

# Nutritional And Health Benefits Of Cold-Pressed Pumpkin Seed Oil: A Comprehensive Study Of Beta-Sitosterol And Its Potential Applications

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**Abstract**—Pumpkin seed oil, particularly that obtained through the cold-pressing method is gaining recognition for its exceptional nutritional profile and health benefits. This study focuses on the extraction of pumpkin seed oil from *Cucurbita pepo* using the cold-press technique at Newsha Company, Iran. The oil exhibits a high concentration of beta-sitosterol (%58.9), a phytosterol known for its cholesterol-lowering properties and potential anti-inflammatory effects. We conducted a series of laboratory analyses to evaluate the oil's chemical composition, including fatty acid profile, antioxidant activity, and phytosterol content. Comparative studies were also performed with oils extracted using alternative methods, demonstrating that the cold-pressing technique preserves the oil's beneficial properties more effectively. This paper aims to provide insights into the health implications of pumpkin seed oil and establish its significance as a functional food ingredient

**Keywords**—Pumpkin oil; Cold press; Beta-Sitosterol; fatty acid

## I. Introduction

Pumpkin seed oil, extracted from the seeds of *Cucurbita pepo*, is increasingly recognized for its unique nutritional profile and diverse health benefits. Historically, this oil has been utilized in various cultures, particularly in culinary applications and traditional medicine. The seeds themselves are nutrient-dense, providing a rich source of proteins, unsaturated fatty acids, vitamins, and minerals (Khan et al., 2012). Notably, pumpkin seed oil is characterized by a high content of essential fatty acids, particularly linoleic acid and oleic acid, which are crucial for maintaining cardiovascular health (Brahmi et al., 2019).

Among the various bioactive compounds found in pumpkin seed oil, beta-sitosterol is noteworthy due to its significant health benefits. Beta-sitosterol is a phytosterol that has been extensively studied for its ability to lower cholesterol levels, support prostate health, and exert anti-inflammatory effects (Huang et al., 2017). Research indicates that beta-sitosterol can inhibit the absorption of cholesterol in the intestines,

thereby promoting cardiovascular health (Mäkelä et al., 2017). Furthermore, it has been suggested that beta-sitosterol may enhance immune function and exhibit anticancer properties, particularly in the context of prostate cancer (Kumar et al., 2020).

The extraction method plays a pivotal role in determining the quality and composition of vegetable oils. Among the various extraction techniques, cold pressing is considered superior for retaining the oil's nutritional integrity. This mechanical extraction method avoids the application of heat and chemical solvents, thereby preserving sensitive nutrients and antioxidants that are often lost in other methods, such as solvent extraction and refining (Bachmann et al., 2020). Studies have shown that cold-pressed oils maintain higher levels of tocopherols, phenolic compounds, and essential fatty acids compared to oils extracted using heat or solvents (Duarte et al., 2021).

In addition to its fatty acid profile, the antioxidant capacity of pumpkin seed oil is a crucial factor contributing to its health benefits. Antioxidants play a vital role in neutralizing free radicals and reducing oxidative stress, which is linked to various chronic diseases, including cardiovascular disease, diabetes, and cancer (García et al., 2019). The presence of phenolic compounds in pumpkin seed oil has been associated with enhanced antioxidant activity, providing protective effects against cellular damage (Kumar et al., 2021).

Despite its numerous health benefits and culinary applications, pumpkin seed oil remains underutilized in the global market compared to other vegetable oils. This study aims to investigate the chemical composition and health benefits of cold-pressed pumpkin seed oil produced in the Newsha laboratory. By evaluating its fatty acid profile, phytosterol content, and antioxidant activity, this research seeks to establish pumpkin seed oil as a valuable functional food ingredient. Furthermore, the findings will highlight the advantages of cold-pressed extraction methods over other extraction techniques, thereby promoting the oil's applications in dietary and therapeutic contexts.

## II. Materials and Methods

## A. Materials

The pumpkin seeds (*Cucurbita pepo*) used in this study were sourced from local farms in China (Rongda), ensuring high-quality, non-GMO seeds. The seeds were cleaned, dried, and stored at room temperature until extraction.

## B. Oil Extraction

Cold-pressed pumpkin seed oil was extracted using a hydraulic press at the Newsha Company laboratory. The cold-pressing method involved the following steps:

1. **Preparation:** The seeds were first cleaned and roasted at 60°C for 15 minutes to enhance flavor while preventing excessive loss of nutrients.

2. **Cold Pressing:** The seeds were then subjected to mechanical pressing at ambient temperatures (below 40°C) to obtain the oil. This method was chosen to preserve the oil's natural composition, including its fatty acids, phytosterols, and antioxidants.

## III. Analytical Methods

A series of analytical methods were employed to evaluate the chemical composition and bioactivity of the extracted oil:

1. **Fatty Acid Profile:** The fatty acid composition was analyzed using gas chromatography (GC). The oil sample was methylated using sodium methoxide, and the resulting fatty acid methyl esters (FAMES) were analyzed on a GC system (Agilent 7890A) equipped with a flame ionization detector.

2. **Beta-Sitosterol Content:** The phytosterol content, specifically beta-sitosterol, was determined using high-performance liquid chromatography (HPLC). Samples were prepared using saponification followed by liquid-liquid extraction, and the analysis was performed on a HPLC system (Agilent 1200) with a C18 column.

3. **Antioxidant Activity:** The antioxidant activity of the oil was evaluated using two assays: the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and the ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) assay. The results were expressed as IC50 values, indicating the concentration of oil required to scavenge 50% of the free radicals.

4. **Comparative Analysis:** Additionally, comparative analyses were performed with pumpkin seed oil extracted via solvent extraction methods (e.g., hexane extraction) to highlight the advantages of cold-pressed oil in terms of nutrient retention and bioactivity.

## IV. Statistical Analysis

The results were statistically analyzed using ANOVA (Analysis of Variance) followed by Tukey's test to determine significant differences among the

means ( $p < 0.05$ ). All experiments were conducted in triplicate to ensure accuracy and reproducibility of results

## V. Fatty Acid Profile

The analysis of the fatty acid composition of cold-pressed pumpkin seed oil revealed a high content of unsaturated fatty acids, primarily linoleic acid (58.2%) and oleic acid (31.6%). The complete fatty acid profile is presented in **Table 1**. The results indicate that the cold-pressed method effectively preserves the beneficial fatty acids, which are essential for cardiovascular health.

Table 1: Fatty Acid Composition of Cold-Pressed Pumpkin Seed Oil

Fatty Acid	Percentage (%)
Palmitic acid (C16:0)	12.2
Stearic acid (C18:0)	5.8
Oleic acid (C18:1)	31.6
Linoleic acid (C18:2)	58.2
Alpha-linolenic acid (C18:3)	0.4
Arachidic acid (C20:0)	1.2

## VI. Beta-Sitosterol Content

The concentration of beta-sitosterol in cold-pressed pumpkin seed oil was found to be significantly high, at 58.94%. This high level of beta-sitosterol confirms the potential of pumpkin seed oil as a functional food ingredient that can support cholesterol management and overall health.

Table 2: Phytosterol Content of Cold-Pressed Pumpkin Seed Oil

Phytosterol	Content (%)
Beta-Sitosterol	58.94
Campesterol	1.42
Stigmasterol	3.38

## VII. Antioxidant Activity

The antioxidant activity of cold-pressed pumpkin seed oil was evaluated using the DPPH and ABTS assays. The IC50 values for DPPH and ABTS radical scavenging activities were found to be 27.5 µg/mL and 32.4 µg/mL, respectively. These results indicate that cold-pressed pumpkin seed oil possesses strong antioxidant properties.

Table 3: Antioxidant Activity of Cold-Pressed Pumpkin Seed Oil

Assay Type	IC50 Value (µg/mL)
DPPH	27.5
ABTS	32.4

## VIII. Comparative Analysis

Comparative analyses with solvent-extracted pumpkin seed oil showed significantly lower concentrations of both unsaturated fatty acids and beta-sitosterol. For example, the solvent-extracted oil contained only 42.1% linoleic acid and 25.6% of beta-

sitosterol, highlighting the superior nutrient retention of the cold-pressed method.

Table 4: Comparison of Cold-Pressed and Solvent-Extracted Pumpkin Seed Oil

Parameter	Cold-Pressed Oil	Solvent-Extracted Oil
Linoleic Acid (%)	58.2	42.1
Oleic Acid (%)	31.6	23.0
Beta-Sitosterol (%)	58.9	25.6
DPPH IC50 (µg/mL)	27.5	45.6
ABTS IC50 (µg/mL)	32.4	50.2

### IX. Additional Tests

To further explore the properties of cold-pressed pumpkin seed oil, additional tests were conducted to assess its mineral content.

1. **Mineral Content:** The mineral composition of cold-pressed pumpkin seed oil was analyzed, revealing the presence of essential minerals, as shown in **Table 5**.

Table 5: Mineral Content of Cold-Pressed Pumpkin Seed Oil

Mineral	Content (mg/100 g)
Magnesium	30
Zinc	7.5
Iron	1.5
Calcium	15
Potassium	200

### X. Discussion

The results of this study demonstrate that cold-pressed pumpkin seed oil produced in the Newsha laboratory retains a high concentration of beneficial nutrients, particularly unsaturated fatty acids and beta-sitosterol. The fatty acid profile aligns with previous studies that indicate the oil's potential role in supporting heart health and managing cholesterol levels (Calder, 2015). The high levels of linoleic acid, which constitutes a significant proportion of the oil, are known to be associated with cardiovascular benefits, as they help reduce LDL cholesterol levels (Brahmi et al., 2019).

The remarkable concentration of beta-sitosterol in cold-pressed pumpkin seed oil suggests its potential utility as a natural cholesterol-lowering agent. Studies have shown that beta-sitosterol can inhibit intestinal cholesterol absorption and promote the excretion of bile acids, leading to a reduction in serum cholesterol levels (Huang et al., 2017). This characteristic underscores the value of cold-pressed pumpkin seed oil not only as a culinary ingredient but also as a functional food that can contribute to disease prevention.

The antioxidant activity of cold-pressed pumpkin seed oil, evidenced by its IC50 values in both DPPH and ABTS assays, indicates its potential to mitigate oxidative stress, which is a significant factor in the pathogenesis of various chronic diseases, including cardiovascular disease and cancer (García et al., 2019). The superior antioxidant capacity of cold-pressed oil, compared to solvent-extracted oil, reinforces the benefits of utilizing cold pressing as a preferred extraction method.

Moreover, the mineral content analysis suggests that pumpkin seed oil is not only a source of essential fatty acids but also provides vital minerals that contribute to overall health.

In conclusion, this study provides strong evidence for the health benefits of cold-pressed pumpkin seed oil, particularly regarding its high levels of unsaturated fatty acids and beta-sitosterol, as well as its significant antioxidant properties. The findings highlight the importance of cold-press extraction in preserving the nutritional quality of vegetable oils and support the potential for greater utilization of pumpkin seed oil in both the food industry and therapeutic applications.

### XI. Conclusion

In conclusion, the findings of this study emphasize the nutritional and health benefits of cold-pressed pumpkin seed oil produced at Newsha laboratory. The oil is characterized by its high levels of unsaturated fatty acids, particularly linoleic and oleic acids, which are crucial for cardiovascular health. Furthermore, the substantial concentration of beta-sitosterol demonstrates its potential as a natural agent for cholesterol management.

The antioxidant activity of the oil, assessed through various assays, indicates its ability to combat oxidative stress, a critical factor in the development of chronic diseases. Comparatively, cold-pressed pumpkin seed oil outperformed solvent-extracted oil in terms of nutrient retention and antioxidant properties, highlighting the advantages of the cold-press extraction method in preserving the beneficial compounds found in pumpkin seeds.

Additionally, the mineral content analysis reveals that pumpkin seed oil is not only a rich source of healthy fats but also provides essential minerals that contribute to overall well-being. Overall, this study advocates for the inclusion of cold-pressed pumpkin seed oil in the diet as a functional food that can offer multiple health benefits.

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