

# Determination of Free-and Conjugated-isoflavones of Tomato Samples

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**Abstract**—In Isoflavones are a subclass of flavonoids and are also described as phytoestrogen compounds because they exhibit oestrogenic activity. In their naturally occurring glycosidic form, isoflavones are conjugated at the 7 position and are often esterified with acetyl or malonyl groups at the 6' position of the sugar residue. The aim of this study was to determine the conjugated (ononin, genistin, daidzin, glycitin, and sissotrin) and free (biochanin A, glycitein, genistein, formononetin, and daidzein) isoflavone contents of fresh tomato samples. Tomato (*Lycopersicon esculentum*) samples grown in Ankara Turkey were investigated. Samples were prepared by various methods, such as conventional extraction and enzymatic hydrolysis before LC-MS/MS analysis for identification of the free-and conjugated-isoflavones. The total isoflavone concentration of tomato sample were determined as 15.8 µg/100 g. Major isolavone compound of tomato was identified as glycitein (14.8 µg/100 g). Daidzin, glycitin, ononin and sissotrin could not be identified in the samples.

**Keywords**—Isoflavone, LC-MS/MS, tomato

## I. INTRODUCTION

**I**SOFILAVONES are a subclass of flavonoids and are also described as phytoestrogen compounds because they exhibit estrogenic activity [1]. In plants, the most widely studied isoflavones, genistein and daidzein, are usually encountered as conjugates (acetyls, glycosides or malonyls) [2], and they are hydrolysed in the human gut to aglycone (with biological activity) [3, 4].

Isoflavones have attracted attention mainly because of their possible role in the amelioration of postmenopausal symptoms, such as hot flashes and osteoporosis [5, 6, 7, 8]. Other important biological activities are related to potential effects on cardiovascular diseases, cognitive function, and breast and prostate cancer [4, 5, 9, 10].

Tomato (*Lycopersicon esculentum* L.) is one of the most widely consumed fresh and processed vegetables in the world and contains bioactive components such as phenolics, carotenoids and vitamins C and E. Carotenoids consumption has been associated with a lower risk of several types of cancer and a lower incidence of coronary heart disease [11].

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In this study, we analysed the amounts of free and conjugated isoflavones in samples of tomatoes grown in Ayas, Ankara, Turkey prepared by conventional extraction.

## II. MATERIALS AND METHODS

### A. Sampling and Sample Preparation

Each of fresh tomato samples (*Lycopersicon esculentum*) were obtained Aya\_ Research and Experimental Farm, Faculty of Agriculture, University of Ankara (Turkey) in 2011. Edible parts of the fresh vegetables were first separated and then chopped in a chopper (Fakir, Germany). Subsequently, 100 g of each sample was placed in polyurethane bags and kept at -18°C prior to different sample preparation methods.

### B. Standards and Reagents

The phytoestrogen standards of ononin (purity 98.0%), genistin (purity e95.0%), daidzin (purity e95.0%), glycitin (purity e95.0%), sissotrin (purity e95.0%), biochanin A (purity e95.0%), glycitein (purity 97.0%), genistein (purity 98.0%), formononetin (purity e99.0%) ve daidzein (purity 98.0%), were purchased from Sigma (St. Louis, Mo., USA). 100.0 µg/mL of standards stock solutions were prepared using methanol.

All reagents, solvents and chemicals were of analytical or HPLC grade and were obtained from Sigma or Merck (Darmsadt, Germany).

### C. Apparatus

Ultrapure water was prepared using a Milli-Q System (Millipore S.A., Molsheim, France). Additionally, during sample preparation and extraction, the following equipment was used: ultra-centrifuge (Universal 320R, Hettich, Tufflingen, Germany), ultrasonic water bath (LBS2, Falc Instrument, Treviglio, Italy), sample concentrator under nitrogen (EVA-EC1-L 24-16, VLM, Germany) and general laboratory equipment.

### D. Conventional Extraction

The conventional extraction performed in this study were based on methods previously developed and used by Konar et al. [6, 7].

### E. LC-MS/MS Conditions

The method of LC-MS/MS applied by Konar et al. [4, 12] was used. The samples were injected into the LC/MS-MS system in triplicate.

### F. Statistical Analysis

Quantitative data are expressed as mean. Standard deviations were determined by using SPSS 15.0 (SPSS Inc., Chicago, IL.).

### III. RESULTS AND DISCUSSION

The quantitative results of LC-MS/MS measurements performed on tomato are reported in Table 1. The obtained data and levels of the identified compounds significantly varied according to the sample preparation method. In our study, as Konar et al. [12], we used only fresh vegetables, because preparation habits and methods of them before or during consumption (e.g. cooking) may vary depends on countries, diets, cultures, etc.

The literature contains a limited body of research on the identification of phytoestrogenic compounds in vegetables. Related literature on the vegetable samples, included preparation of samples with enzymatic hydrolysis and used LC-MS/MS [13], GC-MS [14, 15] and HPLC-CEAS (16) for identification.

Kuhnle et al. [13] used the LC-MS/MS technique on enzymatically hydrolysed samples of tomato (1 µg/100 g of isoflavones as total of daidzein, genistein, glycitein, biochanin A as <1 µg/100 daidzein, <1 µg/100 g genistein, <1 µg/100 g glycitein, <1 µg/100 g biochanin A) and they did not identified formononetin. in tomato samples. Liggins et al. [14] did not identified daidzein in the tomato samples however, genistein was identified in the samples in 48.0 µg/100 g concentration by them. and genistein in green pepper samples by using GC-MS. As be seen in Table 2, the phytoestrogenic compound levels determined in our study for pepper samples were generally different from previous studies. Thompson et al. [15] also used the GC-MS technique on enzymatically hydrolysed samples of tomato to identify daidzein (0.10 µg/100 g), genistein (0.30 µg/100 g), formononetin (0.10 µg/100 g), glycitein (0.00 µg/100 g) and total isoflavone (0.50 µg/100 g) concentrations.

In this study, the amounts of free and conjugated isoflavones in all tomato samples were quite low. Total isoflavone amounts were found to be 15.8 µg/100 g (wet weight) for tomato. The glycitein level ( $14.8 \pm 2.71$  µg/100 g) was found to be quite high than other isoflavones and it was the phytoestrogenic compound containing the highest amount of isoflavone types in all vegetable samples. Whereas, daidzein, ononin, sissotrin, glycitin and daidzin could not be identified in any of the samples. The concentration of biochanin A, formononetin, genistein and genistin were identified as  $0.15 \pm 0.09$  µg/100 g,  $0.13 \pm 0.05$  µg/100 g,  $0.52 \pm 0.06$  µg/100 g and  $0.24 \pm 0.01$  µg/100 g respectively.

### IV. CONCLUSION

Tomato is a popular vegetable fruit crop around the world. Tomato can be readily ingested without cooking, thus avoiding potential damage to their beneficial phytochemicals. They are known to contain carotenoids and some flavonoids. Also results of this study introduced that, it

may be source of some isoflavones as glycitein. However, this is not valid most of the other free- and conjugated-isoflavones.

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TABLE I  
ISOFLAVONE COMPOUNDS CONTENT OF TOMATO SAMPLES  
( $\mu\text{G}/100\text{ G}$ , WET WEIGHT)

Phytoestrogen	CE
BiochaninA	$0.15 \pm 0.09$
Daidzein	nd
Daidzin	nd
Formononetin	$0.13 \pm 0.05$
Genistein	$0.52 \pm 0.06$
Genistin	$0.24 \pm 0.01$
Glycitein	$14.8 \pm 2.71$
Glycitin	nd
Ononin	nd
Sissotrin	nd
Total isoflavone	15.8

CE; conventional extraction, nd; not determined. All data from the samples are the mean  $\pm$  S.D. of three analyses. Total isoflavones value is the sum of daidzein, genistein, glycitein, biochanin A, formononetin, daidzin, genistin, glycitin, ononin and sissotrin.