Enhancement of Cold-pressed Thymoquinone Extraction from Black Seed (*Nigella sativa* L.) using Freeze-thaw Pretreatment

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Quality attributes of the black seed oil depend on various extraction methods. Conventional methods, including cold pressing, provide high quality oil but low in yield and reduced bioactive compound content. To address this, the use of innovative seed pre-treatment procedures has been investigated in order to improve oil extraction efficiency, oil yield, and bioactive compounds recovery. Thymoquinone is the main active compound extract of black seed oil which has antioxidant, anti-diabetic, anti-inflammatory, anti-cancer, anti-viral and anti-microbial properties due to its phenolic compounds. In this study, freeze-thaw pre-treatment was employed on black seeds prior to extracting oil through the cold pressing method, with the primary goal of increasing the thymoquinone content. Black seed samples were subjected to freeze at -17 °C 24 hours and thaw at 50 °C hot water bath for 1 hour and the process was repeated for 1, 2, and 3 cycles at the same temperature and times. The subsequent oil extraction was carried out using the cold-pressing technique. The study encompassed the assessment of thymoquinone content, quantification of the extract yield, and the undertaking of physical and chemical evaluations on the obtained oil. Thymoquinone content in the black seed oil was determined using UV-Visible spectroscopy and this value (1.46%) from 3 cycles freeze-thaw seeds were stated as 73.81% increased than untreated black seeds (0.84%). Other quality parameters, including moisture content, specific gravity, acid value, peroxide value, and iodine value, exhibited comparable properties between untreated and freeze-thaw pre-treated black seed oil ($p < 0.05$). Based on the analysis of thymoquinone content, black seed oil yield, and other related metrics, the 3-cycle freeze-thaw pretreatment emerged as the optimal method for enhancing cold-pressed thymoquinone content in black seed oil.

Keywords: Black seed oil, Cold pressing, Extraction, Freeze-thaw, Thymoquinone

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