Identification of Ergocalciferol (Vitamin D₂) in the Extraction of Almond by Soxhlet Extractor

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ABSTRACT
Background: The almond (Prunus dulcis) is a species of tree native to the Middle East and South Asia. The world produced 2.00 million tons of almonds in 2011 according to Food and Agriculture Organization, with United States the largest producer at 0.73 million tons. Objective: In recent study, in order to measurement the ergocalciferol (vitamin D₂), after sampling and drying in shade and room temperature, sample was extracted with Soxhlet for four hours by n-hexane solvent. Then, sample has been analyzed by high performance liquid liquid chromatography (HPLC). Results: Results of this study identified vitamin D₂ in fruits of almond.

INTRODUCTION

Vitamins are vital food ingredients for maintaining good health in humans; lack of a sufficient amount of any of them can cause serious diseases (Riaz et al., 2009). The human diet does not always contain the amount of vitamins needed for normal development and maintenance of body functions (Gomez and Jose, 2006). For this reason, several food products are fortified with vitamins, mainly milk and milk products.

Vitamin D is a fat-soluble vitamin recognized for its importance in skeletal health (Ceglia, 2009). Vitamin D is present in animal foods as cholecalciferol (vitamin D₃). Ergocalciferol (vitamin D₂) has plant origin, where it is converted from the provitamin, ergosterol. The two vitamin D forms differ by the side chain to the sterol skeleton and in the hydroxylated products (Mawer et al., 1998).

Vitamin D as cholecalciferol was reported in fish and beluga whale (Blanchet et al., 2000; Mattila et al., 1995), and cod liver and cod liver oil are known to be rich in this nutrient (Brustad et al., 2003).

There are a few studies about vitamin D in plants and foods. Furthermore, most of them are about foods. It is typical of vitamin D that variation in its contents occurs in foods; good examples for this are fishes and eggs (Mattila et al., 1997; Mattila et al., 1999).

The aim of the present study was to evaluate the vitamin D₂ in the fruits of almond with Soxhlet, by HPLC method.

1. Methodology:

The fruits of almond were harvested from natural growing plants in Borujerd region, Western Iran. The fruits were dried in the shade and room temperature (25°C) for ten days. One of the most commonly method for extraction of vitamin D is Soxhlet extraction with a variety of solvents. In this study, the extraction used 20 g of almond to pass a 1.0 mm sieve. Then 2.5 g of IBA has been added and extraction was completed with 250 mL of n-hexane containing 1 mg of IBA at 60 °C for 4 h using Soxhlet extractor in a dark place.

After extraction, the hexane extract was evaporated to near dryness using a rotary evaporator at 50°C. Afterward, sample was analyzed by high performance liquid chromatography (HPLC).

The extraction were analyzed using a HPLC model 200, making by Perkin Elmer company from the USA with the separation techniques used with ultraviolet/visible (UV/visible), Fluorescence. The processor of information with Brezz, Column was C18 (Agilent-100 mm, 4.6mm and 3.5 µm) support with isocratic elution. Mobile phase was acetonitrile (5%) with methanol (95%), flow rate 1.0 mL min⁻¹. Collect vitamin D fraction between 2 min before and 2 min after established peak retention time.

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3. Results:
Ergocalciferol is a secosteroid formed by a photochemical bond breaking of a steroid, specifically, by the action of ultraviolet light on ergosterol. Conflicting evidence exists for how similarly D2 and D3 behave in the body and whether they are equally active or efficient in production of 1,25-hydroxyvitamin D (1,25(OH)D), the active hormone. Some preliminary studies indicate D3 is more potent (Trang et al., 1998) while others report equal efficacy (Holick et al., 2011; Biancuzzo et al., 2010). Both forms appear to have similar efficacy in ameliorating rickets (Thacher et al., 2010) and reducing the incidence of falls in elderly patients (Fosnight et al., 2008).

The metabolism of each appears to be different, with the vitamin D binding protein possibly having greater affinity for 25(OH)D3 than for 25(OH)D2, as shown in one study. (Houghton and Vieth, 2006) Cholecalciferol (vitamin D3) is sensitive to UV radiation and rapidly, but reversibly, forms other sterols which can further irreversibly convert to ergosterol.

Fig. 1: A chromatogram of vitamin D$_2$ in the fruits of almond.

4. Discussion and Conclusion:
By use of the results of this research and other investigations about this tree in different areas and even in deferent kind of almond, the best area from the aspect of this chemical composition can be recognized. By researching about the morphological and ecological features of the areas were these trees are grown, the relation between these factors and the amount of effective compositions in plants can be found. The characteristics of agrology are another important factor that should be considered. Also different ways of extract and analysis the extracts, are factors that should be noticed. Further information in this area, needs further research.

REFERENCES


