- **Indicator organisms:** High counts of coliforms or *Enterobacteriaceae* can indicate flaxseed contamination. For instance, the levels of coliforms and *Enterobacteriaceae* were higher in contaminated flax powder samples from a 2013-2014 *Salmonella* outbreak than in clean samples (Tamber et al., 2016b). Thus, routine measurements of coliforms and total aerobic counts are used to indicate the hygiene level of a seed lot (Podravac et al., 2018).

2.3.1 Importance of microbial decontamination for flaxseed

Flaxseed faces significant post-harvest challenges from microbial contamination, which compromises both seed safety and quality. Effective decontamination strategies are necessary to maintain quality, prevent foodborne illnesses, and comply with regulatory standards.

Microbial contamination poses health risks because flaxseed is commonly consumed raw or minimally processed. *Salmonella* infection from contaminated flaxseed can cause gastroenteritis (with diarrhea, fever, and cramps), which is often more severe in vulnerable individuals, including the elderly, infants, and those with impaired immune systems (FSAI, 2022). Moreover, fungal infections in flaxseed can produce harmful mycotoxins, which can threaten consumers' health. Therefore, it is important to implement microbial decontamination strategies for flaxseed before it is used for human and animal consumption (Silva et al., 2022).

Not only pathogenic microorganisms like *Salmonella* spp. and *E. coli*, but also spoilage microflora such as mold and yeasts can occur in flaxseed during processing and storage, where water activity may increase and result in spoilage of flaxseed (Vegi et al., 2016). Lipid oxidation is accelerated by microbial activity, increasing peroxide values and free fatty acids, which degrade nutritional quality (M. Shah et al., 2018). Furthermore, higher microbial counts can affect seed germination and other seed quality characteristics (Findlay et al., 2023). High levels of aerobic microorganisms (>6 log CFU/g) and fungi (>4 log CFU/g) were observed in a study conducted by Silva et al. (2022) for flaxseed, highlighting the need to improve processing, storage, and distribution practices for edible seeds and their derivatives, as well as to implement effective decontamination methods.

2.4 Seed decontamination methods

Microorganisms associated with food spoilage and foodborne diseases pose significant threats to seed quality and consumer health. The use of effective decontamination methods for seeds is essential to ensure food safety and enhance the agricultural productivity of seed-derived food products (Akinsemolu & Onyeaka, 2024; NACMCF, 1999). A range of conventional and non-conventional methods have been explored for seed decontamination, which can be further categorized into chemical and non-chemical approaches.

2.4.1 Conventional methods

a) Chemical methods

Conventional chemical methods for seed decontamination are widespread because of their efficacy and ease of application. Several commercially available chemical compounds, such as chlorine and its derivatives, offer cost-effective and convenient alternatives to more expensive, complex, and less reliable methods that may require continuous monitoring of the decontamination process (Chaidez et al., 2012). Therefore, chlorine-based compounds are the most widely used microbicidal halogens for disinfection purposes (Abdi et al., 2024; Gilbert et al., 2023; McDonnell & Russell, 1999). The primary conventional chemical sanitizer used in the food industry is sodium hypochlorite (NaOCl , bleach) because of its affordability, availability, longstanding use, and excellent cleaning ability. Gilbert et al. (2023) showed that NaOCl solutions (such as 10% bleach for 15 minutes of exposure) can decrease the bacterial load on seeds by more than 3 logs, without significantly affecting germination rates across a variety of seeds. The concentration of chlorine and the solution pH are key factors in determining NaOCl 's effectiveness in cleaning and disinfecting processes (Fukuzaki, 2006). Another widely used chlorine-based compound for decontamination is calcium hypochlorite [$\text{Ca}(\text{OCl})_2$]. The Food and Drug Administration (2022) recommends seed disinfection with $\text{Ca}(\text{OCl})_2$ at 20,000 ppm for seeds intended for sprouting. Nevertheless, chlorine produces toxic byproducts that are carcinogenic and mutagenic, which raise concerns about

environmental impact and worker safety, resulting in the need for better alternative seed decontamination techniques (Ding et al., 2013; McDonald & Komulainen, 2005).

b) Non-chemical methods

Food processors have used conventional non-chemical methods, particularly heat (hot water, aerated steam, or dry heat) and high-pressure treatments, for microbial inactivation. Unlike conventional chemical treatments, these methods are effective on pathogens sheltered in scarified surfaces and the interior of seeds, as physical treatments are more effective at reaching bacteria in those areas due to their better penetration characteristics (Ding et al., 2013). In support of this statement, Ding et al. (2013) reported that heat treatments can achieve approximately a 3.76 log CFU/g reduction in microbial load. Among the available physical and chemical seed disinfection methods, high-pressure treatment was identified as the most effective, achieving a 5.09 log CFU/g microbial reduction, compared with 20,000 ppm calcium hypochlorite, which achieved an average of 3.08 log CFU/g. These methods are also more environmentally friendly than conventional chemical methods. Heat treatment has been the primary alternative to chemical treatment, particularly in Japan (Jiang et al., 2006). Various seeds subjected to dry heat for hours or days showed varying degrees of disinfection (Ding et al., 2013). For example, according to Bari et al. (2009), *Escherichia coli* O157:H7 numbers were reduced to below detectable levels in radish, broccoli, and alfalfa seeds by applying dry heat treatment (50 °C) for 24 hours. However, using the same treatment, the pathogen count was not reduced to below the detectable level in mung bean seeds. In another study on mung bean seeds, Hu et al. (2004) reported that after heat treatment at 55 °C, no pathogenic bacterial populations were detected (<1 log CFU/g). Alfalfa seeds inoculated with five *Salmonella* strains or *E. coli* O157:H7 and exposed to dry heat at 55 °C for up to 8 days achieved a 5-log microbial reduction. According to Koch & Roberts (2014), hot water treatment can be utilized to effectively treat a variety of crops, with protocols tailored to specific seed species to optimize pathogen reduction without compromising germination.

High pressure also appears to be a promising method for seed disinfection. At room temperature, alfalfa seed treatment at 500-600 MPa for 2 minutes reduced the bacterial load by 3.50 log CFU/g or more, as reported by Neetoo et al. (2008). However, there are a limited number of studies that have been conducted to determine the efficacy of these methods.

2.4.2 Non-conventional methods

a) Chemical methods

In the food processing industry, commonly used non-conventional chemical agents for seed decontamination include hydrogen peroxide (H_2O_2), organic acids (e.g., vinegar, peroxyacetic acid, and fatty acid), and ethanol (Beuchat, 1997; Buchholz & Matthews, 2010; Chang et al., 2010; Gilbert et al., 2023; Lang et al., 2000). As alternatives to conventional chemical treatments such as NaOCl, Beuchat, (1997) demonstrated that treating alfalfa seeds with 6% H_2O_2 or 80% ethanol for 10 minutes reduced *Salmonella* populations by more than 1000-fold. Ölmez (2010) studied the antimicrobial activity, the effect of incubation times and temperature on the efficacy of ozone treatment against *E. coli* on lettuce. Ölmez (2010) reported the lowest reduction in *E. coli* after incubation at 22 °C for 18 h. In addition, no significant differences ($P < 0.05$) were observed between lettuce samples treated with ozone and stored at 4 °C for 18 h compared with those incubated at 22 °C for 6 h. Kim et al. (1999) reported that exposing shredded lettuce to 1.3 mM bubbling ozone (~62 ppm) in water for 3 minutes resulted in reductions of 3.9 and 4.6 log CFU in microbial counts in a separate lettuce batch after 5 minutes of treatment. However, the effectiveness of these chemical agents is often limited by their adverse effects on seed germination and quality, particularly at higher concentrations or during prolonged exposure. Therefore, these treatments are less widely adopted than the use of hypochlorite solutions for disinfection (Ding et al., 2013).

b) Non-chemical methods

Non-conventional non-chemical methods include advanced physical and biotechnological approaches such as irradiation, cold plasma, and advanced oxidative processes (AOPs) (Chandrasekaran et al., 2013; Ding et al., 2013; Fan & Song, 2020; Waskow et al., 2018).

I. Advanced Oxidative Processes (AOP)

Advanced oxidative processes (AOPs) combine promising advanced technologies for seed disinfection. AOPs can effectively generate hydroxyl radicals ($\text{OH}\bullet$), which are powerful, non-selective oxidizers (Wang et al., 2017).

II. Principle

Advanced Oxidative Processes (AOPs) generate highly reactive species, primarily hydroxyl radicals, to inactivate microbes and degrade organic contaminants (Fan & Song, 2020; Phornvillay et al., 2022). These processes can be initiated using combinations of ultraviolet (UV) light, pulsed light, H_2O_2 , ozone, or cold plasma (Deng et al., 2020; Devi et al., 2017)

III. Mechanism of AOP decontamination

Various AOPs have been studied; among the most commonly used are ozone and H_2O_2 , as well as those that use photolytic processes such as UV or pulsed light. In the presence of UV or pulsed light, ozone and H_2O_2 generate hydroxyl radicals through several processes, including photolysis and photo-ozonation (Pera-Titus et al., 2004). Photolysis is a chemical process in which molecules are broken down into smaller units, generally into atoms, radicals, or simpler molecules, by the absorption of light in the form of photons (Steinberg & Paul, 2008). Photo-ozonation combines ozone with UV radiation to initiate AOPs that effectively degrade contaminants. When ozone absorbs UV light, it undergoes photolysis, forming reactive oxygen species (ROS) such as hydroxyl radicals and superoxide anions ($\text{O}_2\bullet^-$). These radicals exhibit a

substantially higher oxidation potential than either ozone or UV treatment alone, allowing them to non-selectively attack and decompose a broad spectrum of organic compounds and microbial contaminants (Mehrijouei et al., 2015; Pera-Titus et al., 2004). Hydroxyl radicals ($\text{OH}\bullet$), the neutral form of the hydroxide ion (OH^-), are unstable and highly reactive, rapidly reacting with other substances, including themselves and those in their environment. With a redox potential of 2.80 V, hydroxyl radicals are among the most reactive oxygen-containing chemical species. Oxidation occurs when the hydroxyl radical, with its unpaired electron, strips an electron from other substances.

In the context of microbial decontamination, due to their high reactivity, short-lived hydroxyl radicals are more likely to react with the lipid bilayer of microbial cell membranes, especially with sulfhydryl groups in proteins and double bonds in lipids, before reaching DNA or organelles of a cell. Cell death occurs when the major components of the cell membrane are oxidized (De La Paz & Anderson, 1992; Fan & Song, 2020; Gowenlock et al., 1961).

2.5 AOP used in food decontamination

The application of AOPs for microbial inactivation on food products has been explored in only a limited number of studies. Most AOP research focuses on disinfecting drinking water and treating wastewater. Earlier studies applied AOPs in the aqueous phase as a wash on fresh produce to reduce populations of human pathogens and other bacteria. Compared with a single treatment, it was found to offer a limited set of advantages. For example, the effectiveness of 1% H_2O_2 , 100 ppm chlorine, and 1 ppm aqueous ozone as single chemical oxidants was evaluated for reducing *Enterobacter agglomerans* and *Pseudomonas fluorescens*, common spoilage bacteria on lowbush blueberries. As AOPs, a combination of 1% H_2O_2 / UV, 100 ppm chlorine/ UV, and 1 ppm ozone/ 1% H_2O_2 / UV was used. The combined aqueous H_2O_2 and ozone with UV produced minimal log CFU reductions in the spoilage bacteria, and its effect was comparable to that of the individual treatments. UV light and H_2O_2 were used by Xie et al. (2008) to

inactivate bacteriophage MS2 inoculated onto iceberg lettuce. Again, no significant reduction in phage was observed when H₂O₂ was applied simultaneously with UV light.

In contrast, Guan et al. (2013) reported that inhibiting lesion development in button mushrooms was more effective with a combination of H₂O₂ and UV-C than with either treatment alone, indicating the effectiveness of AOPs in reducing the microbial load of button mushrooms during storage. In another study, the use of UV and H₂O₂ reduced the populations of human pathogens on lettuce, spinach, broccoli, cauliflower, onion, and tomato (Hadjok et al., 2008). In this study, *Salmonella*-inoculated samples were exposed to UV light during H₂O₂ spraying. H₂O₂ concentration, treatment temperature, and time had significant impacts, whereas UV light intensity was less important. A similar study used aqueous H₂O₂ and UV-C at temperatures above ambient to reduce *E. coli* O157:H7 populations and degrade chlorpyrifos on apples. The UV-C dose was the primary factor influencing the degradation of chlorpyrifos on apples, and combining 6% v/v H₂O₂ at 66 °C synergistically enhanced the UV-C effect. The population of *E. coli* O157:H7 was reduced by >6.6 log CFU, and the *Aspergillus niger* spores were reduced by >4.7 log CFU after the same combined treatment, indicating the importance of AOP treatment temperature for the effectiveness of UV/H₂O₂ combinations (Ho et al., 2020a).

Using water-assisted pulsed light/UV systems to wash berries/fresh produce in an H₂O₂ solution and to irradiate them with pulsed light or UV light to inactivate *Salmonella* was evaluated by Guo et al. (2017) and Huang et al. (2015), respectively. The combination of pulsed or UV light with 1% H₂O₂ was effective in reducing *Salmonella* populations on fruits, but the increased efficacy was not always significantly greater than single treatments. The limited effectiveness of any aqueous treatment may be due to water tension preventing aqueous solutions from reaching protected sites (Fan & Song, 2020). As a result, gaseous and aerosolized AOP systems have been created to increase the effectiveness of hydroxyl radicals in recent years (Fan & Song, 2020; Murray et al., 2018).

Abdi et al. (2024) aimed to explore the potential of utilizing UV-C radiation, H₂O₂, and ozone as a low-moisture process compared with aqueous chemical wash processes to reduce fungal and total aerobic

contamination on the surface of wheat kernels. The research showed that H₂O₂-UV-ozone treatment can reduce the microbial load on the surface of wheat kernels. In the study, this combined treatment achieved a maximum efficacy of a 1.3-log CFU reduction in the total aerobic count.

In contrast to the previous studies that used aqueous ozone or liquid H₂O₂, Murray et al. (2018), applied a combination of gaseous ozone, vaporized H₂O₂, which was produced using a vaporizing unit, and UV-C to caramel apples. This approach differs in that it relies on gas-phase and vapour-phase interventions rather than liquid-phase application, potentially enhancing the antimicrobial efficacy by enabling higher hydroxyl radical generation and broader penetration (Guan et al., 2021; Murray et al., 2017). Murray et al. (2018) conducted their experiment using an AOP reactor operated with UV-C light (54 mJ cm⁻²), 6% (v/v) H₂O₂, 23 ppm ozone, and a chamber temperature of 48°C, achieving a 3 log CFU reduction of *Listeria monocytogenes* on both the surface and internally within the scar tissue of the apples.

The findings of Fan et al. (2020) indicated that ozone alone was capable of reducing *Salmonella* populations by around 0.6 log CFU/fruit in both the smooth surface and the stem scar area. The pathogen population on the smooth surface was reduced by 2.1 log CFU/fruit after exposure to aerosolized H₂O₂ (produced using an atomizer) alone, while reducing by 0.8 log CFU/ fruit on the stem scar area. In contrast, with AOP, the population decreased by as much as 5.2 log CFU/fruit on the surface and 4.2 log CFU/fruit on the stem scar.

Although AOPs can inactivate human pathogens on food products, it is essential to determine whether these processes negatively affect the sensory and nutritional quality of food products. Furthermore, the treatments can induce the formation of undesirable chemical byproducts. The hydroxyl radicals produced in AOPs can react with microorganisms and food components. The interaction of hydroxyl radicals with microorganisms has a positive impact, but interactions with foods can negatively affect food quality, such as reactions of hydroxyl radicals with nutrients that produce undesirable chemical compounds (Choe & Min, 2005; Fan & Song, 2020).

2.6 AOP decontamination in food matrices

Initially, AOPs were developed and widely used primarily to degrade persistent organic contaminants in industrial effluents and drinking water, including polychlorinated biphenyls (PCBs), dioxins, per- and polyfluoroalkyl substances (PFAS), organochlorine pesticides, and polycyclic aromatic hydrocarbons (PAHs). Early AOP research therefore focused mainly on disinfection and pollutant removal from aqueous systems such as drinking water and wastewater. However, in recent years, the application of AOPs in the food sector has received increasing attention, particularly as a non-thermal, environmentally sustainable decontamination approach (Ağagündüz et al., 2025; Fan & Song, 2020; Findlay et al., 2023; Ho et al., 2020). Despite this interest, relatively few studies have explored their direct application for the inactivation of microorganisms, degradation of chemical residues, or removal of mycotoxins in complex food matrices, including seeds, grains, and fresh produce (Fan et al., 2020; Hasani et al., 2020; Ho et al., 2020b; Xie et al., 2008).

2.6.1 Effects of AOP decontamination on food quality parameters in food matrices

The generation of hydroxyl radicals ($\bullet\text{OH}$) during AOP reactions plays a key role in microbial inactivation. These radicals exhibit extremely high oxidation potential, allowing rapid degradation of microbial cell walls, membranes, and genetic material (Premjit et al., 2022). However, the same reactivity that supports microbial inactivation can also lead to unintended reactions with food components, potentially compromising product quality (Fan & Song, 2020).

The quality of fresh produce and other food matrices is defined by multiple interrelated parameters, including physicochemical properties, sensory attributes (such as appearance, texture, and flavour), nutritional composition, and overall shelf-life stability. These characteristics collectively determine consumer acceptance and marketability (Barrett et al., 2010). While numerous studies have investigated the individual effects of UV radiation, ozone, or hydrogen peroxide on food quality (Bechlin et al., 2019; Byun et al., 1997; Joanna Brodowska et al., 2014; Kamber et al., 2017), only a limited number have examined

the impact of AOPs combining these treatments (Crowe et al., 2007; Fan et al., 2020; Findlay et al., 2023; Hasani et al., 2020; He et al., 2024). Such studies are critical to understanding how AOP exposure influences physicochemical properties, nutritional composition, sensory attributes, shelf life, and oxidative stability.

a) Physicochemical properties

Numerous studies have investigated the effects of individual UV radiation, ozone, or hydrogen peroxide treatments on food matrices, highlighting physical and chemical modifications as well as alterations in macromolecules such as starch, lipids, and proteins (Pandiselvam et al., 2019). However, research on the effects of AOP treatments on the physicochemical properties of food matrices remains limited.

It has been demonstrated that Findlay et al. (2023), that AOPs have the ability to alter flaxseed's physicochemical characteristics. Specifically, ozone and UV-based AOPs can cause fatty acid values (FAV) to increase and shifts in chemical profiles, primarily due to accelerated lipid oxidation, especially during storage, as demonstrated by Findlay et al. (2023). Even though not an AOP, similar findings were reported by Bechlin et al. (2019), who observed that higher ozone concentrations and longer exposure times increased free fatty acid levels in flaxseed, suggesting oxidation-induced degradation of lipid fractions. Fourier-transform infrared spectroscopy (FTIR) analysis conducted by Findlay et al. (2023) further indicated that flaxseed may be able to resist changes in the molecular structure of lipids. This effect was particularly evident in yellow flaxseed, which is rich in olefins (unsaturated hydrocarbons containing at least one carbon-carbon double bond), α -linolenic acid (ALA), and other unsaturated compounds. These compounds are suggested to play a role in mitigating lipid oxidation and oxidative damage, with supporting evidence from the study reported by Mohanan et al. (2018). Furthermore, Findlay et al. (2023) proposed that the presence of olefins may be associated with other antioxidant systems that contribute to the protection of flaxseed lipids against oxidation. In addition, Findlay et al. (2023) suggested that, despite

being effective for decontamination, AOPs can reduce germination rates and have a subtle effect on physical integrity over time.

In another study, He et al. (2024) reported that combined UV and H₂O₂ treatments notably altered the physicochemical properties of fucoidan, a sulfated polysaccharide with a backbone of fucose sugar units from brown algae. The study used varying exposure times (0 to 180 min), a final H₂O₂ concentration of ~3400 ppm, and a UV irradiation dose of 6500 mJ/cm². The fucoidan sample, degraded for 90 minutes at a UV intensity of 1.2 mW/cm², exhibited the strongest antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. Prolonged exposure (up to 180 min) reduced its molecular weight from 530.7 kDa to 3.4 kDa, indicating strong depolymerization. Total sugar and uronic acid contents in fucoidan decreased by 23.7% and 28.0%, while sulfate content increased by 6.6%, possibly enhancing the bioactivity of fucoidan. Protein levels were mostly unchanged. Monosaccharide analysis showed fucose and galactose as main components, with increased fucose and mannose and reduced galactose and glucuronic acid after treatment. The decrease in total sugar content consequently led to a relative increase in sulphate groups, which are considered a key factor contributing to the biological activities of the degraded fucoidans. The pH dropped from 6.69 to 2.89, indicating acidification during fucoidan degradation. FTIR analysis confirmed that sulfate groups, uronic acids, and β -glycosidic linkages were retained, suggesting the main fucoidan structure remained unchanged. Overall, UV-H₂O₂ treatment modified fucoidan's composition and improved its solubility, which indicates effects to its alterations in physicochemical properties.

b) Nutritional composition

Research on the nutritional implications of AOP treatments has primarily focused on oxidation-sensitive nutrients, such as vitamins and phytochemicals, rather than on macronutrients. Allende et al. (2007) examined the combined effects of UV-C light (dose 100 mJ/cm²) and gaseous ozone (5000 ppm) on the polyphenol and vitamin C content of strawberries. The findings showed a significant reduction in total

phenolic content after the combined treatment. Furthermore, when applied individually, gaseous ozone markedly decreased vitamin C levels after 12 days of storage at 2 °C, compared with untreated or UV-C-treated samples. These results suggest that while AOP oxidants are effective at microbial inactivation, their oxidative strength can compromise sensitive nutrients in food matrices.

Li et al. (2019) reported that AOP combining UV irradiation (intensity 0.35 mW/cm²) with different ozone concentrations (3 to 7 ppm) at varying exposure times (10 to 60 min) has been shown to preserve the nutritional quality of peanuts. Under optimal conditions of 5 ppm ozone and 30 min UV exposure (dose 630 mJ/cm²), the polyphenol content remained largely unaffected, and only minimal changes were observed in acid and peroxide values, indicating limited lipid oxidation. According to Byun et al. (1997), high ozone concentrations (~9.2 ppm) or prolonged exposure (480 min) can accelerate fatty acid oxidation of aloe powders. Furthermore, studies on other foods, particularly dried red peppers, similarly suggest that low ozone doses (40 ppm) or short exposure times (20 min) are more effective for aflatoxin B₁ detoxification and cause less deterioration in product quality compared to higher ozone levels (80 ppm) (Kamber et al., 2017). Overall, these findings highlight the importance of optimizing AOP conditions to balance decontamination efficacy with nutrient preservation across different food systems.

c) Sensory attributes

Although AOPs are highly effective for microbial inactivation, some studies have indicated that these treatments may induce undesirable changes in sensory attributes, particularly after extended storage periods. Such alterations, including reduced seed discolouration and texture degradation, which are often associated with oxidative reactions, especially lipid oxidation, which can compromise visual and structural integrity (Findlay et al., 2023). Despite these potential drawbacks, only a few studies have systematically examined the influence of AOPs on sensory quality, such as visual appearance, colour, texture, and overall

acceptability (Allende et al., 2007b; Gutiérrez et al., 2017; Hadjok et al., 2008; Hasani et al., 2020; Xie et al., 2008).

Gutiérrez et al. (2017) investigated rocket leaves treated with UV-C (dose 500-2000 mJ/cm²) and ozone (1-5 ppm) and found no significant differences in lightness (L*), chroma (C*), or hue values between AOP-treated and control samples throughout storage. Similarly, Hadjok et al. (2008) reported that the visual appearance of combined UV and H₂O₂-treated iceberg lettuce inoculated with bacterial suspensions remained comparable to that of untreated controls. Consistent observations were made by Xie et al. (2008), who treated iceberg lettuce inoculated with MS2 F(+) coliphage (an RNA virus that infects specific *E. coli* strains and serves as a safe model indicator for viral contamination studies) using UV (intensity 0.632 mW/cm²) and 2% (v/v) hydrogen peroxide (initial concentration 20000 ppm). No visible differences were observed after treatment and storage. Allende et al. (2007) also reported that the combined application of UV-C light (dose 100 mJ/cm²) and gaseous ozone (5000 ppm) did not result in significant changes in the sensory quality of strawberries, suggesting that controlled exposure can preserve visual and textural attributes.

Hasani et al. (2019), evaluated an AOP combining hydrogen peroxide (10,000 to 60,000 ppm), UV-C (intensity 0.6-3.6 mW/cm²), and exposure time (20-60 s) for the inactivation of mold spores and bacterial pathogens on lemons. Lemons subjected to prolonged exposure and higher hydrogen peroxide concentrations exhibited surface browning and loss of firmness during 14 days of storage. However, the firmness changes were mainly attributed to moisture loss. Significant differences were found between AOP-treated and untreated lemons in terms of firmness, indicating minimal impact of AOP on textural quality. Hasani et al. (2020) further investigated the use of AOP for the inactivation of *Listeria monocytogenes* and spoilage molds associated with grapes. Excessive treatment conditions, including UV-C doses above 129 mJ/cm² or hydrogen peroxide concentrations exceeding 40000 ppm, caused surface blistering and darkening after 14 days of storage. However, an optimized AOP treatment consisting of 13000 ppm hydrogen peroxide (5 mL per 10 berries), 9 mg ozone gas, and a UV-C dose of 123 mJ/cm² (10 s exposure

at 12.3 mW/cm² intensity) effectively maintained grape colour and firmness. Sensory panel evaluations revealed no significant differences between AOP-treated and untreated grapes, although a few panellists reported subtle flavour variations, suggesting that the optimized AOP treatment can maintain sensory attributes while achieving microbial inactivation.

d) Shelf life and oxidative stability

The influence of AOPs on shelf life and oxidative stability has been examined primarily in fresh produce and also pre-made products. Xie et al. (2008) studied the effect of temperature on AOP treatment effectiveness, and observed that iceberg lettuce treated with a combined UV and H₂O₂ treatment consisting of 10 s exposure to hydrogen peroxide (approximately 1.5 ppm concentration in the reaction chamber) and 20 s of UV exposure (intensity 0.632 mW/cm²) showed no visible differences immediately after treatment compared to controls. However, after six days of storage at 4 °C under aerobic conditions, samples treated at 10 °C or 25 °C displayed noticeable discolouration, whereas samples treated at 50 °C remained visually unchanged. Similarly, Hadjok et al. (2008) observed that lettuce inoculated with *Salmonella* Montevideo or *Escherichia coli* O157:H7 and treated with UV (dose 37.8 mJ/cm²) and hydrogen peroxide (15000 ppm at 50 °C) showed no significant differences in visual quality after storage at 4 °C or 25 °C compared to untreated controls.

In a more recent application, Murray et al. (2018) an AOP combining 60000 ppm hydrogen peroxide, ozone (23 ppm concentration at the treatment bed), and UV-C light (dose 54 mJ/cm²) was employed during the caramel apple-making process to reduce *Listeria monocytogenes* contamination on the surface and scar tissue of apples. AOP-treated samples exhibited low residual *L. monocytogenes* levels on both the surface and internal tissues. Furthermore, no outgrowth was observed during a 19-day storage period at 22 °C. In contrast, caramel apples prepared from untreated control fruits exhibited *L. monocytogenes* growth within

the core but not on the surface, indicating that AOP treatment reduced microbial growth and extended product shelf life.

2.7 Techno-functional properties of flaxseed

2.7.1 Definition and importance of techno-functional properties in oilseeds

Techno-functional properties refer to the physicochemical attributes that influence the behaviour of macromolecules within food systems throughout processing, storage, preparation, and consumption (Bessada et al., 2019; Damodaran & Parkin, 2017). Although proteins are often the primary contributors, other biomolecules, including carbohydrates and lipids, can significantly influence techno-functionality through their inherent chemical characteristics and interactive capabilities (Garcia et al., 2018; Kiskini et al., 2016). Techno-functional properties are governed by both intrinsic and extrinsic factors. Intrinsic factors influencing techno-functionality include elements of physicochemical properties such as macromolecular composition, particle size, molecular structure, surface hydrophobicity, net charge, and molecular flexibility. Extrinsic factors include isolation and modification techniques and environmental parameters such as temperature, pH, and ionic strength (Huamaní-Perales et al., 2024; Zayas, 2012).

a) Water holding capacity (WHC):

WHC, also referred to as hydration capacity, water-binding capacity, or water-absorption capacity, describes the ability of a material to retain water against gravitational forces, including bound, capillary, hydrodynamic, and physically trapped water (Shevkani et al., 2015). Several factors influence WHC, including protein and fibre composition and molecular structure, as well as physicochemical matrix properties such as surface hydrophobicity and porosity. Environmental conditions such as pH, temperature, and ionic strength also affect WHC (Djoullah et al., 2018; Shevkani et al., 2015). In addition, non-protein constituents, including hydrophilic polysaccharides and lipids, can influence the WHC of food materials

(Zhang et al., 2021). WHC contributes to moisture retention and juiciness in meat analogues, and imparts a smooth, creamy texture in dairy alternatives. It is also vital in developing gel-based products, such as energy gels for athletes and gelatinous desserts (Kotecka-Majchrzak et al., 2020). Proteins with WHC values between 1.49 and 4.72 g/g have been reported as suitable for a variety of viscous food formulations (Arise et al., 2015). Furthermore, protein concentrates/isolates with WHC within this range hold potential applications in products such as baked goods, soups, custards and dairy products (Huamaní-Perales et al., 2024; Kaur et al., 2025). Oilseeds' WHC vary in their proportions and types of lipids, proteins, and carbohydrates, depending on species, variety, and cultivar. Because these components differ in polarity, their ability to interact with and retain water also varies, influencing the material's overall WHC. WHC varies not only among oilseed types (e.g., flaxseed, soybean, rapeseed) and their compositional profile but is also significantly influenced by processing methods such as heating, milling, and extrusion (Adinew, 2014; Bárta et al., 2021; Elsorady, 2020; Escobedo-Avellaneda et al., 2025).

b) Oil holding capacity (OHC):

OHC, also referred to as oil (or fat) absorption capacity or oil (or fat) binding capacity, refers to the ability of a material to retain oil through a combination of physical entrapment and physicochemical interactions. It represents the amount of oil that can be immobilized within the matrix's intricate three-dimensional structure, including its pores and capillary spaces (Bárta et al., 2021; Kotecka-Majchrzak et al., 2020), while also binding oil through hydrophobic interactions, hydrogen bonding, and electrostatic forces between oil molecules and nonpolar functional groups (e.g., hydrophobic amino acid side chains). Materials with higher hydrophobicity or more developed structural networks generally exhibit greater OHC (Malik et al., 2017; Q. Zhao et al., 2021a). Additionally, in oilseeds, OHC is influenced by the presence of dietary fibres, starches, and other carbohydrates, as well as the structural and physicochemical properties of soluble and insoluble fibres; such as density, surface characteristics, and polysaccharide structure (Dzuovor et al., 2018; Nevara et al., 2021). In addition, lipid molecules can interact with the nonpolar side

chains of protein molecules (Kinsella, 1979; Sathe & Salunkhe, 1981). Proteins rich in hydrophobic interactions play a role in stabilizing protein-lipid complexes, and proteins containing a greater proportion of hydrophobic regions generally exhibit enhanced OHC. Higher OHCs are vital for flavour and aroma retention, and these are widely used in products such as donuts, pancakes, desserts, beverages, salad dressings, baked goods, sausages, mayonnaise, cake batters, confectioneries, meat extenders, and analogs (Kaur et al., 2025). Its superior OHC helps stabilize formulations, prevent ingredient separation, and enhance flavour, palatability, and shelf life. OHC is an important indicator of flavour retention and mouthfeel in food proteins (Marambe & Wanasundara, 2017). Because of its high OHC, flaxseed protein can be effectively incorporated into emulsion-type meat products, where it helps reduce fat loss during cooking and, consequently, minimizes overall product weight loss (Marambe & Wanasundara, 2017; Martinez-Flores et al., 2006).

c) Emulsifying properties:

An emulsion consists of two immiscible liquids, where one is dispersed as droplets within the other. In food systems, the dispersed and continuous phases typically form oil-in-water (O/W) or water-in-oil (W/O) emulsions. These systems are thermodynamically unstable due to interfacial tension between the two phases. To improve stability, amphiphilic molecules or emulsifiers are used to reduce interfacial tension and prevent phase separation (Damodaran & Parkin, 2017; Zayas, 2012; Zhang et al., 2021). The emulsifying properties of oilseeds are largely attributed to the presence of oleosins (membrane proteins) associated with oil body membranes, phospholipids, and minor lipophilic constituents, including tocopherols and phytosterols (M. Guan et al., 2025; Ishii et al., 2017; Yuan et al., 2024). The amphiphilic nature of proteins enables them to migrate and adsorb at the oil-water interface, forming an interfacial film around oil droplets that prevents coalescence and facilitates the formation of stable food emulsions (Shevkani et al., 2015; Xu et al., 2017). Additionally, emulsifying properties are determined by several factors that influence proteins' surfactant properties, including net charge, structural flexibility, and the

ratio of surface hydrophobicity to hydrophilicity (Gundogan & Can Karaca, 2020; Hojilla-Evangelista et al., 2018; Paiva Gouvêa et al., 2023). Consequently, a higher proportion of soluble proteins facilitates rapid migration to the oil-water interface, thereby enhancing both the formation and stability of emulsions (Huamaní-Perales et al., 2024). Such emulsifying properties are critical in a variety of emulsion-based food systems, including milk, mayonnaise, salad dressings, emulsified condiments, sausages, and ice cream (Loushigam & Shanmugam, 2023; Y. Ma et al., 2023; Xu et al., 2017).

Methods for evaluating the emulsifying properties of food in literature are highly diverse, complicating direct comparisons between studies. The most reported parameters include emulsifying capacity (EC), emulsifying stability (ES), emulsifying activity index (EAI), and emulsifying stability index (ESI). EC quantifies the maximum amount of oil that can be emulsified per gram of material, whereas ES reflects the volume of the emulsified phase that remains after a specified period (Gundogan & Can Karaca, 2020; Pearce & Kinsella, 1978; Stone et al., 2015). ES is influenced by the strength of interactions such as electrostatic repulsion, van der Waals forces, and other molecular forces (Karaca et al., 2011; Z. Ma et al., 2011). The EAI is determined from the stabilized interfacial area per unit protein weight, based on the turbidity of a dilute emulsion, whereas ESI estimates the stability of the diluted emulsion by monitoring changes in turbidity over time (Eckert et al., 2019; Rudra et al., 2016). In addition to these influencing parameters, researchers have used different analytical methods to assess the emulsifying properties of foods. A classical approach to determining EC involves measuring the volume of oil emulsified per unit weight of protein, with oil added incrementally until the emulsion collapses, as indicated by a sharp decrease in conductivity (Stone et al., 2015). EC can also be evaluated by measuring the height of the emulsified layer formed during the process, and ES by measuring the height of the emulsified layer formed after being heated in a water bath (Zhang et al., 2021).

d) Least gelation concentration (LGC)

Gelation describes the ability of proteins to form a semi-solid network between a solid and liquid state, which is defined as a gel (Damodaran, 2017). This process involves heat-induced denaturation, followed by the reorganization of protein molecules into a cohesive three-dimensional matrix stabilized by non-covalent interactions (van der Waals forces, hydrophobic interactions, hydrogen bonds, and electrostatic forces) and covalent sulfhydryl/disulfide linkages (Hermansson, 1979; Liang et al., 2020). LGC is a commonly used index to characterize gelation functionality. It represents the minimum concentration required to form a firm gel, with lower values indicating greater gelling capacity (Peyrano et al., 2016; Piornos et al., 2015). The expression of gelation properties is critical in products such as sausages, cheese, yogurt, and tofu, where they influence both the texture and juiciness of the food matrices (Rudra et al., 2016; Vogelsang-O'Dwyer et al., 2020).

e) Protein solubility (PS)

PS is a techno-functional property that depends on the balance between protein-protein and protein-solvent interactions, which is influenced by surface charge. When proteins have similar charges, they repel each other, reducing protein-protein interactions and increasing protein-water interactions, which helps them stay dissolved (Rao et al., 2023; Varayil & Mitra, 2026). PS is an important property in many food systems because it supports other functions such as emulsification, foaming, and gelation (Hui & Sherkat, 2005). Protein solubility is influenced by intrinsic factors such as amino acid composition, sequence, structure, and protein type, as well as extrinsic factors like pH, ionic strength, temperature, and nonprotein components (Lafarga, 2018; Sathe et al., 2018). Protein denaturation can occur due to heat treatment, which reduces solubility and decreases emulsification and foaming abilities (deWit & Klarenbeek, 1984; Moro et al., 2013). Optimal extraction and processing are crucial for techno-functional applications in food systems because they maintain protein solubility and bioavailability. Protein solubility refers to the extent to which

protein molecules remain dispersed or dissolved in a given solvent. As proteins are typically available in powdered form, solubility assessments are commonly conducted by dispersing the protein powder (usually 1-3% w/w) in an aqueous or buffered medium, adjusting the pH with acid or alkali (typically within the range of 3-8 pH), and allowing sufficient time for hydration, either through brief stirring at ambient temperature or overnight storage under refrigerated conditions. The soluble protein content is then quantified in the supernatant obtained after centrifugation under defined conditions (Zhang et al., 2021). Generally, oilseed proteins possess relatively low solubility. For instance, soybean protein isolate has been reported to exhibit limited solubility, approximately 26.06% at pH 7 (Wang et al., 2023), and between 20% and 30% within the pH range of 4.0-6.5 (Kaur & Ghoshal, 2022).

The wide applicability of oilseeds in food formulation arises from their diverse techno-functional properties, which facilitate the development of plant-based meat analogues, dairy alternatives, bakery items, snacks, and other value-added foods (Kadam et al., 2025). Appropriate selection and optimization of these techno-functional traits enable manufacturers to achieve the desired texture, stability, and sensory qualities in formulated products (Kaur et al., 2025; Shevkani et al., 2024). Çakıtlı & Nurko (2025) further highlight that oilseeds represent ideal alternatives to animal-derived or soy-based proteins, aligning with the increasing consumer demand for sustainable, plant-based diets. In addition, oilseeds provide bioactive compounds, dietary fibre, and high-quality proteins, thereby contributing health-promoting benefits alongside their techno-functional roles (Kadam et al., 2025; Nevara et al., 2021). Understanding these techno-functional characteristics from a processing standpoint allows for the customization or modification of extraction and formulation strategies to optimize product quality (Kadam et al., 2025; Moure et al., 2006). Comprehensively, techno-functional properties bridge food science, industrial innovation, and sustainable nutrition, making oilseeds indispensable to both current and future food systems.

2.8 Major techno-functional properties of flaxseed

Flaxseed proteins comprise both hydrophilic and hydrophobic amino acid residues, enabling interactions with aqueous and lipid phases within complex food matrices. These molecular interactions significantly play a crucial role in determining the sensory, structural, and textural properties of food products (Elsohaimy et al., 2015). Among the various techno-functional properties, water (WHC) and oil (OHC) holding capacities are particularly significant for flaxseed, due to their importance in texture, mouthfeel, and flavour retention (Bessada et al., 2019).

Kaushik et al. (2016) reported that the water and oil absorption capacities of flaxseed protein isolate (FPI) were superior to those of several commonly studied proteins, including soy protein and gelatin. FPI typically exhibits WHC values between 3.0 and 6.1 g/g, indicating a strong water-binding capacity. In their study, Sharma & Saini (2021) reported that the WHC of FPI was 2.85 g/g, whereas Martinez-Flores et al. (2006) reported 2.7 g/g of WHC value. In contrast, Madhusudhan & Singh. (1985) observed water-holding capacities of 7.17, 8.97, and 6.30 g/g of protein for ground flaxseed, boiled ground flaxseed, and soy meal, respectively. In particular, Mohamed et al. (2020) reported a considerably higher WHC of 11.4 g/g for FPI, indicating its strong affinity for water molecules and potential for moisture retention in food matrices. According to Bárta et al. (2021), the carbohydrate composition of flaxseed gum (FG) constituents significantly affects its techno-functional properties. Kaur et al. (2019) and Man et al. (2021) reported that the lipid and dietary fibre fractions of flaxseed influence its WHC. In particular, FG in soluble fibre fractions has been reported to retain substantial amounts of water, further contributing to overall WHC (Basiri et al., 2018; Korus et al., 2015). For FG, WHC values ranged from 16 to 30 g water per g FG (Kajla et al., 2015). The overall high WHC of flaxseed flour (approximately 4.15 g water/g) is largely attributed to its FG content (Bárta et al., 2021). The presence of FG appears to be a major contributor to enhanced water retention due to its highly hydrophilic polysaccharide structure. Collectively, these studies demonstrate that flaxseed and its protein-rich fractions possess substantial WHC compared with many conventional plant protein ingredients.

As previously mentioned (Kaushik et al., 2016), the higher OHC of flaxseed protein isolate (FPI) than that of soy protein and gelatin was attributed to the greater lipophilicity of flaxseed protein molecules. OHC values for FPI have been reported to range from 1.47 g/g (Mohamed et al., 2020) to 3.86 g/g (Sharma & Saini, 2021), exceeding those reported for quinoa and wheat protein isolates (Ashraf et al., 2012). Martinez-Flores et al. (2006) reported an OHC of 1.18 g/g for FPI, whereas Madhusudhan & Singh, (1985b, 1985a) reported even higher OHC values (4.90 g/g), surpassing that of soy protein (3.44 g/g). This strong OHC likely facilitates the storage of flaxseed's high lipid content. Boiling flaxseed protein was found to reduce its OHC to 3.37 g/g, presumably due to heat-induced denaturation and the consequent masking of apolar amino acids (Madhusudhan & Singh, 1985b, 1985a). The high OHC of flaxseed makes it an effective ingredient in emulsion-type meat products (Kaur et al., 2025).

Flaxseed protein exhibits higher emulsifying capacity (EC) compared with whey and canola protein isolates (Karaca et al., 2011). Factors such as pH, solvent, temperature, and processing conditions influence its emulsifying performance, with excessive heat reducing solubility and impairing emulsion formation (Martinez-Flores et al., 2006). Proteins generally exhibit greater emulsifying efficiency when the pH is farther from their isoelectric point (pI) (Cabra et al., 2008; Damodaran & Parkin, 2017). At acidic pH levels, flaxseed protein exhibits a higher EC than that of soy protein, whereas at neutral and alkaline pH values, soy protein demonstrates superior EC compared to flaxseed protein (Madhusudhan & Singh, 1985b). Similarly, Martínez-Flores et al. (2006) reported that flaxseed protein concentrate showed the highest EC (84.8%) at pH 6, while significantly lower values were observed near its pI (pH 4) and at pH 10 (62.91% and 60.92%, respectively). Alkali-solubilized flaxseed protein has also shown high emulsifying performance (Mueller, Eisner, Yoshie-Stark, et al., 2010; Sharma & Saini, 2021; Yoshie-Stark et al., 2011), with Tirgar et al. (2017) the highest EC (87.91%) reported, which was attributed to its carbohydrate content. According to Mueller et al. (2010), the EC of different flaxseed fractions was in the range of 145 to 540 mL/g. Moreover, flaxseed globulin and albumin exhibited enhanced emulsification under alkaline and acidic conditions, respectively (Nwachukwu & Aluko, 2018). EC can be further improved through protein

structural modification (Juodeikiene et al., 2020; Nie et al., 2022; Wanasundara & Shahidi, 1997; Wang et al., 2010). Despite variability among studies, flaxseed proteins consistently exhibit favorable EC.

Martinez-Flores et al. (2006) reported that the emulsifying stability (ES) of flaxseed protein concentrate reached a maximum at pH 8.0 (88.4%) and a minimum at pH 4.0 (50%). Similarly, Dev & Quensel. (1989) demonstrated that flaxseed protein, when combined with flaxseed mucilage in ice cream emulsions, exhibits an emulsion-stabilizing effect comparable to that of gelatin. Rani & Badwaik (2021) further observed that flaxseed cake demonstrated higher emulsion stability (128.46%) compared with defatted flaxseed meal (108.15%), indicating that the presence of native lipids contributes to stabilizing emulsions. The reduction in emulsion stability following defatting suggests that lipid removal alters interfacial interactions within the protein-oil-water matrix. These findings suggest that both protein and lipid fractions contribute to emulsion stabilization in flaxseed systems.

Rani & Badwaik (2021) reported that defatted flaxseed meal exhibited a substantially lower LGC of 8% compared with flaxseed cake (18%), indicating enhanced gelling capacity. Similarly, Elsorady (2020) reported an even lower LGC of 3.6% for FPI. Ahmad Khan et al. (2015) also showed that LGC values varied among samples, including wheat flour and unroasted (URFF) and roasted flaxseed (RFF) flours. No gel formation was observed in wheat flour at concentrations up to 10%, whereas URFF and RFF formed gels at 8% and 6%, respectively. The relatively low LGC values reported for flaxseed ingredients indicate strong gel-forming ability, which is desirable in food systems requiring structural integrity and moisture retention.

Protein solubility is a crucial factor influencing the techno-functionality of flaxseed, and it can affect other techno-functional properties despite having desirable techno-functional properties (Shevkani et al., 2024). The solubility of flaxseed protein is strongly influenced by pH, as variations in pH affect the intramolecular interactions that stabilize the protein's structural conformation (Bekhit et al., 2018). Flaxseed contains two main protein types: albumins and globulins, referred to as linins and colinins based on their solubility characteristics. These proteins were first isolated by Vassel & Nesbitt. (1945) using a

stepwise extraction method, followed by acidification to reach the pI at pH 4.75. In terms of PS, Kaushik et al. (2016) reported that FPI exhibited minimum solubility at pH 4.0, near its pI; and being alkali-soluble, its solubility gradually increased at pH values above 5.0, reaching 57% at pH 7.0 and 76% at pH 9.0. Consistent with these findings, Elsorady (2020) reported that FPI exhibited the least solubility at pH 4.0 (24.54%), whereas the solubility of FPI increased to 53.68% at pH 7.0 and 75.35% at pH 9.0. Tirgar et al. (2017) investigated the solubility of protein concentrates extracted from flaxseed meals using alkali, enzymatic, and enzymatic-solvent methods and reported that all protein concentrates exhibited maximum solubility at pH 8.0, whereas minimum solubility occurred near pI of the flaxseed protein concentrates. Similarly, Martínez-Flores et al. (2006) observed that the PS of flaxseed paste increased markedly above pH 6.0, reaching a maximum at pH 10.0, with the lowest solubility near pH 5.0, corresponding to the pI of flaxseed proteins.

2.9 Factors affecting techno-functional properties of flaxseed

The techno-functional properties of flaxseed are determined by a combination of intrinsic compositional features and extrinsic processing and environmental factors. A comprehensive understanding of these influences is essential for improving the performance and consistency of flaxseed-derived ingredients in industrial applications (Lorenc et al., 2022; Sá et al., 2025).

a) Processing Conditions

Processing technologies and extraction methods significantly affect the structure and functional integrity of flaxseed components. Thermal treatments often cause protein denaturation and aggregation, leading to reduced solubility, emulsifying, and foaming capacities (Huo et al., 2024; Madhusudhan & Singh, 1985b; Tirgar et al., 2017). Prolonged heating can disrupt disulfide linkages and hydrogen bonding, thereby modifying protein structures (Li-Chan & Ma, 2002). Emerging non-thermal technologies, such as

AOPs, offer the potential to enhance techno-functionality while preserving nutritional integrity (Ağagündüz et al., 2025). AOP conditions, which generate reactive oxygen species (ROS), can modify surface hydrophilicity and partially oxidize reactive groups (Feng et al., 2022), enhancing or impairing hydration and emulsification capacities depending on exposure levels. Controlled AOP treatment can induce protein unfolding and accessibility of binding sites, whereas excessive oxidation promotes protein cross-linking and reduces solubility (Pandiselvam et al., 2019). Additionally, mechanical operations such as milling, defatting, and extraction alter particle size and surface area, influencing hydration kinetics and dispersion stability (Kadam et al., 2025).

b) Compositional Factors

The inherent composition of flaxseed proteins plays a crucial role in their techno-functional performance (Mueed et al., 2022). The ratio of hydrophilic to hydrophobic amino acids, degree of polymerization, and the presence of associated non-protein materials such as polysaccharides, lipids, and phenolic compounds directly influence solubility, emulsification, and gelation behaviour (Damodaran, 2005; Jarošová et al., 2024; Kaushik et al., 2016; K.Y. Li et al., 2023). Flaxseed proteins consist primarily of linin and conlinin, two distinct fractions that contribute unique structural and functional attributes (Nwachukwu & Aluko, 2018). Lipids and phenolics also influence techno-functionality by acting as natural emulsifiers and antioxidants that stabilize lipid-protein interfaces (Mueed et al., 2022).

c) Environmental and Physicochemical Conditions

Environmental parameters such as pH, temperature, ionic strength, and solvent polarity significantly affect the techno-functional performance of flaxseed macromolecules. Protein solubility is minimal near the pI, where electrostatic repulsion is reduced, and aggregation is favoured. Moving away from the pI enhances solubility, thereby improving emulsifying and foaming properties. Moderate

temperature increases can expose hydrophobic regions, improving interfacial activity, whereas excessive heating can cause irreversible aggregation and functionality loss (Damodaran, 2005; Karaca et al., 2011; Zayas, 2012)

Ionic strength has a dual influence: low to moderate salt levels enhance solubility through salting-in effects, whereas high salt concentrations cause salting-out. Similarly, variations in solvent polarity and water activity alter hydrogen bonding and electrostatic interactions, affecting hydration and emulsification capacities (Arakawa & Timasheff, 1984; K.Y. Li et al., 2023; Oomah et al., 1994). For flaxseed gum, both pH and ionic composition influence the degree of ionization and chain conformation, which directly impact viscosity and gel-forming behaviour (Wang et al., 2010).

In practical applications, these factors often interact synergistically. For instance, mild heat or oxidative treatments may improve emulsifying performance by exposing hydrophobic sites, whereas combining such treatments with low pH or high ionic strength may lead to aggregation and phase separation. Therefore, optimization of flaxseed techno-functionality requires balancing structural stability with desired techno-functional performance (Lorenc et al., 2022; Madhusudhan & Singh, 1985b; Ye et al., 2025).

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3. Materials and methods

3.1 Conditioning of flaxseed samples

Whole brown flaxseed (*Linum usitatissimum* L., variety: AAC Bravo) was sourced from a commercial supplier in Manitoba, Canada. The initial moisture content (MC) of flaxseed was measured to be 6.0-7.0% (dry weight basis). The seed samples were conditioned to the target moisture content of 9% by adding calculated amounts of distilled water in a batch tumbler. The water quantity required for each adjustment was determined using equation 1.1.

$$\text{Water added} = \text{Weight of seeds} \times \left(\frac{M_f - M_i}{1 - M_f} \right) \times 100 \quad (\text{Eq: 1.1})$$

Where: M_f is the MC to be achieved and M_i the initial MC.

The seeds were conditioned for 2 hours at 36 rpm using a metal-lined batch tumbler (24 cm × 24 cm × 51 cm). At 30-minute intervals, the lid was opened, and any seed clumps were gently broken to ensure even moisture distribution. After tumbling, the seeds were sealed in double plastic bags with adequate headspace to allow further moisture equilibration. These bags were held at room temperature for 6 hours, and the seeds were shuffled every 3 hours.

Subsequently, the bags were transferred to an environmental chamber (Convion CMP3244, Controlled Environments Ltd., Winnipeg, MB, Canada) set at 5 °C for 72 hours. The final moisture content of the flaxseed samples was assessed on a dry-weight basis using the hot-air oven method in accordance with ASAE Standard S352.1 (ASAE, 2012). Analyses were performed in duplicate to ensure accuracy. The conditioned samples were then stored in double-layered polyethylene bags at 5 °C until use in experiments.

3.2 AOP Decontamination

Decontamination of the conditioned flaxseed samples was performed using a Clean Flow Healthcare Mini (CleanWorks Corp., St. Catharines, ON, Canada) with an advanced oxidative process (AOP). For each treatment, 250 g of flaxseed was evenly distributed in a monolayer on a plastic tray. The trays were conveyed through the Clean Flow Healthcare Mini chamber, where the seeds were simultaneously exposed to ozone (O_3), UV-C radiation, and hydrogen peroxide (H_2O_2). The seeds were divided into different treatment groups, including an untreated control, as summarized in Table 2.

Table 2. Treatment conditions of AOP decontamination.

Treatment groups	Hydrogen peroxide flow rate (mL/min)	Ozone concentration (ppm)	UV-C light intensity (mW/cm²)	Exposure time (s)
Control	0	0	0	0
T1-30	20	5-9	8-12	30
T1-40	20	5-9	8-12	40
T2-30	30	5-9	8-12	30
T2-40	30	5-9	8-12	40

During treatment, O_3 concentrations of 5-9 ppm were used, and samples were irradiated with UV-C light at 8-12 mW/cm². In parallel, a 3% H_2O_2 solution was sprayed as a fine mist at flow rates of 20 mL/min and 30 mL/min. The conveyor speed was adjusted to achieve exposure times of 30 and 40 seconds for the respective treatments. Flaxseed samples were processed under five treatment conditions (Control, T1-30, T1-40, T2-30, and T2-40), with each treatment prepared in duplicate (R1 and R2). The selected AOP treatment conditions were designed to achieve effective microbial decontamination with minimal or no impact on flaxseed's physicochemical and techno-functional properties. Ozone concentrations (5-9 ppm), UV-C light intensity (8-12 mW/cm²), and H_2O_2 (3% solution at 20 and 30 mL/min) were chosen based on previously reported AOP studies on food and agricultural commodities, where these ranges were shown to achieve significant microbial reductions (Crowe et al., 2007; Guan et al., 2013; Hadjok et al., 2008; Phornvillay et al., 2022; Abdi et al., 2024; Murray et al., 2018). The selected O_3 and UV-C ranges reflect the operational ranges recommended for safe and effective use of the Clean Flow Healthcare Mini system

and are in line with conditions reported to be effective for surface decontamination of agricultural commodities (Hamid et al., 2024; Templalex et al., 2023; J. Wang et al., 2021). Hydrogen peroxide flow rates of 20 and 30 mL/min were selected to evaluate the effect of oxidant dose on flaxseed while remaining within the spraying capacity of the Clean Flow Healthcare Mini system. Exposure times of 30 and 40 seconds were achieved by adjusting conveyor speed and were selected to represent short, industrially feasible treatment durations, allowing assessment of time-dependent effects without prolonged oxidative stress. Overall, the selected treatments enabled systematic evaluation of oxidant intensity and exposure time under equipment constraints while remaining relevant to scalable, environmentally friendly decontamination strategies for flaxseed processing.

The treated seeds were collected separately into polyethylene bags and manually shuffled for 1 minute to ensure thorough mixing and to prevent clumping. Prior to storage, 50 g of flaxseed were collected from each of the five treatments to characterize the initial physicochemical and techno-functional properties. All analyses followed the procedures outlined in Section 1.4 to ensure consistent, reliable results. Rest of the flaxseed were used in storage studies.

3.3 Flaxseed Storage

For the storage study, 750 g of conditioned flaxseed was placed in a polyester mesh bag (0.8 mm mesh size) as the primary sample, along with an additional 250 g sample designated for microbial analyses. All bags were placed inside a cylindrical plastic pail (26.0 cm in diameter, 36.0 cm in height, 20 L capacity). To minimize moisture loss from the sampling bag during routine pail opening for sample collection, 1 kg of buffer seeds in polyester mesh bags (0.8 mm mesh size) were positioned both above and below the sampling bag, following the approach of Findlay et al. (2023) and Mundhada et al. (2022). The sampling and buffer bags rested on a perforated grid separator positioned above a saturated NaCl solution, supported by three PVC columns (approximately 8 cm in diameter and 6 cm in height), as illustrated in Figure 2.

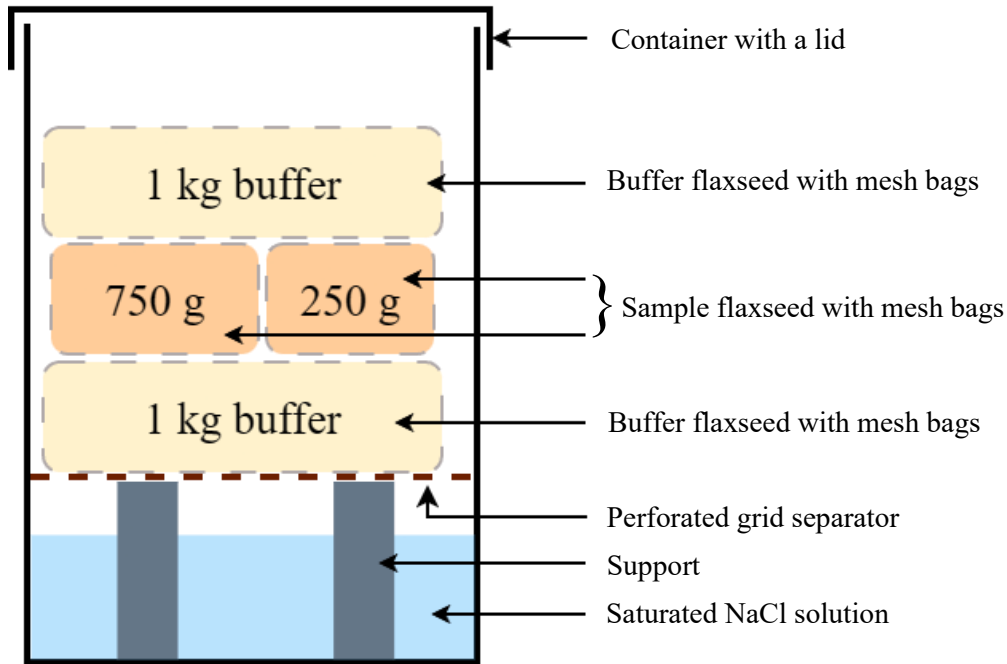


Figure 2. Schematic model of a pail.

Approximately half of the height of each PVC support was submerged in the saturated NaCl solution. The pail was loosely covered with a lid to allow air exchange. The flaxseed was also shuffled at each acquisition interval to minimise anaerobiosis and accumulation of gaseous metabolites. Each treatment consisted of two replicates, resulting in a total of 10 pails stored in an environmental chamber (Conviron CMP4030, Controlled Environments Ltd., Winnipeg, MB) maintained at 20 ± 2 °C and an RH of 60%. Temperature and relative humidity sensors (HOBO Temp/RH 2.5% data logger, model UX100-011A; LI-COR Inc., Bourne, MA, USA) were placed inside each pail to verify that the expected environmental conditions (20 ± 2 °C and 75% RH) were achieved. The seeds in the sampling bags were thoroughly mixed prior to sampling, and 50 g were collected from each pail at monthly intervals over a 16-week period for subsequent analyses.

3.4 Sample preparation

The collected flaxseed samples were separately ground using a Retsch ultra-centrifugal mill (Model ZM 200, Retsch GmbH, Haan, Germany). A sieve size of 500 μm was used in the mill, and samples were milled in small 5 g portions to prevent clogging and unintended oil exudation. The temperature was carefully monitored with an infrared thermometer during milling to ensure it did not exceed 30 °C, thereby avoiding any negative impact on the sample quality. Milled flaxseed tended to adhere to the sieve surface; therefore, the sieve was carefully cleaned between uses to maintain consistency and prevent clogging. After milling, the samples were immediately sealed in individual zip lock bags and stored at 4 °C for subsequent analyses.

3.5 Proximate chemical composition

Moisture content of ground flaxseed samples was measured according to the AOCS official method Ba 2a-38 (AOCS, 2024). For each treatment, 2 g of ground samples were dried in a convection oven at 130 °C (± 2 °C) for 2 hours, with analyses performed in triplicate to ensure accuracy. Crude protein content was quantified using a Rapid N Exceed® nitrogen and protein analyzer (Elementar, Langenselbold, Germany), following the procedures outlined in AACC combustion method 46-30.01 (AACC International, 2011). Protein content was calculated by multiplying the measured nitrogen content by the conversion factor of 6.25. Crude fat (Method 30-25.01) and crude ash (Method 08-01.01) contents were analyzed according to AACC International (2011). Moisture content, crude protein, and crude ash were each measured in triplicate, while crude fat content was assessed in duplicate. Total carbohydrate content was calculated as the remainder after subtracting the measured moisture, crude protein, crude fat, and crude ash values from 100%.

3.6 Techno-functional properties

Ground whole flaxseed samples stored at 4 °C were used and analyzed for their techno-functional properties, with all values corrected for their moisture content.

3.6.1 Water and oil holding capacities

Water and oil holding capacities (WHC and OHC) were assessed following the procedure outlined by Stone et al. (2015) with some adaptations. Briefly, 0.5 g of ground whole flaxseed samples were weighed into 50 mL centrifuge tubes and mixed with 5 g of either water or canola oil for the WHC and OHC determinations, respectively. The tubes were tightly closed and vortexed using a vortex mixer (Model G-560, Scientific Industries, Bohemia, NY, USA) at speed 10 for 10 seconds at 5-minute intervals over 30 minutes. After mixing, the samples were centrifuged (Model RC6 Plus, Thermo Fisher Scientific, Asheville, NC, USA) at $10,155 \times g$ for 15 minutes to measure WHC and OHC. After centrifugation, excess liquid was decanted by tilting the tubes for 30 seconds, and the remaining solids were weighed. WHC as g water/ g sample and OHC as g oil/ g sample were determined using the following equations 1.2 and 1.3, respectively.

$$\text{WHC} = \frac{W_f - W_i}{W_i} \quad (\text{Eq. 1.2})$$

$$\text{OHC} = \frac{W_f - W_i}{W_i} \quad (\text{Eq. 1.3})$$

Where, W_i is the initial weight of the sample prior to analysis, and W_f is the weight of the solids after centrifugation.

3.6.2 Emulsion properties

Emulsion capacity (EC) and emulsion stability (ES) were evaluated based on Ahmedna et al. (1999) with minor adaptations. For EC determination, 0.5 g of each sample was transferred into 15 mL centrifuge tubes, and 5 mL of distilled water was added. The tubes were tightly closed and mixed on a vortex mixer at 10 for 30 seconds every 5 minutes for a total of 30 minutes. Subsequently, 3 mL of canola oil was added, and the mixtures were homogenised (T 18 basic Ultra turrax, IKA, Germany) at 24000 rpm for 2 minutes. After homogenization, the samples were centrifuged at $4000 \times g$ for 20 minutes. ES was determined using a similar procedure. Following homogenization, the samples were incubated in a water bath (Model MW-11260-1, Blue M Electric Company, Illinois, USA) at 45 °C for 30 minutes as a thermal stability treatment, then cooled in water at 20 °C for 10 minutes. The samples were then centrifuged at $4000 \times g$ for 20 minutes. Emulsion capacity (EC, %) was calculated as the ratio of the height of the emulsified phase to the total height of the whole liquid using equation 1.4.

$$\text{Emulsion Capacity (\%)} = \frac{H_e}{H_t} \times 100 \quad (\text{Eq. 1.4})$$

Where, H_e is the height of the emulsified phase immediately after homogenization, and H_t is the total height of the whole liquid. Emulsion stability (ES, %) was expressed as the ratio of the height of the emulsified phase after thermal stability treatment and centrifugation to the total height of the liquid, as given by equation 1.5.

$$\text{Emulsion Stability (\%)} = \frac{H_s}{H_t} \times 100 \quad (\text{Eq. 1.5})$$

Where H_s is the height of the emulsified phase after the thermal stability treatment followed by centrifugation.

3.6.3 Least gelation concentration

The least gelation concentration (LGC) was determined following the method described by Coffmann & Garcia (1977) with minor modifications. Solutions of flaxseed at varying concentrations (0.4 to 0.9 g per 5 mL) were prepared in 50 mL centrifuge tubes using three different buffers (pH 4, 5.5, and 7). The tubes were tightly closed and mixed on a vortex mixer set at speed 10 for 1 minute to ensure proper mixing. Thereafter, the tubes were placed in a water bath at 95 °C for 1 hour, after which the mixtures were immediately cooled to room temperature in cold water and then stored at 4 °C for 2 hours. Following storage, the tubes were inverted, and the LGC was defined as the lowest concentration of flaxseed at which the solution remained intact and did not flow or slip from the tube. All measurements were conducted in duplicate. LGC values were expressed as weight per volume (% w/v).

3.6.4 Protein solubility

Protein solubility (PS) was determined following the method described by Barac et al. (2010) with some adaptations. Briefly, 1.8 g of ground flaxseed was weighed and transferred into 50 mL centrifuge tubes. Flaxseed dispersions were prepared using three different buffers (pH 4, 5.5, and 7). Fifteen millilitres of the respective buffer were added, and the mixtures were vortexed at speed 10 for 15 seconds. The dispersions were then stirred on a shaker (Thermo Scientific MaxQ 5000 Shaker, Thermo Fisher Scientific, Asheville, NC, USA) at 350 rpm for 15 minutes at 20 °C and stored overnight at 4 °C to allow complete hydration. The samples were vortexed at speed 10 again for 15 seconds, stirred on a shaker at 350 rpm for 1 hour at 20 °C, and the pH was checked and adjusted as necessary, followed by an additional hour of stirring. The suspensions were centrifuged at $10,155 \times g$ for 15 minutes, and the supernatants were carefully filtered through multilayered muslin cloth to obtain soluble protein solutions, thereby removing the mucilage-rich layers. All measurements were performed in triplicate.

The nitrogen content of flaxseed extracts was quantified using a Rapid N Exceed® nitrogen and protein analyzer (Elementar, Langenselbold, Germany), following the procedures outlined in AACC

combustion method 46-30.01 (AACC International, 2011). Soluble protein content was calculated by multiplying the measured nitrogen content by the conversion factor of 6.25.

Thereby, the protein solubility (%) was calculated using equation 1.6.

$$\text{Protein Solubility (\%)} = \frac{\text{Soluble protein content}}{\text{Protein content in the initial sample}} \times 100 \quad (\text{Eq. 1.6})$$

3.6.5 Statistical Analysis

Statistical differences among flaxseed's techno-functional properties at different AOP treatments were determined by two-way ANOVA followed by Tukey's test ($p < 0.05$) to compare all, considering treatments and storage time as independent variables using OriginPro statistical computing software (Version 2025b. OriginLab Corporation, Northampton, MA, USA).

4. Results and discussion

4.1 Proximate composition

The moisture content, crude protein, crude fat, total ash and carbohydrate contents of the ground whole flaxseed are summarized in Table 3. All values are expressed on a dry basis.

Table 3. Proximate composition of whole ground flaxseed.

Component (%)	Proximate composition
Moisture	6.87 ±0.24
Crude Protein	25.80 ±0.14
Crude Fat	41.10 ±0.05
Total Ash	3.37 ±0.04
Carbohydrates*	29.72

* Calculated by difference

Values are mean ± standard deviation (n = 3).

The initial moisture content of whole ground flaxseed was 6.87%, measured before seed conditioning for the storage study, which is within the range reported in the literature. According to Pradhan et al. (2010), flaxseed is typically harvested at moisture levels between 11% and 16%. After harvest, the moisture content is reduced to approximately 5%-8% to ensure storage stability and seed quality (Tarek Fouda et al., 2021). Reported crude protein values for flaxseed vary widely in the literature because of differences in sample form (e.g., whole, ground, etc.) and processing methods (e.g., roasted, defatted, etc.). Protein content differs among whole ground flaxseed, other flaxseed flours (e.g., defatted, dehulled, etc.), seed cakes, defatted meals, and protein isolates, as oil removal, mechanical processing, and fractionation can concentrate or dilute protein fractions to varying degrees. The crude protein content of the whole ground flaxseed in this study (25.80%) is consistent with values reported in the literature, including 23.78% reported by Khan and Saini (2016), 22.58% by Marpalle et al. (2014), and 22.4% by Anjum et al. (2006).

The crude fat content observed in the present study (41.10%) reflects the characteristic composition of oilseeds and falls within the range reported in the literature. Comparable values include 40.5% reported by Marpalle et al. (2014), 32.27% by Khan and Saini (2016), and 37% by Anjum et al. (2006). Slight variations in protein and fat contents may be attributed to differences in the relative proportions of other seed constituents, particularly the fibre fraction, as well as cultivar differences, growing conditions, and processing methods (Y. Lan et al., 2020; Zou et al., 2017).

4.2 Techno-functional properties

4.2.1 Water holding capacity (WHC)

The WHC of whole flaxseed during the 16-week storage study ranged from 3.32 to 3.71 g water/g dry flaxseed, indicating relatively stable hydration properties throughout storage. The ANOVA in Table 4 showed that treatment ($p = 0.0169$), storage time ($p = 0.0078$), and their interaction ($p = 0.0327$) significantly affected WHC. The significant interaction effect indicates that the treatment's influence on WHC varies depending on storage duration.

Table 4. ANOVA table for water holding capacity (WHC) of AOP-treated and stored flaxseed.

Independent variable	Dependent variable	DF	F Value	p Value
Treatment	WHC	4	3.6984	0.01692
Storage time		4	4.41	0.0078
Treatment × Storage time		16	2.2615	0.03268

To further examine differences among the means of different AOP treatments and storage times, a Tukey post hoc test was conducted (Figure 3)¹. The results showed that most means shared the same

¹ Supplementary information is provided in Appendix, Table 14.

grouping letters, indicating no significant ($p > 0.05$) differences among them. The only exception was observed between T1-40 before storage and T2-40 after 3 months, which differed significantly ($p < 0.05$) in pairwise comparison. For the T2-40 treatment, WHC showed a slight decreasing trend as storage time progressed. Overall, across all the treatments and storage times studied, the magnitude of variation in WHC was small (approx. 12%). Despite the statistically significant treatment $\times$ storage interaction, most treatment-time combinations were not significantly different from one another ($p < 0.05$) according to Tukey's test. The significant ANOVA result appears to reflect small treatment-specific fluctuations rather than a major deterioration in WHC during storage. Therefore, the following discussion focuses on possible compositional and structural factors contributing to the overall stability of WHC.

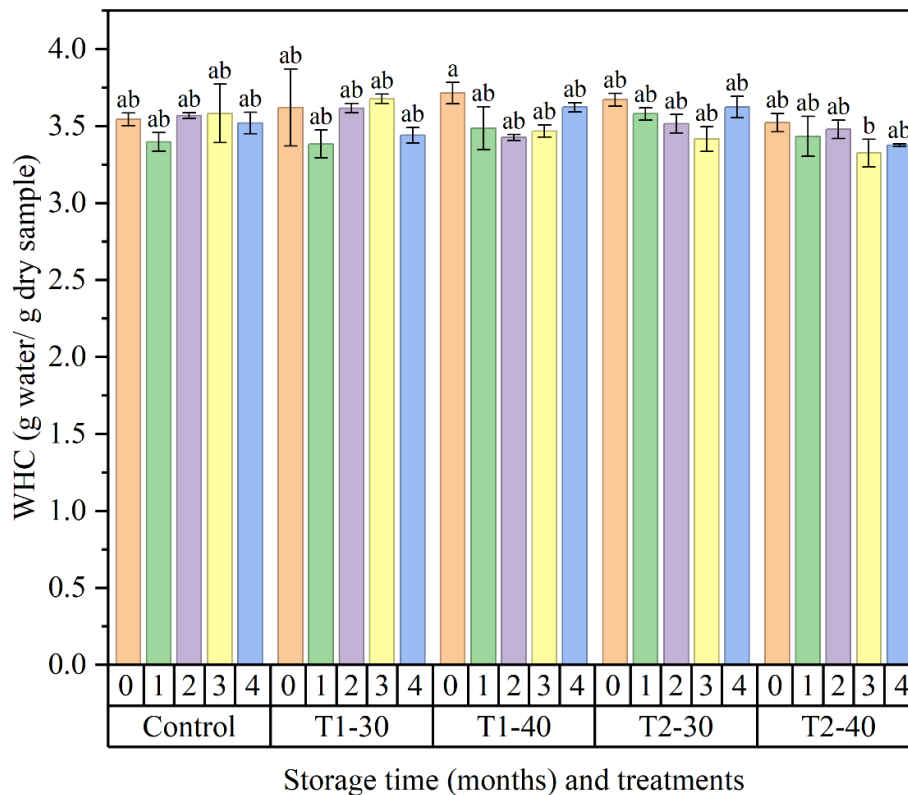


Figure 3. Water holding capacity (WHC) of whole ground flaxseed as a function of storage time (0, 1, 2, 3, and 4 months) and treatment (Control, T1-30, T1-40, T2-30 and T2-40). Error bars represent $\pm$ standard deviation ($n=3$). Bars assigned with different letters are significantly different ($p < 0.05$).

The WHC values observed in the present study, approximately 3.0- 3.7 g water/g dry flaxseed, indicate relatively high water retention ability compared with other oilseed materials, such as canola, sunflower, and mustard flours (Bárta et al., 2021; Rani & Badwaik, 2021). These values were also within a similar range to those reported in the literature for other flaxseed flours (e.g., defatted, dehulled, etc.) and meal fractions. For instance, Mueller et al. (2010) reported WHC values ranging from 3 to 5 g water/g dry flaxseed meal fractions, including soluble protein with fibre fractions and insoluble fibre fractions. Similarly, Bárta et al. (2021) reported WHC values of 3.17- 4.15 g water/g dry flaxseed for different flaxseed cake flour fractions, while Elsorady (2020) reported a WHC of 3.05 g water/g flaxseed protein isolate (FPI). Therefore, the WHC values observed in the present study are consistent with previous reports and confirm the strong WHC of whole ground flaxseed. In contrast, Khan & Saini (2016) reported a WHC of 1.47 g water/ g dry flaxseed for whole ground flaxseed, whereas Teh et al. (2014) reported a WHC of 5.0 g water/ g dry flaxseed for defatted flaxseed flour. The observed variability in WHC among studies may be explained by differences in processing methods, particle-size distributions, and compositional characteristics of flaxseed materials.

For the control, WHC remained stable during storage, suggesting that the natural water-holding ability of whole flaxseed was largely maintained. Similarly, the AOP-treated flaxseed samples showed only minor fluctuations, indicating that the applied AOP conditions did not cause major deterioration of the components responsible for water retention. Although research on the effect of AOP treatments on the WHC of flaxseed is limited, these findings are supported by Abdi et al. (2024), who applied AOP treatment (10 mL/ min of 3% H₂O₂, 9.46 mW/ cm² UV and 2 g/h O₃) to wheat. They reported reduced microbial counts in wheat kernels, with no significant changes in protein aggregation or enzymatic activity, suggesting that AOP can improve microbial safety without substantially altering key techno-functional properties. Similarly, Zhao et al. (2025) reported that WHC of soy protein isolate (SPI) had insignificant changes when H₂O₂ concentration was low to moderate (0- 5 mM, approx. 0-170 ppm) in a hydroxyl radical generation system. The small differences observed among treatments may be due to minor structural

changes caused by AOP exposure or storage. Those mild AOP treatments may slightly modify proteins or polysaccharide-protein interactions, which could influence water binding (Yiasmin et al., 2026). However, the absence of a clear downward trend suggests that these changes were insufficient to substantially reduce WHC. This relatively stable WHC may be mainly related to the presence of flaxseed gum (mucilage), dietary fibres, proteins, and other hydrophilic components in flaxseeds (Dzuovor et al., 2018). Flaxseed gum (FG), a soluble dietary fibre and polysaccharide mainly associated with the seed coat, is considered a major contributor to water retention. Lorenc et al. (2022) reported that FG significantly contributes to the high WHC of flaxseed samples. Similarly, Hu et al. (2020) demonstrated that FG maintains its techno-functional properties and exhibits good storage stability, supporting the notion that mucilage, a polysaccharide-rich hydrocolloid, plays a major role in maintaining WHC over time. This interpretation is further supported by Alfaro-Rodríguez et al. (2024), who reported minimal structural changes in flaxseed fibres under controlled storage conditions. In addition to polysaccharides, flaxseed protein fractions, especially albumins, may be responsible for enhanced WHC owing to their high content of polar amino acids that promote strong protein-water interactions (Kajla et al., 2023).

Mild protein oxidation may expose hydrophobic groups on the protein surface, thereby reducing the availability of hydrophilic sites for water binding and decreasing WHC (Shen et al., 2023). Therefore, the slight decreasing trends in WHC observed for the T2-40 treatment could be partially explained by mild protein oxidation. This effect may be associated with oxidative structural modifications of proteins, which can occur either directly through reactive oxygen species (ROS) or indirectly through reactions with lipid oxidation by-products (Shacter, 2000). Since lipids are highly susceptible to oxidation, lipid peroxidation can generate free radicals, lipid hydroperoxides, and reactive aldehydes, which may subsequently interact with proteins and further contribute to protein oxidation (Refsgaard et al., 2000). Findlay et al. (2023) observed that yellow flaxseed had higher oxidation resistance than brown flaxseed, as indicated by differences in Fourier transform infrared (FTIR) spectra acquired during storage following AOP treatment. They suggested that the higher antioxidant content in yellow flaxseed may help counteract lipid oxidation

and oxidative damage potentially induced by AOP treatment during storage. Although the present study did not directly assess lipid oxidation, these findings suggest that oxidative reactions involving lipids and proteins could partly explain the slight WHC reductions observed in some AOP-treated samples.

Overall, the relatively narrow WHC range indicates that AOP-treated whole ground flaxseed maintained its WHC during storage, likely due to the stability of flaxseed mucilage, dietary fibre, and protein-based water binding sites. Further studies might be needed to assess the changes in WHC in relatively more severely AOP-treated, e.g., T2-40, flaxseed samples if such treatments are required from a microbiological perspective.

4.2.2 Oil holding capacity (OHC)

The OHC of control and AOP-treated flaxseed samples during the 16-week storage period ranged from approximately 1.80 to 2.00 g oil/g dry whole ground flaxseed, indicating relatively stable lipid-binding properties throughout storage. These narrow values suggest that the structural components responsible for oil retention were largely preserved during storage.

Table 5. ANOVA table for oil holding capacity (OHC) of AOP-treated and stored flaxseed.

Independent variable	DF	F Value	p Value
Treatment	4	2.79417	0.0480
Storage time	4	5.98226	0.0016
Treatment × Storage time	16	1.66418	0.1232

The ANOVA results presented in Table 5 showed that OHC was significantly ($p < 0.05$) influenced by treatment ($p = 0.048$) and storage time ($p = 0.0016$). However, the interaction between treatment and storage time was not statistically significant ($p = 0.1232$). In other words, treatment effects on OHC did not depend on storage time. Since the interaction term was not significant, interpretation focused on the main effects. Mean comparisons were therefore conducted separately for treatment and storage time.

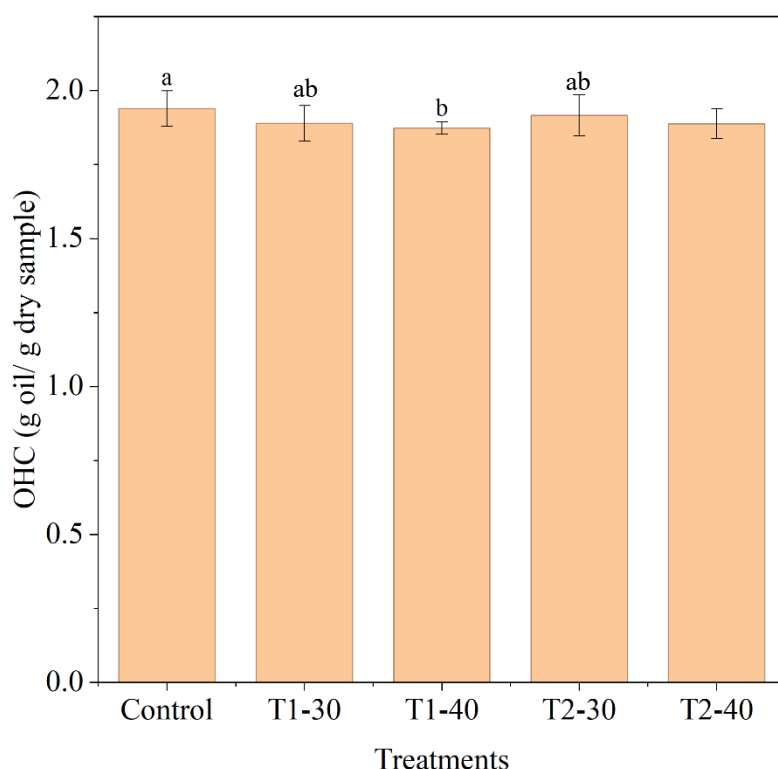


Figure 4. Mean oil holding capacity (OHC) values of whole ground flaxseed as a function of treatment (Control, T1-30, T1-40, T2-30 and T2-40). OHC values were reported as the means of all storage times. Error bars represent $\pm$ standard deviation ($n=3$). Bars assigned with different letters are significantly different ($p < 0.05$).

The OHC values for different treatments ranged from approximately 1.88 to 1.93 g oil/g dry ground whole flaxseed, indicating a narrow variation across treatments (Figure 4)². However, the pairwise comparison of means indicated a small but statistically significant ($p < 0.05$) treatment effect on the OHC of flaxseed. The control and T1-40 treatments were assigned different grouping letters, with the T1-40 treatment's OHC being slightly lower. The small differences among treatments, with the p-value being very close to 0.05, indicate that AOP processing did not substantially alter the structural components responsible for oil retention in flaxseed.

² Supplementary information is provided in Appendix, Table 15.

This limited treatment-related variation agrees with previous flaxseed studies showing that OHC can remain within a modest range unless the seed matrix is substantially modified. For instance, reported OHC values of 1.27 and 1.31 g oil/g dry for roasted full-fat and defatted flaxseed flours are somewhat lower than the values observed in the present study. The slightly higher OHC observed here may be related to the use of whole flaxseed rather than processed flour fractions. Whole ground flaxseed retains lipids, proteins, fibre, and mucilage-containing components, whereas processing steps such as oil extraction, dehulling, or fractionation can alter the relative abundance and structural organization of these components (Shim et al., 2014). Flaxseed contains substantial amounts of lipid, dietary fibre, and protein, with oil mainly stored in the cotyledon tissue (Rubilar et al., 2010). In addition, flaxseed mucilage located in the outer hull contributes to structure-forming and stabilizing (Lorenc et al., 2022; Puligundla & Lim, 2022). Therefore, these components in whole ground flaxseed may help preserve physical sites for oil entrapment and contribute to its OHC (Khan & Saini, 2016). In contrast, studies on isolated flaxseed protein or protein concentrates have reported quite variable OHC values depending on extraction and processing conditions, including around 1.47 g oil/g dry flaxseed protein isolate (Mohamed et al., 2020), about 1.50 to 1.18 g oil/g dry flaxseed protein concentrate (Martínez-Flores et al., 2006), and up to 3.86 g oil/g dry flaxseed protein isolate (Sharma & Saini, 2021). These variations further indicate that OHC in flaxseed depends heavily on sample form and processing steps.

Zhao et al. (2025) reported that soybean protein isolate samples exposed to increasing concentrations of H₂O₂ solutions (0 to 15 mM) showed a gradual and significant reduction in OHC. Liu et al. (2023) suggested that this decline may be attributed to oxidative damage that collapses both the surface and internal porous structures of the protein matrix, thereby reducing OHC. Similarly, Zhang et al. (2022) reported that OHC of walnut protein isolates decreases significantly when protein and lipid oxidation occur, disrupting protein-lipid complexes and reducing hydrophobic binding sites. The limited variation observed in the present study therefore indicates that such AOP-induced structural disruption was minimal. Importantly, these latter studies were conducted on isolated proteins, whereas the present study applied

treatments to whole ground seeds, where the native seed components may offer greater structural protection against AOP.

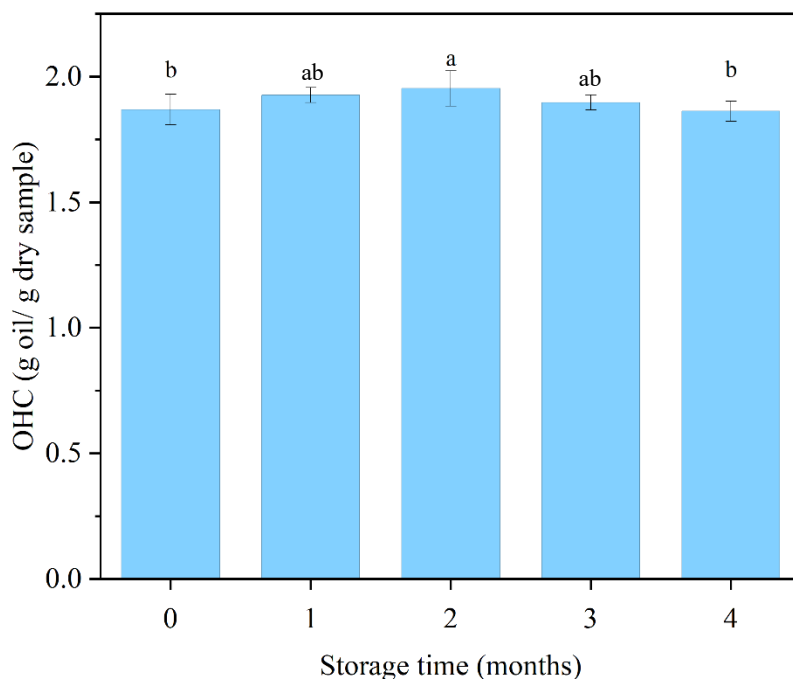


Figure 5. Mean oil holding capacity (OHC) values of whole ground flaxseed as a function of storage time. OHC values are presented over 0, 1, 2, 3, and 4 months as the means of all treatments. Error bars represent $\pm$ standard deviation ($n=3$). Bars assigned with different letters are significantly different ($p < 0.05$).

In Figure 5, the OHC values ranged narrowly between approximately 1.86 and 1.95 g oil/g dry flaxseed³. OHC increased slightly from month 0 to month 2, reaching its highest value in month 2, then gradually decreased toward month 4. However, the overall changes were small, suggesting that storage had a limited overall effect on the OHC of flaxseed. Statistical analysis higher showed that OHC in month 2 was significantly ($p < 0.05$) higher than in months 0 and 4, while months 1 and 3 did not differ significantly from either group.

These statistically detectable yet small fluctuations in OHC values suggest that subtle structural or compositional modifications may have occurred during storage. Although directly related literature on the

³ Supplementary information is provided in Appendix, Table 16.

effects of AOP treatment and storage on the OHC of flaxseed or other oilseeds is limited, related studies on flaxseed ozonation and storage provide useful context. Bechlin et al. (2019) investigated ozonated flaxseed stored under different packaging conditions and reported that grain quality parameters, including moisture content and microbial content, as well as oil quality, were not strongly influenced by ozonation or storage, except for slight changes in lipid content. In another study without any AOP or other decontamination treatment, Malcolmson et al. (2000) reported no significant lipid oxidation in milled flaxseed stored at 23 ± 2 °C for 128 days. This suggests that untreated flaxseed can remain relatively stable during storage, which may partly explain the minor OHC fluctuations observed in the present study. Similarly, reported stable olefinic peaks in ATR-FTIR spectra of AOP-treated ground flaxseed during storage, suggesting minimal structural alteration of lipid components. These olefinic peaks are associated with C=C bonds in unsaturated lipids; therefore, their stability suggests that the degree of lipid unsaturation was largely maintained during storage. This indicates limited lipid oxidation or structural alteration of lipid components, which supports the present finding that storage caused only minor fluctuations in OHC rather than substantial loss of OHC. Collectively, these findings suggest that the structural and compositional characteristics of flaxseed may provide some resistance to storage-induced oxidative or conformational changes, thereby helping maintain OHC during storage.

4.2.3 Emulsion properties

I. Emulsion capacity (EC)

The emulsion capacity (EC) of whole flaxseed was evaluated for the effects of AOP treatments and storage time over a 16-week period. The two-way ANOVA (Table 6) revealed that treatment had a significant effect on EC ($p = 0.0040$), whereas storage time did not ($p = 0.1446$). However, a significant

interaction between treatment and storage time was observed ($p = 0.0112$), indicating that the effect of storage on EC depended on the treatment applied.

Table 6. ANOVA table for emulsion capacity (EC) of AOP-treated and stored flaxseed.

Independent variable	DF	F Value	p Value
Treatment	4	5.0469	0.0040
Storage time	4	1.8841	0.1446
Treatment $\times$ Storage time	16	2.7605	0.0112

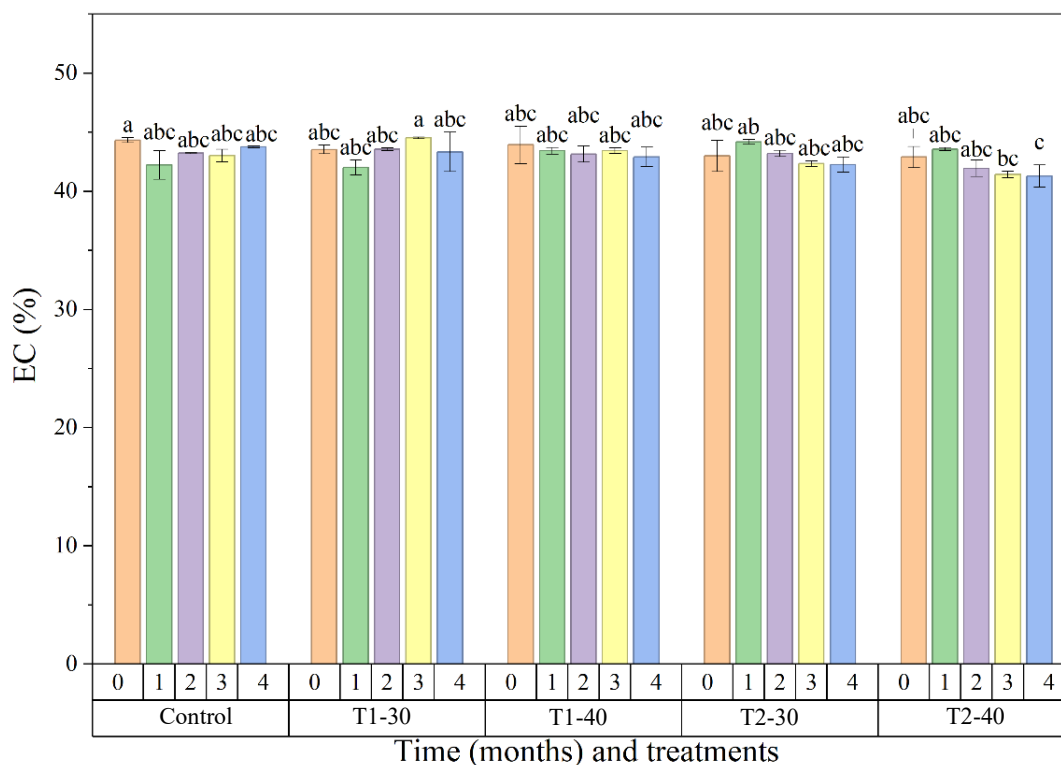


Figure 6. Emulsion capacity (EC) values of whole ground flaxseed as a function of storage time (0, 1, 2, 3, and 4 months) and treatment (Control, T1-30, T1-40, T2-30 and T2-40). Error bars represent $\pm$ standard deviation ($n=3$). Bars assigned with different letters are significantly different ($p < 0.05$).

Because of the significant interaction, Tukey's test was used to conduct mean comparisons for all treatment and storage combinations. EC values ranged from 41.3 to 44.5% across all treatments and storage periods, indicating relatively little variation.

Before storage (0 months), EC values were comparable among treatments, ranging from 42.9% to 44.3%, with no statistically significant differences (all sharing the same grouping letters), as shown in Figure 6.⁴ The control sample exhibited one of the highest EC values (44.3%) before storage (0 month), though it was not significantly different from the treated samples ($p < 0.05$). During storage, EC fluctuated slightly between 42.2% and 43.8% over 1 to 4 months, remaining statistically similar across all time points. Similarly, the T1-30, T1-40, and T2-30 treatments maintained relatively stable EC values during storage, with minor fluctuations that were not statistically significant ($p > 0.05$). However, some localized

⁴ Supplementary information is provided in Appendix, Table 17.

differences emerged at specific treatment-time combinations. For example, T1-30 in 3 months showed the highest EC value (44.5%), similar to that of the control before storage. In general, T2-30 and T2-40 treatments showed slightly lower EC values at later storage stages, particularly at 3 and 4 months, suggesting minor treatment-dependent changes over time. T2-40 in 4 months exhibited the lowest EC value (41.3%), indicating a significant reduction compared to the highest value.

The highest EC value was observed for the T1-30 sample after 3 months of storage, together with the slight increase in EC for T1-30 up to 3 months, suggesting that mild oxidative modification of proteins by AOP may have improved emulsifying capacity during storage. Such changes may be explained by protein oxidation, which involves structural modifications induced either directly by reactive oxygen species (ROS) or indirectly through reactions with lipid peroxidation by-products (Shacter, 2000). Because lipids are highly susceptible to oxidation, lipid peroxidation can generate free radicals, lipid hydroperoxides, and reactive aldehydes, which may interact with proteins and contribute to protein oxidation (Refsgaard et al., 2000). As flaxseed contains a high lipid content, limited oxidation products formed during storage may have interacted with proteins, causing mild structural modifications, such as partial protein unfolding. This may have exposed hydrophobic groups and increased surface hydrophobicity, allowing proteins to adsorb more effectively at the oil-water interface and thereby improving EC (Wang et al., 2025). However, the lower EC values observed in the 4th month compared to earlier storage periods, particularly for T2-40, may be associated with more extensive oxidative changes, such as a reduction in free sulfhydryl groups and the formation of large, insoluble protein aggregates during prolonged storage (Le Priol et al., 2022). Despite these statistically significant differences among certain mean comparisons, the overall variation in EC remained small. Collectively, these results suggest that although AOP treatments and storage time influenced EC in a statistically detectable manner through their interaction effect, the EC of whole flaxseed remained largely stable over the 16-week storage period, with only minor treatment-specific variations which were more apparent at the most severe AOP treatment condition (i.e., T2-40).

Although few studies have investigated the emulsion capacity of whole flaxseed after oxidative decontamination treatments during storage, findings from flaxseed and other oilseed protein isolate systems can help explain the mechanisms underlying the observed EC changes. Zhao et al. (2025) observed that moderate H₂O₂ oxidation (1 mM, approx. 34.01 ppm) of soy protein isolate (SPI) slightly improved EC from 20.8% to 21.1%, while higher H₂O₂ concentrations resulted in reduced EC. Similar results were obtained by Kong et al. (2013) for hydroxyl radical-stressed whey protein isolate (WPI), where emulsion properties improved with increasing H₂O₂ concentrations from 5 to 10 mM (approx. 170 to 340 ppm, respectively). Similarly, Chen et al. (2013) reported that moderate oxidation of SPI could generate soluble protein aggregates with more flexible structures, which improved emulsion properties. Pham et al. (2019) reported that complexation between flaxseed protein isolate and phenolic compounds altered emulsifying properties of flaxseed proteins, indicating that structural modification of flaxseed proteins can influence their adsorption at the oil-water interface and thereby affect emulsion properties. Although EC was not the main focus of their study, Wang et al. (2024) investigated alkyl peroxy radical oxidation in walnut protein emulsions and reported improved emulsifying performance under moderate oxidation, followed by a reduction at higher oxidation levels. Similar observations were reported by Liu et al. (2015), who oxidized SPI using a hydroxyl radical-generating system. In their study, they assessed emulsifying ability using the emulsifying activity index (EAI). These findings support the present observation that mild oxidative modification may enhance EC, while more extensive oxidation may reduce emulsifying ability.

II. Emulsion Stability (ES)

The emulsion stability (ES) of control and AOP-treated flaxseed was significantly ($p < 0.05$) affected by both AOP treatment ($p < 0.0001$) and storage time ($p < 0.0001$). The treatment $\times$ storage time interaction was not significant ($p = 0.774$).

Table 7. ANOVA table for emulsion stability (ES) of AOP-treated and stored flaxseed.

Independent variable	DF	F Value	p Value
Treatment	4	9.6028	<0.0001
Storage time	4	15.6600	<0.0001
Treatment $\times$ Storage time	16	0.6942	0.7735

Since the treatment $\times$ storage time interaction was not significant, and the main effects of treatment and storage time were significant ($p < 0.05$), Tukey's mean comparison test was conducted separately for the main effects. The ES values ranged from 49.72% to 51.97% across all treatments and storage periods, indicating relatively narrow variation despite statistically significant differences among treatments and storage times.

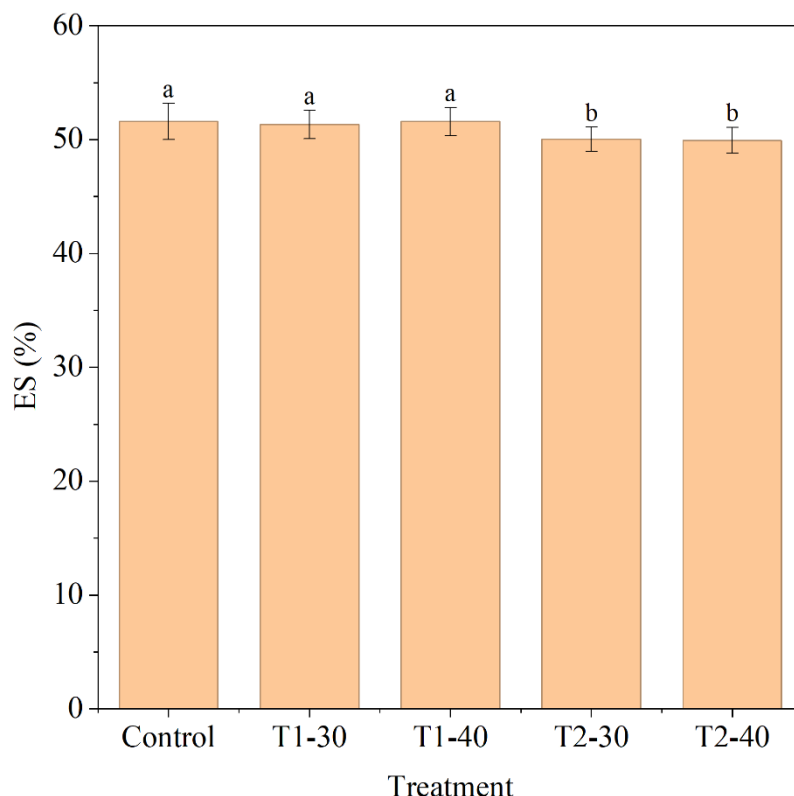


Figure 7. Mean emulsion stability (ES) values of whole ground flaxseed as a function of treatment (Control, T1-30, T1-40, T2-30 and T2-40). ES values were reported as the means of all storage times. Error bars represent $\pm$ standard deviation (n=3). Bars assigned with different letters are significantly different ($p < 0.05$).

As shown in Figure 7, the control, T1-30, and T1-40 treatments, which were not statistically different, had significantly higher ES values than T2-30 and T2-40 ($p < 0.05$).⁵ This suggests that the higher H₂O₂ flow-rate treatments may have caused limited changes in the components responsible for emulsion stabilization. However, the absolute difference between the highest and lowest treatment means was less than 2%, indicating that the practical impact of treatment on ES would be minimal. The slight decrease in ES observed for T2-30 and T2-40 (Figure 7) may be related to increased oxidative exposure under higher-intensity AOP conditions. Mild oxidation may expose hydrophobic groups of proteins and improve

⁵ Supplementary information is provided in Appendix, Table 18.

interfacial behaviour in emulsions (i.e. protein adsorption, interfacial tension, and bond formation at the oil-water interface), whereas stronger oxidative modification may promote protein aggregation, reduce protein solubility, or weaken the ability of proteins to migrate and adsorb at the oil-water interface (Li et al., 2021; M. Zhang et al., 2022; S. Zhang et al., 2025). Therefore, the lower ES values in T2-30 and T2-40 may reflect the oxidation-induced changes in flaxseed proteins or protein-mucilage interactions. However, because the reduction was minimal, these changes were likely minor rather than severe in techno-functional aspects.

Although the atmospheric pressure plasma jet (APPJ) is not identical to the AOP treatment used in the present study, it provides a useful comparison because both technologies generate ROS that can affect proteins and other cellular components. In flaxseed protein, Yu et al. (2020) reported that short APPJ exposure improved ES, which was attributed to mild oxidation-induced conformational changes. In contrast, longer APPJ exposure reduced protein solubility, increased particle size, and promoted disulphide bond formation, indicating protein aggregation. Therefore, the findings of Yu et al. (2020) support the possibility that mild oxidative modifications may enhance or preserve emulsifying techno-functionality, whereas excessive oxidative exposure may reduce ES through protein aggregation or reduced solubility. This is further supported by Chen et al. (2013), who evaluated the emulsifying properties of soy protein isolate after oxidation with peroxy radicals generated by 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) and storage for 15 days. Although AAPH oxidation is not identical to AOP treatment, peroxy radicals are relevant secondary oxidative species that may form during lipid oxidation following AOP treatments (Bouzayani et al., 2025). Chen et al. (2013) reported that moderate oxidation of soy protein isolate at 0.04 mmol/L AAPH (approximately 10.85 ppm) improved emulsion properties, whereas excessive oxidation at higher AAPH levels, such as 25 mmol/L AAPH (approximately 6780 ppm), promoted insoluble aggregate formation and reduced emulsion stability. This may suggest that the AOP treatment conditions used in the present study produced relatively low concentrations of hydroxyl radicals, thereby limiting oxidative modifications of the flaxseed components involved in emulsion stabilization.

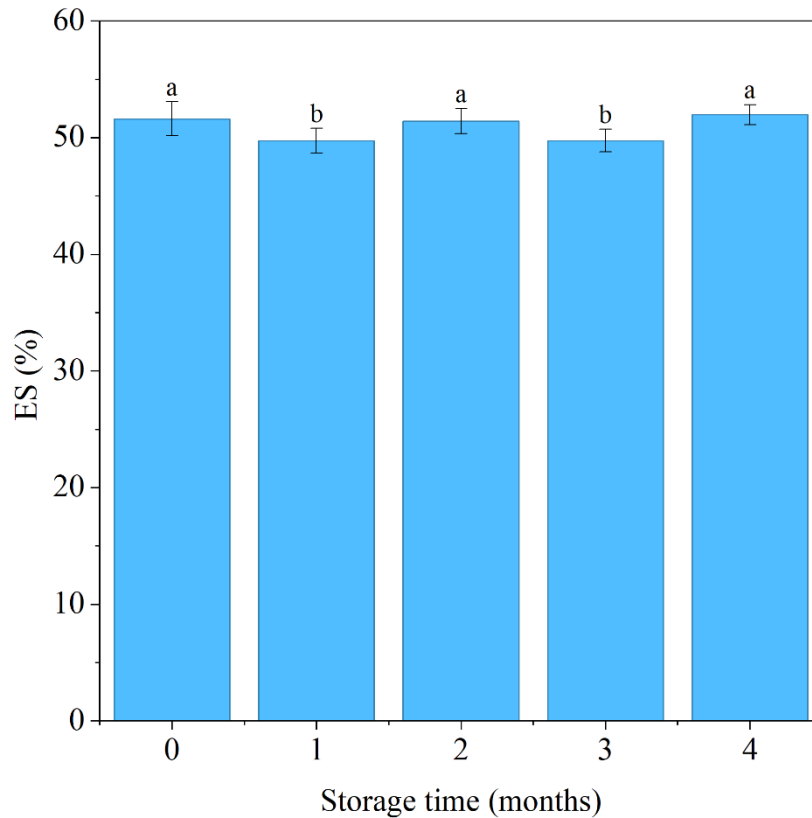


Figure 8. Mean emulsion stability (ES) values of whole ground flaxseed as a function of storage time. ES values are presented over 0, 1, 2, 3, and 4 months as the means of all treatments. Error bars represent $\pm$ standard deviation (n=3). Bars assigned with different letters are significantly different ($p < 0.05$).

Storage time also significantly affected ES, as shown by comparisons of mean ES values across treatments within each storage month. In Figure 8, the ES values before storage, after 2 months, and after 4 months were not significantly ($p < 0.05$) different from each other, with mean values of $51.61 \pm 1.46\%$, $51.41 \pm 1.08\%$, and $51.97 \pm 0.86\%$, respectively.⁶ These values were significantly ($p < 0.05$) higher than those observed after 1 and 3 months of storage, which were $49.72 \pm 1.06\%$ and $49.75 \pm 0.97\%$, respectively. However, the storage-related changes did not follow a continuous increasing or decreasing trend. Instead, ES fluctuated during storage and remained within a narrow range of approximately 49.7% to 52.0%. This fluctuation may indicate minor storage-related changes in the organization or interactions of proteins,

⁶ Supplementary information is provided in Appendix, Table 19.

mucilage, lipids, and other components in the flaxseed matrix. Because lipids are highly susceptible to oxidation, they can produce lipid peroxidation-derived free radicals, lipid hydroperoxides and reactive aldehydes, which may interact with protein oxidation (Refsgaard et al., 2000). However, since ES remained within a narrow range of approximately 49.7-52.0%, the storage-related variation appears to be limited. The absence of a clear downward trend suggests that the ES of whole ground flaxseed was largely maintained over the 4-month storage period. To investigate protein oxidation and its stability during storage, Ye et al. (2015) evaluated peanut protein isolate which was oxidized by peroxy radicals derived from AAPH. Their results showed that oxidation generally reduced the physical stability of peanut protein-stabilized emulsions, as reflected by increased mean droplet size, indicating reduced ES. However, the response was not entirely linear, indicating that the relationship between oxidation intensity and emulsion stability can be complex. This may help explain the present findings, where ES did not decline continuously during storage, possibly because the AOP conditions caused only limited oxidative modification of the protein components involved in emulsion stabilization.

The ES of flaxseed-related systems is mainly associated with the combined techno-functionality of proteins and mucilage (Nasrabadi et al., 2019). Flaxseed proteins can stabilize emulsions by adsorbing at the oil-water interface and forming a protective layer around oil droplets (Nwachukwu & Aluko, 2018; Yang et al., 2026). FG, as a polysaccharide-rich hydrocolloid, may also contribute to ES through its hydrocolloid behaviour. As a polysaccharide-rich fraction, FG can increase the viscosity of the aqueous phase in emulsion systems, which may reduce oil droplet movement and separation, as reported for FG and other polysaccharide-stabilized emulsions (Hellebois et al., 2022; Q. Liu et al., 2019). Furthermore, Wang et al. (2010) reported that flaxseed protein concentrate containing mucilage was effective in stabilizing oil-in-water emulsions, while Nasrabadi et al. (2019) showed that flaxseed protein-mucilage bioparticles enhanced the physical and oxidative stability of flaxseed oil emulsions. Similarly, Sun et al. (2019) Hu et al. (2020), reported and Lorenc et al. (2022) reviewed the contribution of FG to ES. These findings could

support the idea that both protein and mucilage contribute to the relatively stable ES values that were observed in the present study.

4.2.4 Least Gelation Concentration (LGC)

LGC represents the minimum concentration required for a sample to form a stable gel. It is commonly used as an indicator of gel-forming capacity. Generally, lower LGC values indicate better gelation capacity because less material is needed to form a continuous three-dimensional gel network (Asen & Aluko, 2022; Dapčević-Hadnađev et al., 2018). Flaxseed hydrocolloids, particularly polysaccharides and proteins, contribute to gel formation. In whole-ground flaxseed, FG may play an important role in gelation because its hydration and thickening properties can support gel network formation, while the relatively high denaturation temperature of flaxseed proteins may limit their unfolding under the applied heating conditions (Kaushik et al., 2016; Min et al., 2022).

pH can influence interactions among flaxseed proteins, polysaccharides, and other components involved in gel formation; thus, LGC was measured at pH 4.0, 5.5, and 7.0. These pH levels were selected to represent acidic (pH 4.0), mildly acidic (pH 5.5), and neutral (pH 7.0) conditions commonly encountered in food systems. Measuring LGC across this pH range provides a better understanding of the gel-forming behaviour of whole ground flaxseed and helps determine whether AOP treatment and storage affect this property differently under different pH conditions.

Table 8. ANOVA table for least gelation concentration (LGC) of AOP-treated and stored flaxseed at pH 4.0.

Independent variable	DF	F Value	p Value
Treatment	4	5.2194	0.0034
Storage time	4	6.6403	<0.0001
Treatment × Storage time	16	0.9121	0.5662

The LGC of whole ground flaxseed at pH 4.0 was significantly ($p < 0.05$) affected by both AOP treatment and storage time. The two-way ANOVA (Table 8) showed significant main effects of treatment ($p = 0.0034$) and storage time ($p < 0.0001$), while the treatment $\times$ storage time interaction was not significant ($p = 0.5662$). This indicates that the effect of AOP treatment on LGC was generally consistent throughout storage, and storage-related changes followed a similar pattern across treatments. Therefore, mean comparisons were interpreted in terms of the main effects of treatment and storage time.

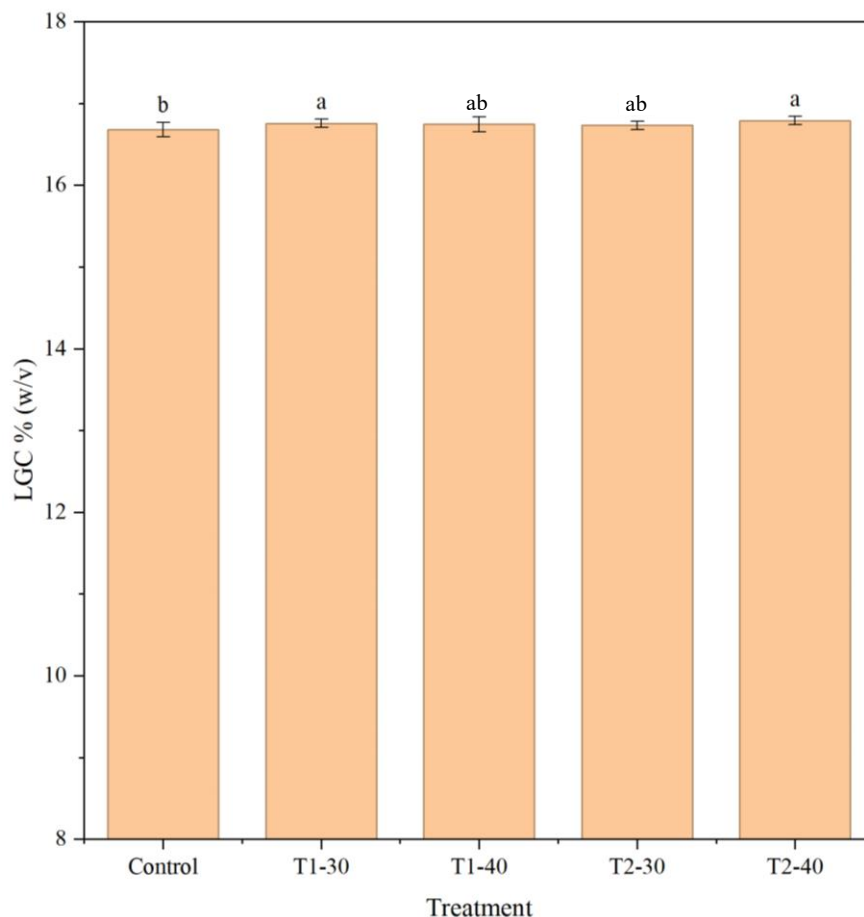


Figure 9. Mean least gelation concentration (LGC) values of whole ground flaxseed at pH 4.0 as a function of treatment (Control, T1-30, T1-40, T2-30 and T2-40). LGC values were reported as the means of all storage times. Error bars represent $\pm$ standard deviation ($n=3$). Bars assigned with different letters are significantly different ($p < 0.05$).

For the treatment effect, LGC values at pH 4.0 ranged from approximately 16.68 to 16.79% (w/v) across AOP treatments (Figure 9).⁷ The Control sample had the lowest LGC value, whereas T1-30 and T2-40 had significantly higher LGC values ($p < 0.05$). However, T1-40 and T2-30 were not significantly ($p < 0.05$) from either the Control or the T1-30 and T2-40 treatments, as indicated by their shared grouping letters. Although these differences were statistically significant, the numerical variation among treatments

⁷ Supplementary information is provided in Appendix, Table 20.

was very small, suggesting that AOP treatment caused only limited changes in the gelation behaviour of whole ground flaxseed at pH 4.0.

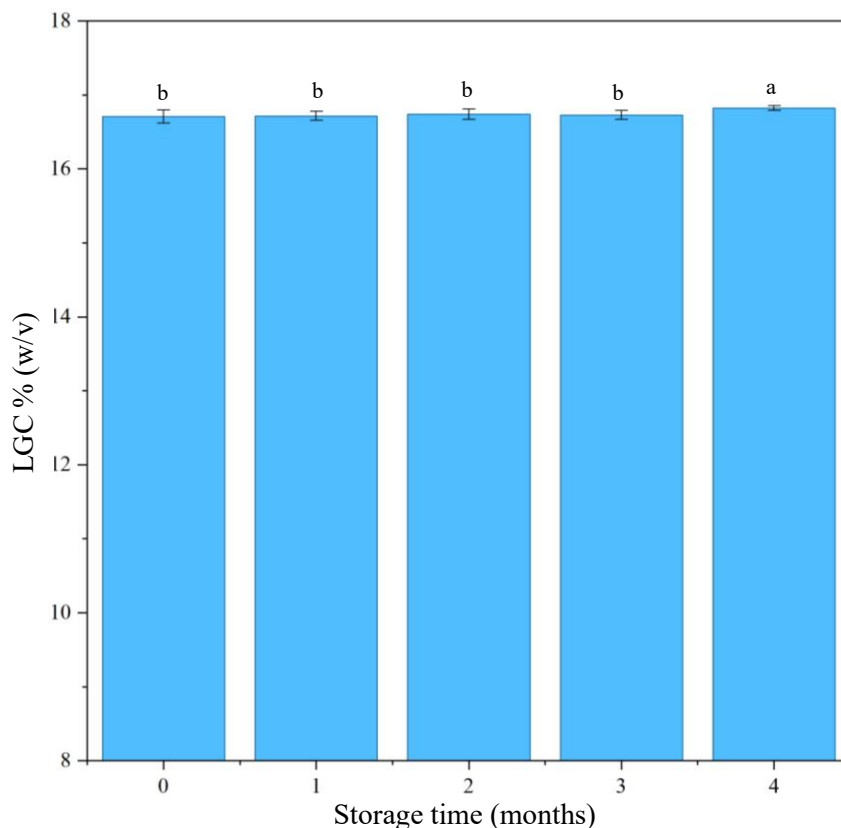


Figure 10. Mean least gelation concentration (LGC) values of whole ground flaxseed at pH 4.0 as a function of storage time. LGC values are presented over 0, 1, 2, 3, and 4 months as the means of all treatments. Error bars represent $\pm$ standard deviation ($n=3$). Bars assigned with different letters are significantly different ($p < 0.05$).

Storage time also significantly affected LGC at pH 4.0. The LGC values remained relatively stable from before storage to 3 months, ranging from 16.71 to 16.74% (w/v), with no significant differences among these time points (Figure 10).⁸ After 4 months of storage, LGC increased significantly ($p < 0.05$) to 16.82% (w/v). This suggests that extended storage may have caused small structural or compositional changes that slightly reduced the gel-forming ability of whole ground flaxseed. These changes could be related to gradual

⁸ Supplementary information is provided in Appendix, Table 21.

protein conformational changes, oxidation-related aggregation, or interactions between proteins and oxidized lipid products during storage. However, the increase was very small, indicating that flaxseed's gelation capacity was largely maintained throughout the 4-month storage period.

The LGC of whole ground flaxseed at pH 5.5 was significantly ($p < 0.05$) influenced by both AOP treatment ($p = 0.0077$) and storage time ($p = 0.0073$) (Table 7). The treatment $\times$ storage time interaction was not significant ($p = 0.4955$). This indicates that the effect of AOP treatment on LGC at pH 5.5 was generally consistent throughout the storage period, and that changes during storage were similar among treatments. Therefore, mean comparisons were interpreted in terms of the main effects of treatment and storage time.

Table 9. ANOVA table for least gelation concentration (LGC) of AOP-treated and stored flaxseed at pH 5.5.

Independent variable	DF	F Value	p Value
Treatment	4	4.425	0.0077
Storage time	4	4.4698	0.0073
Treatment $\times$ Storage time	16	0.9899	0.4955

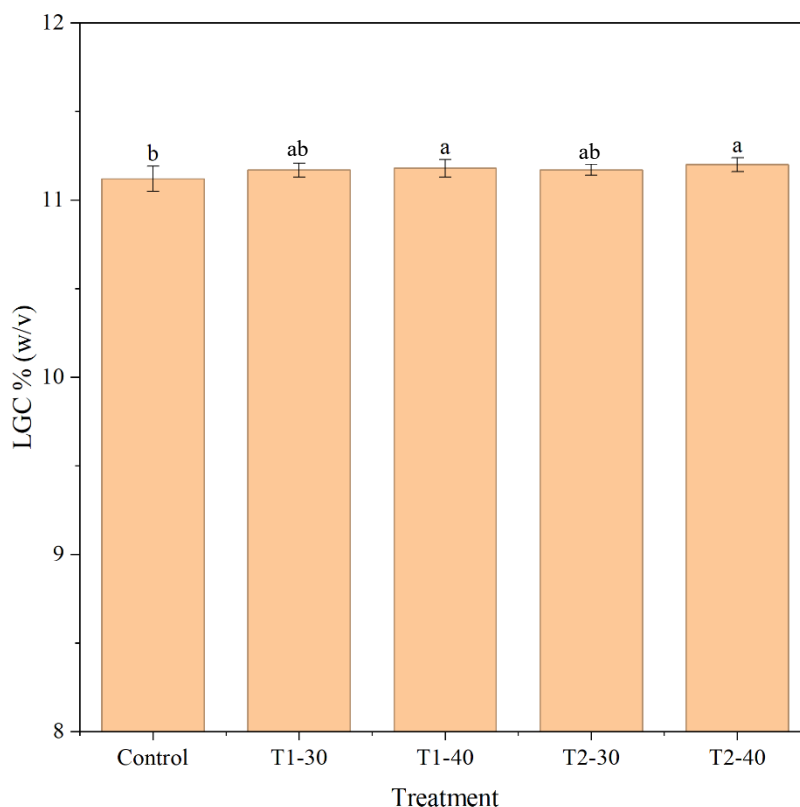


Figure 11. Mean least gelation concentration (LGC) values of whole ground flaxseed at pH 5.5 as a function of treatment (Control, T1-30, T1-40, T2-30 and T2-40). LGC values were reported as the means of all storage times. Error bars represent $\pm$ standard deviation ($n=3$). Bars assigned with different letters are significantly different ($p < 0.05$).

For the treatment effect, LGC values ranged from 11.12 to 11.19% (w/v) (Figure 11).⁹ The control sample showed the lowest LGC value of 11.12% (w/v), while both T1-40 and T2-40 showed significantly higher LGC values ($p < 0.05$) of 11.18% and 11.19% (w/v), respectively. The latter differed significantly ($p < 0.05$) from the control, as indicated by different distinct Tukey grouping letters. In contrast, T1-30 and T2-30 shared letters with the control, T1-40, and T2-40 treatments, indicating that these treatments were not significantly ($p < 0.05$) different from any of these groups. Although the treatment effect was statistically significant, the numerical difference between the lowest and highest treatment means was very small, approximately 0.08% (w/v). This suggests that AOP treatment caused only minor changes in the gelation behaviour of whole ground flaxseed at pH 5.5.

⁹ Supplementary information is provided in Appendix, Table 22.

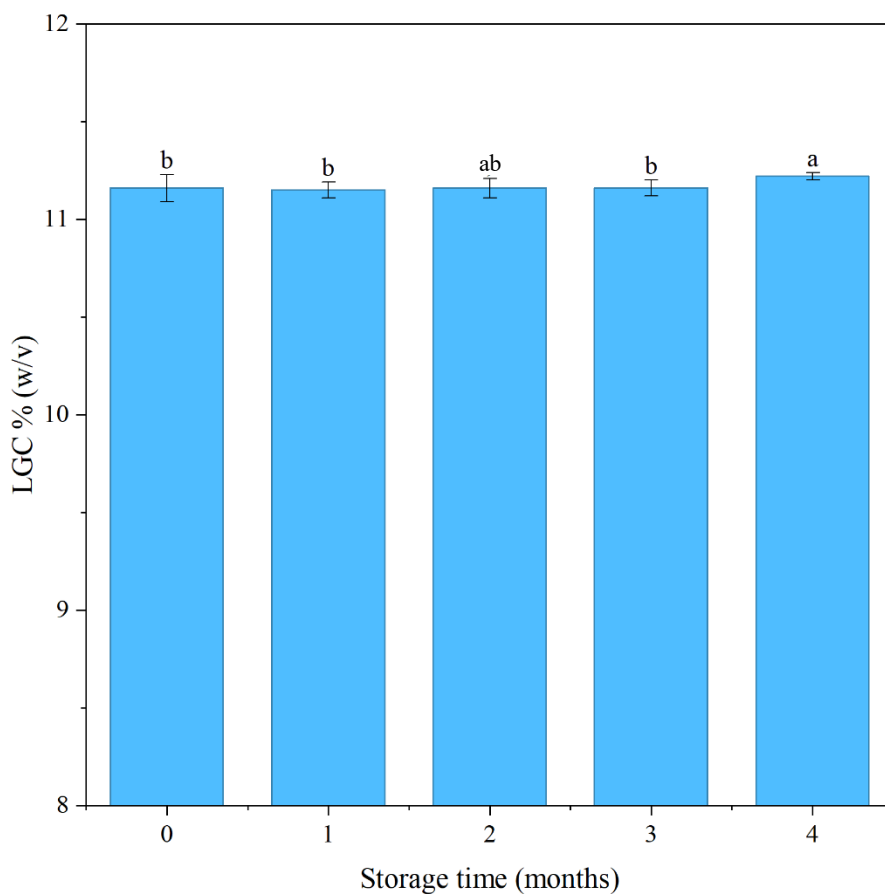


Figure 12. Mean least gelation concentration (LGC) values of whole ground flaxseed at pH 5.5 as a function of storage time. LGC values are presented over 0, 1, 2, 3, and 4 months as the means of all treatments. Error bars represent $\pm$ standard deviation ($n=3$). Bars assigned with different letters are significantly different ($p < 0.05$).

Storage time also significantly affected LGC at pH 5.5 (Figure 12).¹⁰ The LGC values remained relatively stable from before storage to 3 months, ranging from 11.15 to 11.16% (w/v), with no major numerical variation among these time points. The highest LGC value was observed after 4 months of storage, with a mean of 11.22% (w/v), which was significantly higher ($p < 0.05$) than the values at 0, 1, and 3 months. Month 2 showed an intermediate response and was not significantly different ($p < 0.05$) from

¹⁰ Supplementary information is provided in Appendix, Table 23.

either the earlier storage months or month 4. Similar to the treatment effect, the storage-related increase in LGC was statistically significant but small in magnitude.

Table 10. ANOVA table for least gelation concentration (LGC) of AOP-treated and stored flaxseed at pH 7.0.

Independent variable	DF	F Value	p Value
Treatment	4	3.4227	0.0231
Storage time	4	6.6193	<0.0001
Treatment × Storage time	16	1.6241	0.1346

The LGC of whole ground flaxseed at pH 7.0 was significantly ($p < 0.05$) affected by AOP treatment ($p = 0.0231$) and storage time ($p < 0.0001$), while the treatment × storage time interaction was not significant ($p = 0.1346$) (Table 10), indicating that the effect of AOP treatment on LGC was generally consistent across the storage period. Therefore, mean comparisons were performed for the main effects of treatment and storage time.

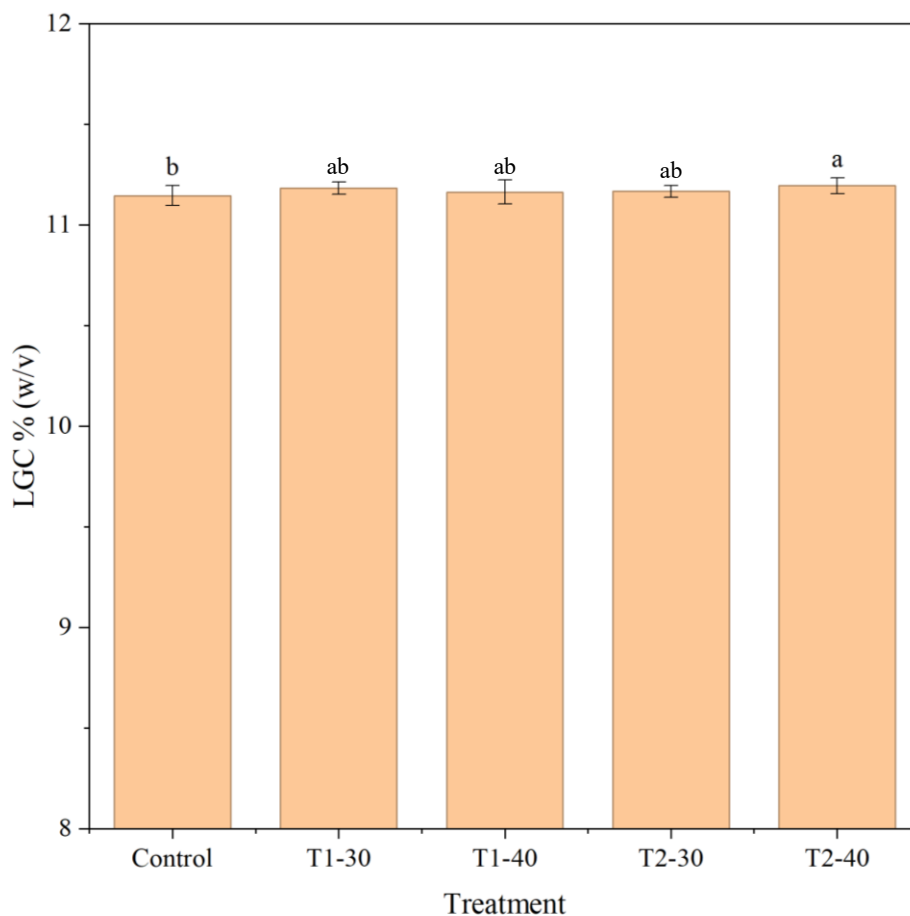


Figure 13. Mean least gelation concentration (LGC) values of whole ground flaxseed at pH 7.0 as a function of treatment (Control, T1-30, T1-40, T2-30 and T2-40). LGC values were reported as the means of all storage times. Error bars represent $\pm$ standard deviation ($n=3$). Bars assigned with different letters are significantly different ($p < 0.05$).

For the treatment effect, mean LGC values were approximately 11.15-11.20% (w/v) (Figure 13).¹¹ The control sample showed the lowest LGC value, (11.15% w/v), while T2-40 showed the highest (11.20% w/v). These two treatments did not share the same grouping letter, indicating a significant difference. In contrast, T1-30, T1-40, and T2-30 shared grouping letters with both the control and T2-40, indicating that these treatments did not differ significantly ($p < 0.05$) from either. Although the treatment effect was

¹¹ Supplementary information is provided in Appendix, Table 24.

statistically significant, the numerical difference among treatment means was very small, approximately 0.05% (w/v). This suggests that AOP treatment caused only minor changes in the gelation behaviour of whole ground flaxseed at pH 7.0.

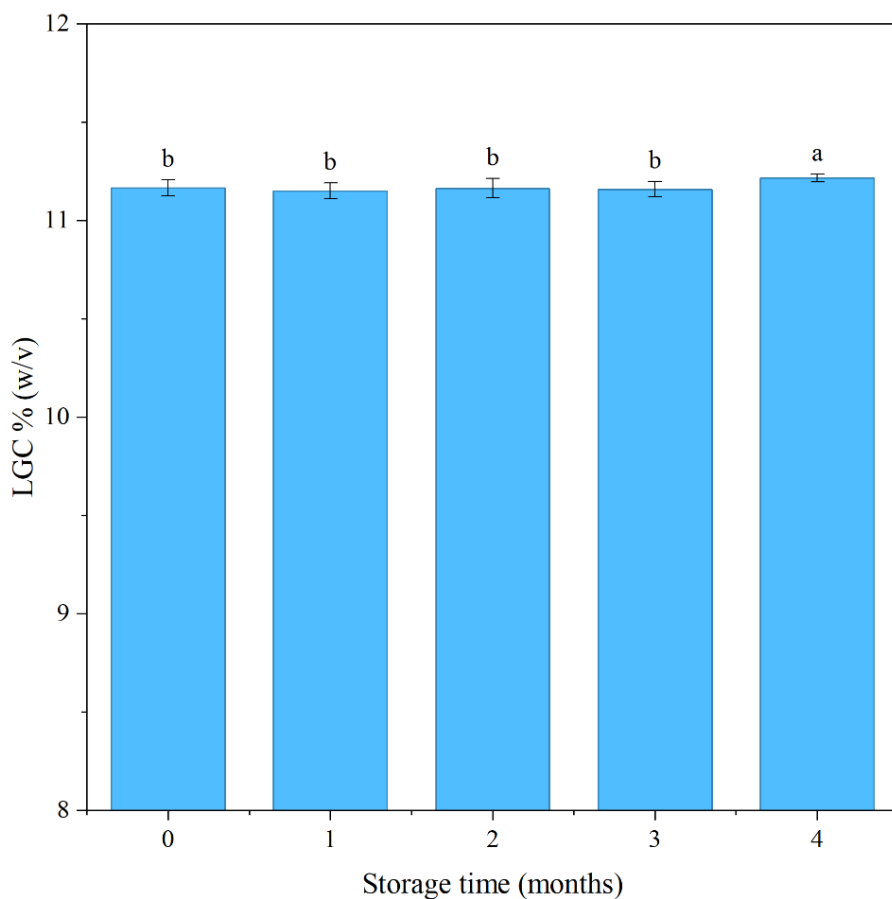


Figure 14. Mean least gelation concentration (LGC) values of whole ground flaxseed at pH 7.0 as a function of storage time. LGC values are presented over 0, 1, 2, 3, and 4 months as the means of all treatments. Error bars represent $\pm$ standard deviation ($n=3$). Bars assigned with different letters are significantly different ($p < 0.05$).

Storage time also had a significant effect on LGC at pH 7.0 (Figure 14).¹² The LGC values remained relatively stable from storage through 3 months, ranging from 11.15% to 11.17% (w/v). These storage periods shared the same grouping letter, indicating no significant differences among them. However, after 4 months of storage, the LGC increased slightly to 11.22% (w/v), and this time point did not share the same grouping letter with the earlier storage periods. This indicates that the LGC after 4 months was significantly

¹² Supplementary information is provided in Appendix, Table 25.

higher ($p < 0.05$) than the values observed from 0 to 3 months. Despite this significant difference, the overall increase during storage was small, with a difference of approximately 0.07% (w/v) between the lowest and highest mean LGC values.

In the present study, LGC was significantly ($p < 0.05$) influenced by AOP treatment and storage time at pH 4.0, 5.5, and 7.0; however, the treatment $\times$ storage time interaction was not significant at any tested pH level. This suggests that the effect of AOP treatment on gel-forming capacity was generally consistent during storage, and that storage-related changes followed a similar pattern among all treatment groups. Across all pH levels, the treatment effect was statistically significant ($p < 0.05$), but the actual numerical differences among treatments were very small. At pH 4.0, LGC values ranged from approximately 16.68 to 16.79% (w/v), whereas at pH 5.5 and 7.0, they ranged from approximately 11.12 to 11.20% (w/v). The LGC values observed in the present study were lower than those reported by Rani and Badwaik (2021) for flaxseed cake, mustard seed cake, and soybean seed cake, which were 18.00% (w/v). However, the same study reported a lower LGC value of 8.00% (w/v) for defatted flaxseed cake. Similarly, Elsorady (2020) reported considerably lower LGC values for flaxseed and sesame protein isolates at 3.60% and 5.00% (w/v), respectively. These differences may reflect variations in sample composition, especially protein concentration, fat content, mucilage, and other fibre contents across studies. Protein isolates generally contain a higher proportion of protein and fewer non-protein components, which can promote gel network formation at lower concentrations. In contrast, whole ground flaxseed contains a complex mixture of protein, soluble fibre (mucilage), insoluble fibres, and lipids, which may require a higher sample concentration to form a stable gel.

The isoelectric point (pI) is the pH at which proteins have no net electrical charge and are least soluble, making them more likely to aggregate (Pereira et al., 2024) and thereby limiting their ability to unfold and form a uniform gel network. The pI of flaxseed proteins is approximately pH 4.2 (Hellebois et al., 2022; Y. Lan et al., 2020; Sá et al., 2025). However, in whole flaxseed systems, interactions between proteins and carbohydrate components, particularly FG, may alter protein solubility and aggregation near

the pI, thereby influencing gelation behaviour (Kaushik et al., 2016; Y. Lan et al., 2020). The higher LGC observed at pH 4.0 compared with pH 5.5 and 7.0 may be related to the pH-dependent behaviour of flaxseed proteins and other hydrocolloids. As FG is an anionic polysaccharide with negative charges from ionised carboxyl groups, it can form gels by repelling similar charges, thereby promoting molecular chain stretching and interaction to form a gel network (Chen et al., 2006). At low pH, the negative charge is reduced, so the chains interact less and form weaker gels. Also, gel formation depends on the balance between protein solubility, unfolding, aggregation, and intermolecular interactions (Pang et al., 2025). Therefore, the higher LGC at pH 4.0 may reflect the combined effects of reduced FG ionization and reduced protein solubility near the isoelectric region, both of which could limit the formation of a continuous gel network.

In contrast, the lower LGC values at pH 5.5 and 7.0 indicate better gelation ability under near-neutral conditions. This may be due to improved hydration, dispersion, and molecular interactions of flaxseed proteins and polysaccharides away from the isoelectric pH region (Tirgar et al., 2017). In agreement with this, Chen et al. (2006) reported that flaxseed gum gel strength was affected by pH, with stronger gelation generally observed around pH 6.0-9.0 compared with more acidic conditions (pH 4.0). Furthermore, flaxseed contains both proteins and other hydrocolloids, and the techno-functional properties of flaxseed ingredients are strongly influenced by the composition and structure of the whole material. Lan et al. (2020) showed that flaxseed proteins extracted from whole flaxseed and flaxseed meal differed in structural and functional properties, indicating that the original matrix and processing can strongly affect protein techno-functionality. Since whole ground flaxseed contains protein, mucilage, fibre, and lipids, the observed gelation behaviour likely reflects the combined contribution of these components.

The slight increase in LGC after 4 months of storage, observed across the tested pH levels, may suggest a minor reduction in gel-forming capacity over storage. Since higher LGC indicates that more material is required to form a stable gel, this increase may reflect minor structural or compositional changes in the flaxseed. Possible mechanisms include gradual protein conformational changes, limited oxidation-induced aggregation, or interactions between proteins and lipid oxidation products during storage. Protein

oxidation can modify amino acid side chains, reduce sulfhydryl groups, promote disulphide bond formation, and induce aggregation, all of which may influence hydration, solubility, and thereby gel network formation (Liao et al., 2026; L. Xiong et al., 2025). In addition to protein-related changes, possible changes in mucilage hydration or protein-polysaccharide interactions during storage may also have contributed to the slight increase in LGC.

The potential role of oxidation is particularly relevant because AOP treatments produce radicals that can react with food components, including lipids and proteins. Although studies directly examining the effects of AOP treatment on gelation properties are limited, previous studies using other non-thermal or modification treatments have shown that structural changes in oilseed proteins can alter gelation behaviour. For instance, Malik et al. (2017) investigated the effects of gamma irradiation on sunflower protein isolates and found that the treatment improved gelation properties. Similarly, Karabulut & Yemiş (2022) showed that high-intensity ultrasound-treated hemp seed protein isolate exhibited improved gelling capacity, as evidenced by low LGC values. Flores-Jiménez et al. (2019) also showed partial denaturation of canola protein isolates by high-intensity ultrasound, leading to improved gelation properties. However, the very small LGC changes observed within each pH level suggest that the AOP conditions used were not severe enough to cause major deterioration in the gel-forming components of whole ground flaxseed. The absence of a significant treatment $\times$ storage time interaction further suggests that AOP-treated flaxseed maintained relatively stable gelation ability throughout the 4-month storage period.

4.2.5 Protein Solubility (PS)

Protein solubility (PS) of whole ground flaxseed was measured at pH 4.0, 5.5, and 7.0 to evaluate changes in PS under different pH conditions after AOP treatment and storage. Unlike protein isolates or concentrates, whole ground flaxseed contains proteins together with lipids, mucilage, fibre, and other seed components, all of which may influence PS. Therefore, measuring PS at different pH levels provides a realistic understanding of how AOP-treated flaxseed may behave in food systems with acidic to neutral pH conditions.

AOP treatment, storage time, and their interaction significantly affected the PS of whole ground flaxseed at pH 4.0 ($p < 0.05$) (Table 11). The significant treatment $\times$ storage time interaction ($p = 0.0198$) indicated that the effect of AOP treatment on PS varied across storage periods. Therefore, mean comparisons were interpreted using simple-effects analyses rather than discussing treatment and storage effects independently.

Table 11. ANOVA table for protein solubility (PS) of AOP-treated and stored flaxseed at pH 4.0.

Independent variable	DF	F Value	p Value
Treatment	4	4.4404	0.0076
Storage time	4	111.0604	<0.0001
Treatment $\times$ Storage time	16	2.4914	0.0198

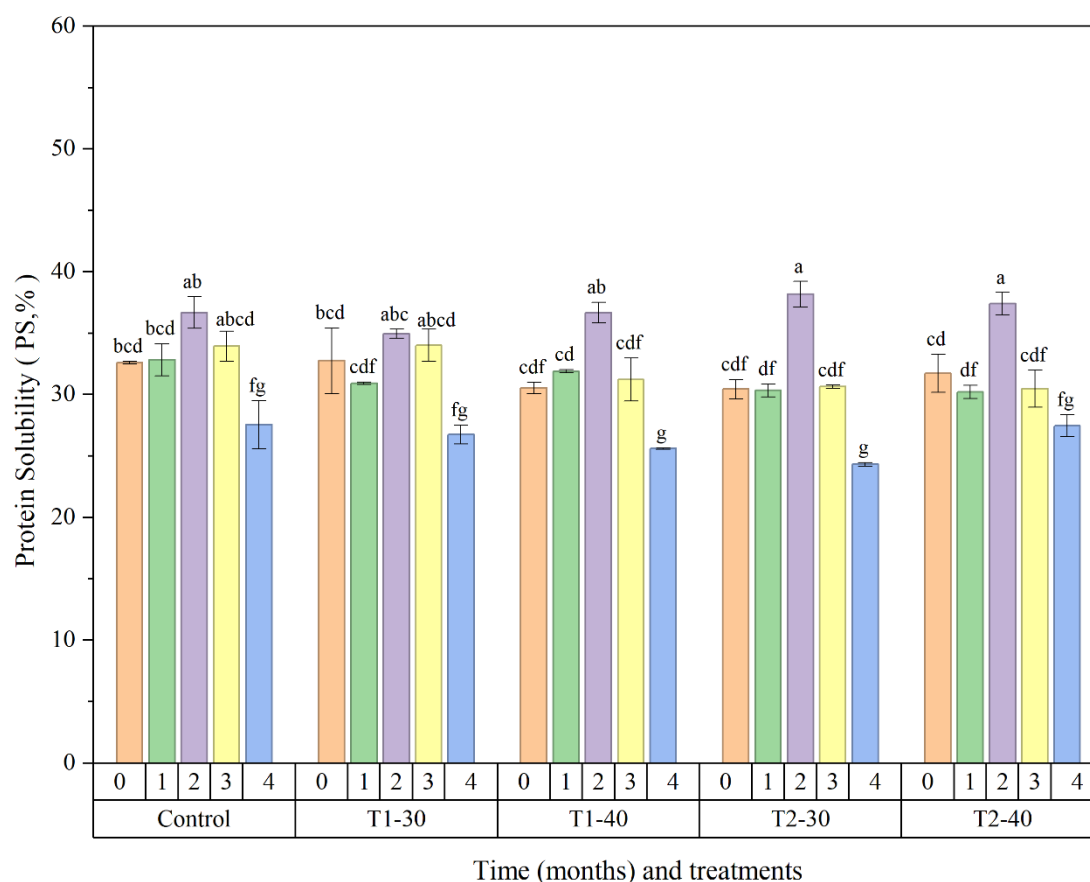


Figure 15. Protein solubility (PS) values of whole ground flaxseed at pH 4.0 as a function of storage time (0, 1, 2, 3, and 4 months) and treatment (Control, T1-30, T1-40, T2-30 and T2-40). Error bars represent $\pm$ standard deviation ($n=3$). Bars assigned with different letters are significantly different ($p < 0.05$).

As shown in Figure 15, PS values ranged from 24.3 to 38.2% across all treatments and storage periods.¹³ Before storage, PS values were relatively similar among the control and AOP-treated samples, ranging from 30.4 to 32.8%. During storage, all treatments showed a noticeable increase in PS at 2 months, where the highest values were observed. Among these, T2-30 at 2 months showed the highest PS value (38.2%), followed by T2-40 (37.4%), Control and T1-40 (36.7%), and T1-30 (34.9%). These values were significantly higher ($p < 0.05$) than those for several other treatment-storage combinations, as indicated by the Tukey grouping letters. After 2 months, PS generally decreased in all samples. At 3 months, PS values declined to approximately 30.5-34.0%, depending on treatment. The control and T1-30 samples maintained slightly higher PS values at this stage, whereas higher AOP-intensity treatments showed lower values. By

¹³ Supplementary information is provided in Appendix, Table 26.

4 months, PS decreased markedly across all treatments, ranging from 24.3 to 27.5%. The lowest PS values were observed in T2-30 (24.3%) and T1-40 (25.6%) in 4 months, while the control and T2-40 had slightly higher values, both around 27.5%. The temporary increase in PS during the early storage period, particularly at 2 months, may indicate partial structural loosening or improved extractability of protein components during storage (Kaur et al., 2025). However, the later decrease by month 4 suggests that prolonged storage may have promoted lipid and protein oxidation, protein aggregation, or other oxidative changes that could reduce soluble protein levels (Drożdowska et al., 2021; Yadav et al., 2018; N. Zhao et al., 2026).

PS at pH 5.5 was significantly ($p < 0.05$) affected by storage time and by the treatment $\times$ storage time interaction, while the main effect of treatment was not significant (Table 12). Therefore, mean comparisons were interpreted based on the combined effects of treatment and storage time rather than on treatment or storage time alone.

Table 12. ANOVA table for protein solubility (PS) of AOP-treated and stored flaxseed at pH 5.5.

Independent variable	DF	F Value	p Value
Treatment	4	0.3096	0.8688
Storage time	4	52.8189	<0.0001
Treatment $\times$ Storage time	16	4.9035	<0.0001

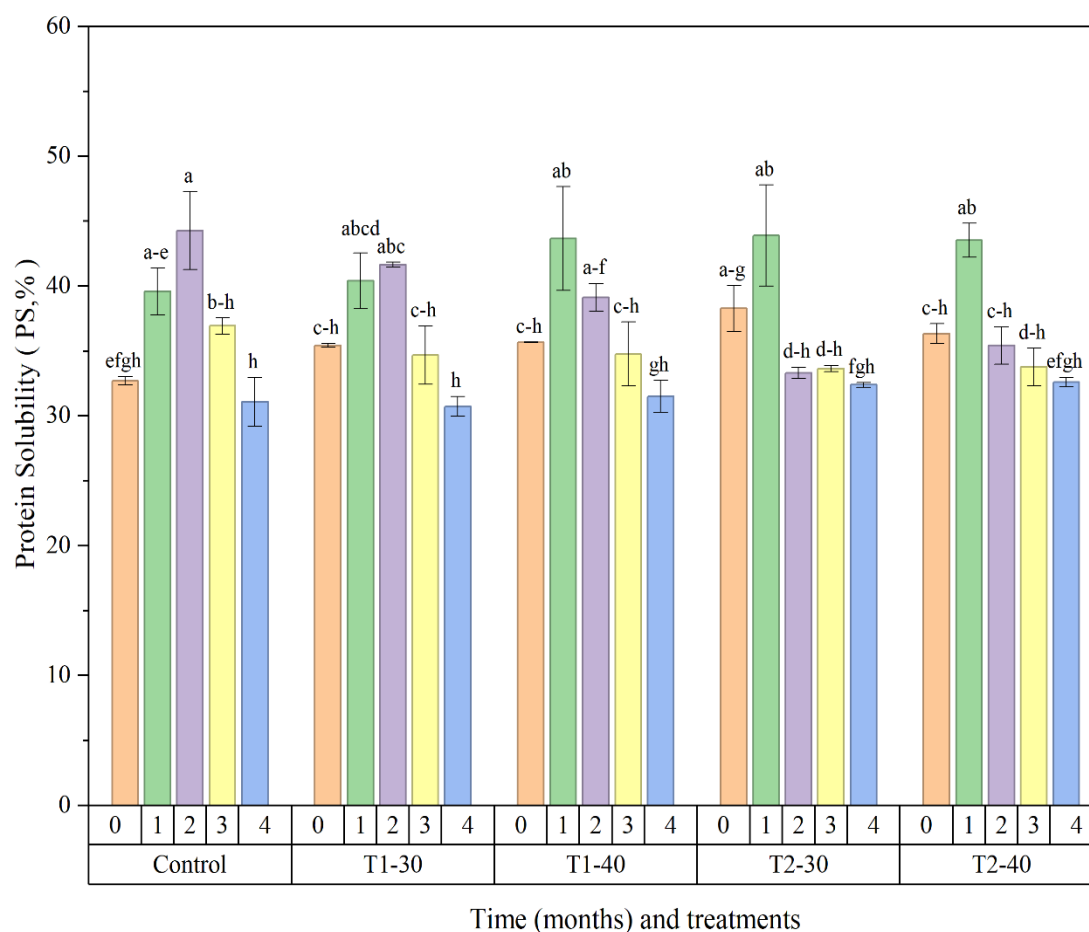


Figure 16. Protein solubility (PS) values of whole ground flaxseed at pH 5.5 as a function of storage time (0, 1, 2, 3, and 4 months) and treatment (Control, T1-30, T1-40, T2-30 and T2-40). Error bars represent $\pm$ standard deviation ($n=3$). Bars assigned with different letters are significantly different ($p < 0.05$).

At pH 5.5, PS values ranged from 30.7 to 44.3% across all treatments and storage periods (Figure 16).¹⁴ Before storage, the PS values were 32.7, 35.4, 35.7, 38.3, and 36.3% for the control and treatment samples T1-30, T1-40, T2-30, and T2-40, respectively. During storage, PS generally increased early on, particularly peaking at 1 month, then declined toward month 4. The control sample increased from 32.7% before storage to 39.6% at month 1, then reached a peak of 44.3% at month 2, before decreasing to 36.9% at month 3 and 31.1% at month 4. A similar early increase was observed for T1-30, with PS increasing with PS rising from 35.4% before storage to 40.4% and 41.6% in months 1 and 2, respectively, then decreasing

¹⁴ Supplementary information is provided in Appendix, Table 27.

to 34.7% in month 3 and 30.7% in month 4. The T1-40 sample showed a slightly different trend, with PS increasing from 35.7% before storage to 43.6% at month 1, then gradually declining to 39.1% at month 2, 34.8% at month 3, and 31.5% at month 4. A similar trend was observed for T2-30 and T2-40.

At pH 7.0, PS of AOP-treated and stored flaxseed was significantly ($p < 0.05$) affected by storage time, while the effect of treatment and the treatment $\times$ storage time interaction were not significant (Table 13). Therefore, mean comparisons were interpreted in terms of the main effect of storage time.

Table 13. ANOVA table for protein solubility (PS) of AOP-treated and stored flaxseed at pH 7.0.

Independent variable	DF	F Value	p Value
Treatment	4	1.3424	0.2820
Storage time	4	13.2792	<0.0001
Treatment $\times$ Storage time	16	1.1999	0.3324

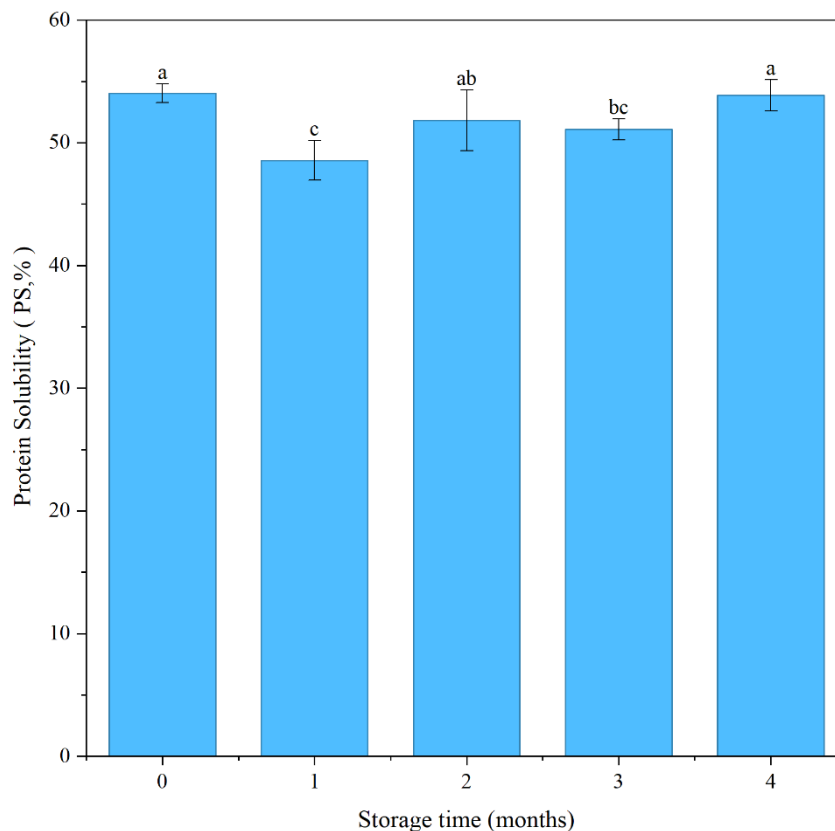


Figure 17. Mean protein solubility (PS) values of whole ground flaxseed at pH 7.0 as a function of storage time. PS values are presented over 0, 1, 2, 3, and 4 months as the means of all treatments. Error bars represent $\pm$ standard deviation ($n=3$). Bars assigned with different letters are significantly different ($p < 0.05$).

As shown in Figure 17, PS values at pH 7.0 ranged from 48.6 to 54.0% during storage.¹⁵ The highest PS values were observed before storage and after 4 months of storage (54.0 and 53.9%, respectively). These two time points did not differ significantly, suggesting that PS at pH 7.0 was largely retained by the end of the storage period. In contrast, PS decreased significantly after 1 month of storage, reaching a minimum of 48.6%. This value was significantly lower than those observed at 0 and 4 months. At 2 months, PS increased to 51.8%, while at 3 months it was 51.1%. These results indicate that storage time caused moderate fluctuations in PS at pH 7.0 but it later recovered by the end of storage. PS value of 68.3% was reported for

¹⁵ Supplementary information is provided in Appendix, Table 28.

whole flaxseed at around pH 7.0 by Oomah & Mazza (1998), and Martínez-Flores et al. (2006) reported similar values around pH 7.0 for flaxseed protein concentrates. However, the PS values observed at pH 7.0 in the present study were higher than the 33% reported for flaxseed protein isolate by Yang et al. (2026).

Overall, PS results indicate that pH and storage time influenced PS, while the effect of AOP treatment depended on pH. Overall, PS was lowest at pH 4.0, intermediate at pH 5.5, and highest at pH 7.0. This trend is expected because flaxseed proteins show reduced solubility near their isoelectric region, where the net protein charge is low and protein-protein attraction, or aggregation, is favored. Flaxseed proteins have been reported to have a pI around pH 4.0-4.75 (Krause et al., 2002; Sá et al., 2025). Previous studies on flaxseed protein isolate reported the lowest solubility around pH 4.0. At pH values above the isoelectric region, proteins develop a greater net negative charge, increasing electrostatic repulsion between protein molecules and reducing aggregation. This allows more protein molecules to remain dispersed and interact with surrounding water through their exposed polar and charged groups, thereby increasing PS (Bekhit et al., 2018; Lan et al., 2020). Elsorady (2020) reported that flaxseed protein isolate had the lowest solubility at pH 4.0 and much higher solubility at pH 7.0, consistent with the PS observed at pH levels in the present study. Similarly, different flaxseed protein concentrates prepared from flaxseed meal showed the lowest PS at approximately pH 4.0 and the highest solubility at pH 7.0 (Tirgar et al., 2017). Similar trends were reported for flaxseed protein concentrates and isolates by Martínez-Flores et al. (2006) and Krause et al. (2002), respectively. Furthermore, similar pH-dependent PS trends have been reported in other oilseed protein systems. Flores-Jiménez et al. (2019), Malomo et al. (2014), and Zhao et al. (2021) reported similar solubility patterns for canola protein isolates, hemp protein isolates, and perilla protein isolates, respectively.

Compared with pH 4.0, the higher PS at pH 5.5 may be due to a greater distance from the isoelectric region, allowing more protein hydration and increasing solubility. However, our findings differ slightly from Martínez-Flores et al. (2006), who reported the lowest PS at approximately pH 4.8, whereas solubility at pH 4.0 remained around 30% for flaxseed protein concentrates. This difference may be attributed to

compositional differences between the samples, as whole-ground flaxseed and flaxseed protein concentrates can differ substantially in protein content, mucilage, and other components. The lower PS values at pH 5.5 compared with pH 7.0 may be explained by pH 5.5 being closer to the isoelectric range, where reduced net protein charge can limit protein solubility.

However, Sakai et al. (2022) reported that H₂O₂ improved PS of soy protein isolates, although the pH at which PS was measured was not specified. Yao et al. (2019) observed a similar PS improvement in proteins extracted from *Camellia oleifera* seed cake at around pH 4.0. Flores-Jiménez et al. (2019) reported that high-intensity ultrasound, which can generate ROS (Mashiko et al., 2018), similar to AOP treatment, improved PS of canola protein isolate across the evaluated pH range of 2.0 to 12.0. This may help explain the temporary increase in PS observed during the early storage period in the present study, as mild oxidative modification can expose hydrophilic groups and improve PS (Guo et al., 2022). However, stronger or prolonged oxidation may promote aggregation and reduce solubility. Furthermore, Guo et al. (2022) directly treated protein extracts with chemical oxidants, whereas the present study applied AOP treatment to whole ground flaxseed. Thus, differences in sample matrix and oxidation method should be considered when comparing the findings.

Flaxseed gum mucilage, as a hydrocolloid, can increase medium viscosity and interfere with protein extraction or dispersion, which may influence PS (Kaushik et al., 2016; Lorenc et al., 2022). Similarly, Nasrabadi et al. (2019) reported that flaxseed mucilage improved PS. Lan et al. (2020) suggested that the lower pI of flaxseed protein concentrate (pH 3.0) compared with flaxseed protein isolate (pH 4.2) was associated with the presence of mucilage and the protein composition in the concentrate. The authors further noted that mucilage can form soluble complexes with flaxseed proteins, thereby enhancing PS within certain pH ranges. The decline in PS observed at later storage periods, especially at pH 4.0 and 5.5, may partly reflect storage-induced oxidation-related changes rather than a direct effect of AOP treatment alone. Flaxseed is rich in unsaturated lipids, particularly α -linolenic acid (Parikh et al., 2019), which makes it susceptible to lipid oxidation during storage, which can promote protein oxidation and aggregation. Primary

and secondary lipid oxidation products can promote protein oxidation, leading to structural changes and altered surface hydrophobicity. These modifications may affect techno-functional properties including PS (Berton-Carabin et al., 2016; Geng et al., 2023; Y. L. Xiong & Guo, 2021). Also, defatting of flaxseed oilseeds elevates their relative protein content, which may contribute to higher measured PS (Galves et al., 2019; Safdar et al., 2020).

Overall, the results show that pH was the key factor influencing PS, with the highest PS at pH 7.0 and lower PS at pH 4.0 and 5.5. The main changes in PS were more closely associated with storage time, particularly an early increase followed by a later decrease at pH 4.0 and 5.5.

5. Conclusion

This study investigated the effects of advanced oxidative process (AOP) decontamination, employing O₃, H₂O₂, and UV-C radiation on the techno-functional properties of whole brown flaxseed during four months of storage. The research addressed an important gap in understanding how AOP treatment affects the techno-functional quality of flaxseed for a wide range of food applications.

Overall, the findings demonstrated that the AOP conditions and storage period studied had statistically significant ($p < 0.05$) effects on techno-functional properties; however, the magnitude of these changes was generally small. Throughout storage, WHC, OHC, EC, ES, LGC, and PS remained within relatively narrow ranges, indicating that the techno-functional attributes of flaxseed were largely preserved following AOP treatment. Although some treatment- and storage-related differences were observed, they were not sufficient to substantially alter the techno-functionality of whole ground flaxseed under the conditions investigated. The highest treatment intensity (T2-40) generally exhibited slightly greater deviations than the other treatments, suggesting that increased oxidative exposure may induce minor changes to flaxseed protein and protein-polysaccharide interactions while maintaining overall techno-functional qualities. PS was influenced mainly by pH rather than AOP treatment, with the lowest values at pH 4.0 and higher solubility at pH 5.5 and 7.0. Similarly, LGC was strongly dependent on pH, demonstrating improved gelation ability under near-neutral conditions. These findings indicate that the physicochemical

characteristics of flaxseed proteins remained the primary determinants of techno-functionality after AOP treatment.

Collectively, the results demonstrate that AOP treatment preserved the techno-functional characteristics of whole brown flaxseed while introducing only minor techno-functional modifications. These findings indicate that combining O₃, H₂O₂, and UV-C radiation is a promising non-thermal decontamination approach that can maintain the quality attributes required for food processing applications. The outcomes of this study contribute to the growing body of knowledge on sustainable non-thermal decontamination technologies and provide valuable scientific evidence supporting the potential application of AOP as an effective microbial decontamination strategy for flaxseed without compromising its techno-functional performance.

6. Study limitations and recommendations for future research

This study provides valuable insights into the effects of advanced oxidative process (AOP) decontamination on the techno-functional properties of whole brown flaxseed during storage. However, we considered several limitations when interpreting the findings and discuss them below as directions for future research.

The present study evaluated a single brown flaxseed cultivar (AAC Bravo) under controlled storage conditions. Flaxseed composition can vary depending on type, cultivar, growing environment, and harvest year. However, between brown and yellow flaxseed, yellow flaxseed generally contains lower concentrations of phenolic compounds and pigments in the seed coat and may differ in mucilage and protein composition and lipid profile. These compositional differences could influence flaxseed's response to oxidative treatments and affect its techno-functional properties. Similarly, cultivar, growing environment, harvest year and other related factors could cause compositional differences in flaxseed. Therefore, future research could investigate the effects of AOP treatment across multiple flaxseed types and cultivars

produced under different environmental and agronomic conditions and harvest years to determine impacts on techno-functionality and establish the broader applicability of the AOP decontamination strategy.

The storage evaluation was limited to four months under controlled conditions. In Manitoba, flaxseed is typically harvested between mid-September to end of October (Canadian Grain Commission, 2019) and may be stored for several months before processing, marketing, or export, depending on market demand and storage logistics. Therefore, the four-month storage period evaluated in this study represents a post-harvest storage interval during which flaxseed may remain in storage before utilization. However, commercial flaxseed can also be stored for considerably longer periods under certain conditions. Extending the storage study duration would provide a more comprehensive understanding of the long-term stability of AOP-treated flaxseed and the persistence of its techno-functional properties over time.

The research focused on selected techno-functional properties, including WHC, OHC, EC, ES, LGC, and PS. Future studies should incorporate additional quality assessments, including lipid and protein oxidation, rheological behaviour, thermal properties, nutritional composition, bioactive compound retention, and colour and sensory characteristics, to provide a more comprehensive evaluation of the effects of AOP treatment on flaxseed techno-functionality.

A limitation of the present study is that microbial decontamination efficacy and lipid oxidation were not directly evaluated as part of the study. Microbial reduction is a critical parameter for determining the effectiveness of AOP as a decontamination technology, while lipid oxidation is particularly important for flaxseed because of its high content of polyunsaturated fatty acids and susceptibility to oxidative deterioration. Consequently, the absence of microbial reduction data limits the ability to directly relate the AOP treatment conditions to their decontamination effectiveness, while the absence of lipid oxidation measurements limits the assessment of whether the oxidative treatment may promote undesirable lipid deterioration. Future studies should therefore simultaneously evaluate microbial reduction and lipid oxidation, together with techno-functional properties, to establish the balance between microbial safety and

quality preservation. Measurements of lipid oxidation indicators, such as peroxide value, and/or thiobarbituric acid reactive substances (TBARS), along with appropriate microbial analyses, would provide a more comprehensive assessment of AOP treatment efficacy and its potential effects on flaxseed quality.

Although the AOP treatment conditions were selected based on prior AOP decontamination studies, future investigations should integrate microbial reduction, physicochemical testing, nutritional composition testing, and techno-functional performance into a single experimental framework to establish optimized AOP processing conditions that maximize microbial safety while maintaining desirable flaxseed quality attributes.

Finally, this study was conducted at the laboratory scale. The instrument's treatment capacity, was approximately 90 kg per hour. However, pilot-scale and industrial-scale validation studies are required to evaluate the scalability, operational feasibility, economic viability, and commercial implementation of AOP technology for flaxseed processing. Furthermore, assessing the performance of AOP-treated flaxseed in finished food products, such as bakery products, beverages, and plant-based food formulations, would provide valuable information regarding its techno-functionality under practical processing conditions and support the adoption of AOP as a sustainable and efficient decontamination technology within the food industry.

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Appendices

Table 14. Mean comparison of WHC values of whole ground flaxseed for interactions.

Treatment	Time period	WHC	SD	CV%	Interactions' grouping letters
Control	0	3.544	0.04	1.13	ab
Control	1	3.397	0.06	1.77	ab
Control	2	3.568	0.02	0.56	ab
Control	3	3.583	0.19	5.30	ab
Control	4	3.519	0.07	1.99	ab
T1-30	0	3.621	0.25	6.90	ab
T1-30	1	3.384	0.09	2.66	ab
T1-30	2	3.617	0.03	0.83	ab
T1-30	3	3.677	0.03	0.82	ab
T1-30	4	3.44	0.05	1.45	ab
T1-40	0	3.7141	0.07	1.76	a
T1-40	1	3.486	0.14	4.02	ab
T1-40	2	3.426	0.02	0.58	ab
T1-40	3	3.4674	0.04	1.06	ab
T1-40	4	3.622	0.03	0.83	ab
T2-30	0	3.671	0.04	1.09	ab
T2-30	1	3.579	0.04	1.12	ab
T2-30	2	3.514	0.06	1.71	ab
T2-30	3	3.4158	0.08	2.30	ab
T2-30	4	3.623	0.07	1.93	ab
T2-40	0	3.523	0.06	1.70	ab
T2-40	1	3.434	0.13	3.79	ab
T2-40	2	3.478	0.06	1.73	ab
T2-40	3	3.324	0.09	2.71	b
T2-40	4	3.376	0.01	0.30	ab

Table 15. Mean comparison of OHC values of whole ground flaxseed for treatments.

Treatments	Mean $\pm$ SD	Groups
Control	1.939 $\pm$ 0.06	a
T1-30	1.889 $\pm$ 0.06	ab
T1-40	1.874 $\pm$ 0.02	b
T2-30	1.917 $\pm$ 0.07	ab
T2-40	1.888 $\pm$ 0.05	ab

Means that do not share a letter are significantly different.

Table 16. Mean comparison of OHC values of whole ground flaxseed for storage time.

Storage time	Mean $\pm$ SD	Groups
0	1.869 $\pm$ 0.06	b
1	1.926 $\pm$ 0.03	ab
2	1.952 $\pm$ 0.07	a
3	1.897 $\pm$ 0.03	ab
4	1.862 $\pm$ 0.04	b

Means that do not share a letter are significantly different.

Table 17. Mean comparison of EC values of whole ground flaxseed for interactions.

Treatment	Time period	EC	SD	CV%	Interactions' grouping letters
Control	Before storage	44.33	0.22	0.50	a
Control	After 1 month	42.24	1.21	2.87	abc
Control	After 2 month	43.22	0.05	0.12	abc
Control	After 3 month	43.03	0.52	1.22	abc
Control	After 4 month	43.76	0.09	0.21	abc
T1-30	Before storage	43.54	0.36	0.83	abc
T1-30	After 1 month	42.01	0.64	1.52	abc
T1-30	After 2 month	43.56	0.11	0.25	abc
T1-30	After 3 month	44.52	0.08	0.18	a
T1-30	After 4 month	43.33	1.66	3.83	abc
T1-40	Before storage	43.93	1.59	3.62	abc
T1-40	After 1 month	43.44	0.28	0.64	abc
T1-40	After 2 month	43.16	0.69	1.60	abc
T1-40	After 3 month	43.44	0.24	0.55	abc
T1-40	After 4 month	42.92	0.81	1.89	abc
T2-30	Before storage	43	1.33	3.09	abc
T2-30	After 1 month	44.17	0.21	0.48	ab
T2-30	After 2 month	43.2	0.27	0.63	abc
T2-30	After 3 month	42.34	0.24	0.57	abc
T2-30	After 4 month	42.26	0.65	1.54	abc
T2-40	Before storage	42.9	0.9	2.10	abc
T2-40	After 1 month	43.57	0.14	0.32	abc
T2-40	After 2 month	41.95	0.69	1.64	abc
T2-40	After 3 month	41.42	0.27	0.65	bc
T2-40	After 4 month	41.29	0.95	2.30	c

Table 18. Mean comparison of ES values of whole ground flaxseed for treatments.

Treatment	Mean	Groups
Control	51.59 ±1.6	a
T1-30	51.323 ±1.24	a
T1-40	51.58 ±1.24	a
T2-30	50.022 ±1.07	b
T2-40	49.933 ±1.13	b

Means that do not share a letter are significantly different.

Table 19. Mean comparison of ES values of whole ground flaxseed for storage time.

Storage time	Mean	Groups
0	51.605 ±1.46	a
1	49.724 ±1.06	b
2	51.408 ±1.08	a
3	49.746 ±0.97	b
4	51.965 ±0.86	a

Means that do not share a letter are significantly different.

Table 20. Mean comparison of LGC values of whole ground flaxseed at pH 4.0 for treatments.

Treatment	Mean ±SD	Groups
Control	16.682 ±0.09	b
T1-30	16.758 ±0.05	a
T1-40	16.746 ±0.09	ab
T2-30	16.732 ±0.05	ab
T2-40	16.792 ±0.05	a

Means that do not share a letter are significantly different.

Table 21. Mean comparison of LGC values of whole ground flaxseed at pH 4.0 for storage time.

Storage time	Mean ±SD	Groups
0	16.706 ±0.09	b
1	16.716 ±0.06	b
2	16.739 ±0.07	b
3	16.729 ±0.06	b
4	16.820 ±0.03	a

Means that do not share a letter are significantly different.

Table 22. Mean comparison of LGC values of whole ground flaxseed at pH 5.5 for treatments.

Treatment	Mean $\pm$ SD	Groups
Control	11.123 $\pm$ 0.07	b
T1-30	11.173 $\pm$ 0.04	ab
T1-40	11.18 $\pm$ 0.05	a
T2-30	11.172 $\pm$ 0.03	ab
T2-40	11.199 $\pm$ 0.04	a

Means that do not share a letter are significantly different.

Table 23. Mean comparison of LGC values of whole ground flaxseed at pH 5.5 for storage time.

Storage time	Mean $\pm$ SD	Groups
0	11.156 $\pm$ 0.07	b
1	11.149 $\pm$ 0.04	b
2	11.164 $\pm$ 0.05	ab
3	11.159 $\pm$ 0.04	b
4	11.219 $\pm$ 0.02	a

Means that do not share a letter are significantly different.

Table 24. Mean comparison of LGC values of whole ground flaxseed at pH 7.0 for treatments.

Treatment	Mean $\pm$ SD	Groups
Control	11.146 $\pm$ 0.03	b
T1-30	11.184 $\pm$ 0.01	ab
T1-40	11.164 $\pm$ 0.07	ab
T2-30	11.167 $\pm$ 0.04	ab
T2-40	11.195 $\pm$ 0.03	a

Means that do not share a letter are significantly different.

Table 25. Mean comparison of LGC values of whole ground flaxseed at pH 7.0 for storage time.

Storage time	Mean $\pm$ SD	Groups
0	11.166 $\pm$ 0.04	b
1	11.15 $\pm$ 0.04	b
2	11.164 $\pm$ 0.05	b
3	11.159 $\pm$ 0.04	b
4	11.217 $\pm$ 0.02	a

Means that do not share a letter are significantly different.

Table 26. Mean comparison of PS values of whole ground flaxseed for interactions at pH 4.0.

Treatment	Storage time	PS	±SD	CV	Interactions' grouping letters
Control	0	32.6	0.1	0.3	bcd
Control	1	32.84	1.31	3.98	bcd
Control	2	36.68	1.28	3.49	ab
Control	3	33.93	1.23	3.63	abcd
Control	4	27.54	1.96	7.11	fg
T1-30	0	32.76	2.67	8.16	bcd
T1-30	1	30.91	0.11	0.34	cdf
T1-30	2	34.94	0.37	1.05	abc
T1-30	3	34.01	1.32	3.87	abcd
T1-30	4	26.74	0.76	2.86	fg
T1-40	0	30.53	0.46	1.46	cdf
T1-40	1	31.89	0.15	0.47	cd
T1-40	2	36.67	0.85	2.31	ab
T1-40	3	31.23	1.75	5.59	cdf
T1-40	4	25.59	0.06	0.22	g
T2-30	0	30.44	0.79	2.6	cdf
T2-30	1	30.33	0.52	1.7	df
T2-30	2	38.17	1.04	2.72	a
T2-30	3	30.64	0.14	5.82	cdf
T2-30	4	24.32	0.15	0.61	g
T2-40	0	31.73	1.55	4.88	cd
T2-40	1	30.22	0.54	1.8	df
T2-40	2	37.39	0.91	2.42	a
T2-40	3	30.48	1.5	4.92	cdf
T2-40	4	27.47	0.88	3.19	fg

Table 27. Mean comparison of PS values of whole ground flaxseed for interactions at pH 5.5.

Treatment	Storage time	PS	±SD	CV	Interactions' grouping letters
Control	0	32.7	0.32	0.97	efgh
Control	1	39.58	1.8	4.54	a-e
Control	2	44.27	3.01	6.8	a
Control	3	36.92	0.61	1.66	b-h
Control	4	31.08	1.87	6.03	h
T1-30	0	35.43	0.13	0.36	c-h
T1-30	1	40.4	2.14	5.29	abcd
T1-30	2	41.64	0.19	0.46	abc
T1-30	3	34.67	2.25	6.48	c-h
T1-30	4	30.73	0.77	2.51	h
T1-40	0	35.67	0.01	0.02	c-h
T1-40	1	43.64	4	9.17	ab
T1-40	2	39.13	1.08	2.77	a-f
T1-40	3	34.76	2.47	7.09	c-h
T1-40	4	31.5	1.22	3.88	gh
T2-30	0	38.27	1.77	4.62	a-g
T2-30	1	43.88	3.9	8.9	ab
T2-30	2	33.31	0.44	1.32	d-h
T2-30	3	33.65	0.23	0.69	d-h
T2-30	4	32.38	0.18	0.55	fgh
T2-40	0	36.32	0.78	2.14	c-h
T2-40	1	43.51	1.31	3.01	ab
T2-40	2	35.41	1.45	4.09	c-h
T2-40	3	33.77	1.44	4.28	d-h
T2-40	4	32.61	0.36	1.11	efgh

Table 28. Mean comparison of PS values of whole ground flaxseed at pH 7.0 for storage time.

Storage time	Mean	Groups
0	54.04 ±0.76	a
1	48.56 ±1.63	c
2	51.81 ±2.48	ab
3	51.08 ±0.85	bc
4	53.86 ±1.26	a

Means that do not share a letter are significantly different.