

**DEVELOPMENT OF GREEN PROCESS FOR
EXTRACTION OF BIOACTIVE COMPOUNDS FROM
FLAXSEED**

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by

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DEDICATED TO
MY LATE GRANDMOTHER
&
MY PARENTS

Certificate

This is to certify that the thesis entitled “**Development of Green Process for Extraction of Bioactive Compound from Flaxseed**” being submitted by **Ms Vidhi Hitesh Bhimjiyani** to the Indian Institute of Technology Delhi for the award of the degree of **Doctor of Philosophy** is a record of bonafide research work carried out by her under my guidance and supervision in conformity with the rules and regulations of Indian Institute of Technology, Delhi. The research report and results presented in this thesis have not been submitted, in part or in full, to any other university or institute for the award of any degree or diploma.

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Abstract

In today's world, the interest of people is growing towards the consumption of healthy and nutritious food. Consumption of functional foods is growing day by day due to their health benefits; on account of this, the production of safer and bioactive compound-rich food is the need of the hour. This can be possible by developing green solvent-free extraction processes for bioactive compounds and knowing the chemistry of bioactive compounds in terms of their applicability in the proper food system. Researchers, scientists, and food manufacturers now have a greater obligation to provide consumers with naturally antioxidant-rich foods. Due to the constant expansion of the population and the need for adequate nutrition, the search for nutritious foods addresses malnutrition, vitamin and mineral deficiencies, and the treatment of chronic diseases. Functional nutrients and nutraceuticals have paved the way for resolving these problems. Nonetheless, new nutritional pressures, such as nutrition transition, are emerging.

Flax is an oilseed praised for high omega-3 oil, fibre and lignan content. Supercritical carbon dioxide (SCo₂) can be used as a green solvent alternative to extract flax bioactive compounds from flaxseed. In this work, the green process is designed to develop different bioactive compounds from flaxseed, i.e., mucilage, oil and lignan. The process of enrichment of flaxseed oil is developed to increase the nutritive value of flaxseed oil. The flaxseed oil is extracted using four different extraction techniques like supercritical fluid extraction, solvent extraction, mechanical extraction and ultrasound-assisted extraction. All characteristics of extracted flaxseed oil are analysed using AOCS analytical methods. It was found that supercritical extracted flaxseed oil has high alpha-linolenic acid content as compared to other extracted oil.

All the physical properties, including length, width, thickness, weight, geometric diameter, bulk density, true density, porosity, and angle of repose, are studied with respect to moisture content. The effect of moisture content on the physical properties by adding different moisture in the range of 2-15% is studied in detail.

The flaxseed mucilage is the gel layer that is present in the flaxseed, and it can be extracted when the seed is soaked in water. The polysaccharide at the epidermal cell layer of the seed coat then becomes viscous in contact with water. The mucilage is extracted by using flax seeds (*Linum usitatissimum* L.) by using hot water in a 1: 10 (w/v) ratio. The effects of different drying methods (oven drying, vacuum drying, spray drying, and drying with ethanol precipitated) on the functional properties of flaxseed mucilage were evaluated. The chemical composition, yield percentage, physicochemical properties, functional properties, and gel strength of mucilage were studied and compared with those of untreated samples. This study shows that the different drying method has significant effects on the properties of dry mucilage. It is found that spray-dried mucilage powder shows a better yield as compared to the mucilage powder obtained by other drying methods. The oven-dried sample showed the best foaming capacity and foam stability. These results provide guidelines for selecting the best drying method for flaxseed mucilage. The final product flaxseed mucilage powder (dry form) can be utilized as a stabilizer in the food and cosmetic industry.

Flaxseed is the principal source of the compound secoisolariciresinol diglucoside (SDG). Flaxseed is a significant source of functional food or nutraceutical components because of its advantageous fatty acid composition and high fibre content. In this work, pressurised liquid extraction of SDG lignan from flaxseed was studied to determine the most efficient extraction conditions. In the aim to do this, the effects of extraction factors, including temperature (150, 160, and 170 °C), extraction time (5, 15, and 30 minutes), and sample amount (5 and 10 g), were investigated. SDG lignan analysis has been carried out using HPLC. It was established that the SDG lignan concentration of water extracts was significantly influenced by temperature, extraction duration, and sample size. The maximum concentration (12.94 mg/g) and extraction yield (72.57%) were obtained using 5 g of flaxseed deoiled cake at 160 °C for 15 min. with 40% fresh water.

Due to its high concentration of omega-3 fatty acids, flaxseed oil is now utilised as a significant functional dietary component. Carotenoids, however, which could improve the oil's oxidative stability,

are absent from flaxseed oil. In this part of study, cold-pressed flaxseed oil is enriched with carotenoids from Seabuckthorn pomace using ultrasound-assisted extraction technique. The carotenoid content of the flaxseed oil was increased by optimising the process parameters using the Box-Behnken design method. Due to its high concentration of -3 fatty acids (-linolenic acid), flaxseed oil is becoming a significant functional dietary component. But the oxidative stability-enhancing carotenoids are absent from natural flaxseed oil. The ultrasound-assisted extraction extracts carotenoids from Seabuckthorn pomace and enrich it directly in the cold-pressed flaxseed oil. A by-product of the Seabuckthorn business that is high in carotenoid concentration is Seabuckthorn pomace, is utilized for enrichment. Through Box-Behnken design, the process parameters (time, feed ratio, and Amplitude%) were enhanced to maximise the amount of carotenoid in the enriched flaxseed oil. According to the statistical analysis's findings, a yield of 14.02 mg/L of carotene was obtained at parameters time-75.56 min, feed oil ratio-19.94, and amplitude-80.81%. The process optimised parameters showed that, when the same enrichment conditions were used for both the ultrasound-assisted extraction approach and traditional extraction (mixing), the yield from the ultrasound-assisted extraction technique was found to be increased by 46.96%. This shows that the ultrasound-assisted extraction approach greatly accelerates the extraction of carotenoid in a less amount of time as compared to tradition extraction method. The enhancement of flaxseed oil's nutritional content through the use of ultrasound technology is a quick and efficient process that does not require the use of solvents. UAE can take the place of the traditional method of oil enrichment. Using the metrics of peroxide value and rancimat, the quality and stability of oil were investigated. It is contrasted with their unenriched oils. Foods can be supplemented with enriched flaxseed oil, or it can be utilised in medications and nutraceuticals. For the purpose of comparing the quality and stability of flaxseed oil before and after enrichment, thorough physicochemical characterization was done.

सारांश

आज के समय में लोगों की रुचि स्वस्थ और पौष्टिक भोजन के सेवन की ओर बढ़ रही है। कार्यात्मक खाद्य पदार्थों की खपत उनके स्वास्थ्य लाभों के कारण दिन-ब-दिन बढ़ रही है; इसके कारण, सुरक्षित और बायोएक्टिव यौगिक युक्त भोजन का उत्पादन समय की मांग है। यह बायोएक्टिव यौगिकों के लिए हरित विलायक-मुक्त निष्कर्षण प्रक्रियाओं को विकसित करने और उचित खाद्य प्रणाली में उनकी प्रयोज्यता के संदर्भ में बायोएक्टिव यौगिकों के रसायन विज्ञान को जानने से संभव हो सकता है। शोधकर्ताओं, वैज्ञानिकों और खाद्य निर्माताओं का अब उपभोक्ताओं को प्राकृतिक रूप से एंटीऑक्सीडेंट से भरपूर खाद्य पदार्थ उपलब्ध कराने का बड़ा दायित्व है। जनसंख्या के निरंतर विस्तार और पर्याप्त पोषण की आवश्यकता के कारण, पौष्टिक खाद्य पदार्थों की खोज कुपोषण, विटामिन और खनिज की कमी और पुरानी बीमारियों के उपचार को संबोधित करती है। कार्यात्मक पोषक तत्वों और न्यूट्रास्यूटिकल्स ने इन समस्याओं के समाधान का मार्ग प्रशस्त किया है। बहरहाल, पोषण संक्रमण जैसे नए पोषण संबंधी दबाव उभर रहे हैं।

अलसी एक ऐसा तिलहन है जिसकी उच्च ओमेगा-3 तेल, फाइबर और लिगनेन सामग्री के लिए प्रशंसा की जाती है। अलसी से बायोएक्टिव यौगिकों को निकालने के लिए सुपरक्रिटिकल कार्बन डाइऑक्साइड (एससीओ₂) का उपयोग हरित विलायक विकल्प के रूप में किया जा सकता है। इस कार्य में, हरित प्रक्रिया को अलसी से विभिन्न बायोएक्टिव यौगिकों, यानी, श्लेष्म, तेल और लिगनेन को विकसित करने के लिए डिज़ाइन किया गया है। अलसी के तेल के पोषक मूल्य को बढ़ाने के लिए अलसी के तेल के संवर्धन की प्रक्रिया विकसित की गई है। अलसी का तेल चार अलग-अलग निष्कर्षण तकनीकों जैसे सुपरक्रिटिकल द्रव निष्कर्षण, विलायक निष्कर्षण, यांत्रिक निष्कर्षण और अल्ट्रासाउंड-सहायता निष्कर्षण का उपयोग करके निकाला जाता है। निकाले गए अलसी के तेल की सभी विशेषताओं का विश्लेषण एओसीएस विश्लेषणात्मक तरीकों का उपयोग करके किया जाता है। यह पाया गया कि सुपरक्रिटिकल निकाले गए अलसी के तेल में अन्य निकाले गए तेल की तुलना में अल्फा-लिनोलेनिक एसिड की मात्रा अधिक होती है। नमी की मात्रा के संबंध में लंबाई, चौड़ाई, मोटाई, वजन, ज्यामितीय व्यास, थोक घनत्व, वास्तविक घनत्व, सरंध्रता और विश्राम के कोण सहित सभी भौतिक गुणों का अध्ययन किया जाता है। 2-15% की सीमा में विभिन्न नमी जोड़कर भौतिक गुणों पर नमी की मात्रा के प्रभाव का विस्तार से अध्ययन किया गया है।

अलसी का श्लेष्मा जेल की परत है जो अलसी में मौजूद होती है, और जब बीज को पानी में भिगोया जाता है तो इसे निकाला जा सकता है। बीज आवरण की एपिडर्मल कोशिका परत पर पॉलीसेकेराइड पानी के संपर्क में चिपचिपा हो जाता है। 1:10 (w/v) अनुपात में गर्म पानी का उपयोग करके अलसी के बीज (लिनम यूसिताटिसिमम एल.) का उपयोग करके श्लेष्मा निकाला जाता है। अलसी के श्लेष्म के कार्यात्मक गुणों पर विभिन्न सुखाने के तरीकों (ओवन सुखाने, वैक्यूम सुखाने, स्प्रे सुखाने और अवक्षेपित इथेनॉल के साथ सुखाने) के प्रभावों का मूल्यांकन किया गया था। श्लेष्मा की रासायनिक संरचना, उपज प्रतिशत, भौतिक रसायन गुण, कार्यात्मक गुण और जेल शक्ति का अध्ययन किया गया और अनुपचारित नमूनों की तुलना की गई। इस अध्ययन से पता चलता है कि अलग-अलग सुखाने की विधि का शुष्क श्लेष्मा के गुणों पर महत्वपूर्ण प्रभाव पड़ता है। यह पाया गया है कि स्प्रे-सूखे म्यूसिलेज पाउडर अन्य सुखाने के तरीकों से प्राप्त म्यूसिलेज पाउडर की तुलना में बेहतर उपज दिखाता है। ओवन में सुखाए गए नमूने में सर्वोत्तम फोमिंग क्षमता और फोम स्थिरता दिखाई दी। ये परिणाम अलसी के श्लेष्मा के लिए सर्वोत्तम सुखाने की विधि का चयन करने के लिए दिशानिर्देश प्रदान करते हैं। अंतिम उत्पाद अलसी म्यूसिलेज पाउडर (सूखा रूप) का उपयोग खाद्य और कॉस्मेटिक उद्योग में स्टेबलाइजर के रूप में किया जा सकता है।

अलसी यौगिक सेकोआइसोलैरिसिरेसिनॉल डिग्लुकोसाइड (एसडीजी) का प्रमुख स्रोत है। अलसी अपने लाभकारी फैटी एसिड संरचना और उच्च फाइबर सामग्री के कारण कार्यात्मक भोजन या न्यूट्रास्युटिकल घटकों का एक महत्वपूर्ण स्रोत है। इस कार्य में, सबसे कुशल निष्कर्षण स्थितियों को निर्धारित करने के लिए अलसी से एसडीजी लिगनेन के दबावयुक्त तरल निष्कर्षण का अध्ययन किया गया था। ऐसा करने के उद्देश्य से, तापमान (150, 160, और 170 डिग्री सेल्सियस), निष्कर्षण समय (5, 15, और 30 मिनट), और नमूना राशि (5 और 10 ग्राम) सहित निष्कर्षण कारकों के प्रभावों की जांच की गई। एसडीजी लिग्रान विश्लेषण एचपीएलसी का उपयोग करके किया गया है। यह स्थापित किया गया था कि पानी के अर्क की एसडीजी लिग्रान सांद्रता तापमान, निष्कर्षण अवधि और नमूना आकार से काफी प्रभावित थी। 15 मिनट के लिए 160 डिग्री सेल्सियस पर 5 ग्राम अलसी के तेल रहित केक का उपयोग करके अधिकतम सांद्रता (12.94 मिलीग्राम/ग्राम) और निष्कर्षण उपज (72.57%) प्राप्त की गई। 40% ताजे पानी के साथ।

ओमेगा-3 फैटी एसिड की उच्च सांद्रता के कारण, अलसी का तेल अब एक महत्वपूर्ण कार्यात्मक आहार घटक के रूप में उपयोग किया जाता है। हालाँकि, कैरोटीनॉयड, जो तेल की ऑक्सीडेटिव स्थिरता में सुधार कर सकता है, अलसी के तेल में अनुपस्थित है। अध्ययन के इस भाग में, अल्ट्रासाउंड-सहायता प्राप्त निष्कर्षण तकनीक का उपयोग करके कोल्ड-प्रेसड

अलसी के तेल को सीबकथॉर्न पोमेस से कैरोटीनॉयड से समृद्ध किया जाता है। बॉक्स-बेनकेन डिज़ाइन पद्धति का उपयोग करके प्रक्रिया मापदंडों को अनुकूलित करके अलसी के तेल की कैरोटीनॉयड सामग्री को बढ़ाया गया था। -3 फैटी एसिड (-लिनोलेनिक एसिड) की उच्च सांद्रता के कारण, अलसी का तेल एक महत्वपूर्ण कार्यात्मक आहार घटक बन रहा है। लेकिन प्राकृतिक अलसी के तेल में ऑक्सीडेटिव स्थिरता बढ़ाने वाले कैरोटीनॉयड अनुपस्थित होते हैं। अल्ट्रासाउंड-सहायता प्राप्त निष्कर्षण सीबकथॉर्न पोमेस से कैरोटीनॉयड निकालता है और इसे सीधे कोल्ड-प्रेस्ड अलसी के तेल में समृद्ध करता है। सीबकथॉर्न व्यवसाय का एक उप-उत्पाद जो कैरोटीनॉयड सांद्रता में उच्च है, सीबकथॉर्न पोमेस है, जिसका उपयोग संवर्धन के लिए किया जाता है।

बॉक्स-बेनकेन डिज़ाइन के माध्यम से, समृद्ध अलसी के तेल में कैरोटीनॉयड की मात्रा को अधिकतम करने के लिए प्रक्रिया मापदंडों (समय, फ़ीड अनुपात और आयाम%) को बढ़ाया गया था। सांख्यिकीय विश्लेषण के निष्कर्षों के अनुसार, पैरामीटर समय-75.56 मिनट, फ़ीड तेल अनुपात-19.94, और आयाम-80.81% पर 14.02 मिलीग्राम/लीटर कैरोटीन की उपज प्राप्त हुई। प्रक्रिया अनुकूलित मापदंडों से पता चला कि, जब अल्ट्रासाउंड-सहायता प्राप्त निष्कर्षण दृष्टिकोण और पारंपरिक निष्कर्षण (मिश्रण) दोनों के लिए समान संवर्धन स्थितियों का उपयोग किया गया था, तो अल्ट्रासाउंड-सहायता निष्कर्षण तकनीक से उपज में 46.96% की वृद्धि पाई गई थी। इससे पता चलता है कि अल्ट्रासाउंड-सहायता प्राप्त निष्कर्षण दृष्टिकोण पारंपरिक निष्कर्षण विधि की तुलना में कम समय में कैरोटीनॉयड के निष्कर्षण को काफी तेज कर देता है। अल्ट्रासाउंड तकनीक के उपयोग के माध्यम से अलसी के तेल की पोषण सामग्री को बढ़ाना एक त्वरित और कुशल प्रक्रिया है जिसमें सॉल्वेंट्स के उपयोग की आवश्यकता नहीं होती है। संयुक्त अरब अमीरात तेल संवर्धन की पारंपरिक पद्धति का स्थान ले सकता है। पेरोक्साइड मूल्य और रैनसीमैट के मेट्रिक्स का उपयोग करके, तेल की गुणवत्ता और स्थिरता की जांच की गई। इसकी तुलना उनके अनसमृद्ध तेलों से की जाती है। खाद्य पदार्थों को समृद्ध अलसी के तेल से पूरक किया जा सकता है, या इसका उपयोग दवाओं और न्यूट्रास्यूटिकल्स में किया जा सकता है। संवर्धन से पहले और बाद में अलसी के तेल की गुणवत्ता और स्थिरता की तुलना करने के उद्देश्य से, संपूर्ण भौतिक-रासायनिक लक्षण वर्णन किया गया था।

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List of Abbreviation

ALA -Alpha-linolenic acid

AOCS- American Oil Chemists' Society

ASE- Accelerated solvent extraction

EP-Ethanol precipitated

FG-Flaxseed gum

FD- Freeze-drying

FO- Flaxseed oil

GC- Gas chromatography

MAE- Microwave-assisted extraction

OD-Oven drying

PFE -Pressurized fluid extraction

PHSE -Pressurized hot solvent extraction

PLE- Pressurized liquid extraction

SCFE- Supercritical fluid extraction

SDG- Secoisolariciresinol diglucoside

SD-Spray drying

UAE- Ultrasound-assisted extraction