Evaluation of Antioxidant Activity of Pomegranate Molasses by 2,2-Diphenyl-l-Picrylhydrazyl (DPPH) Method

A. Akpinar-Bayizit, T. Ozcan, L. Yilmaz-Ersan, and E.Yildiz

***Abstract*—In Turkish cuisine the pomegranate molasses (PM) are used as a condiment and believed to have significant effects for arteriosclerosis, cholesterol levels and cancer prevention due to the antioxidant potential of pomegranate fruit itself. In this study, we measured the total polyphenols content, of which varied from 118.28 to 828.15 mg of gallic acid equivalent per gram** of **PM, and antioxidant activity by DPPH assay, of which were found to be between 560.23 to 1885.23 µmol trolox equivalent per gram of sample. The chemical composition of PM samples were found as: the water soluble dry matter content 62.40-75.00 g 100g^{-1}; viscosity 176 and 2900 mPa.s.; total acidity 4.70-9.73 g 100g^{-1}; pH of the samples changed 1.71 and 2.96; invert sugar and total sugar 23.71-56.95 g 100g^{-1} and 30.33-70.94 g100g^{-1}, respectively.**

***Index Terms*—Pomegranate molasses, antioxidant activity.**

I. Introduction

In recent years, scientific interest has focused on understanding the mechanisms of traditional remedy applications based on nutraceutics and functional food nutrients. The pomegranate fruit (*Punica granatum* L.), one of the oldest edible fruits that widely grows in many tropical and subtropical countries has been concidering as a health-promoting food [1]. It has been featured prominently in all the major religions of the world and has been used for centuries for the treatment of various ailments [2]. The unique chemistry of pomegranate tree have led many studies to be conducted to evaluate the functional efficacy of this fruit due to the claims related to wellbeing. Since the traditional importance of pomegranate as a medicinal plant is well-established by scientific researches, market demand and production of pomegranate products thereof showed a considerable increase [3]-[6]. The compounds in different parts of the fruit have been shown to possess preventive and attenuating activities against numerous chronic and health threatening maladies such as prostate cancer, atherosclerosis and cardiovascular diseases, oxidation of low density lipoprotein (LDL), high density lipoprotein (HDL) and cholesterol, type 2 diabetes, hypertension, arthritis, anemia and Alzheimer's disease [7]-[16].

The chemical composition of the pomegranate and its products depends on the cultivar, growing region and climate, the fruit's stage of maturity, cultural practices and manufacturing systems [7], [17]-[19], [20].

The edible part of the pomegranate fruit consists of 40% arils and 10% seeds. The juice of arils (pomegranate juice) is comprised of 85% water, 10% total sugars (glucose, sucrose, and fructose), and 1.5% pectin, organic acids (citric, malic, tartaric, succinic, fumaric, ascorbic acid), fatty acids (i.e. conjugated linoleic acid, linoleic acid, punicic acid and eleostearic acid) and amino acids (i.e. proline, valin, and methionine) and bioactive compounds (phenolics and flavonoids). The seeds are a rich source of total lipids; and pomegranate seed oil comprises 12% to 20% of total seed weight. The predominant fatty acid in seed oil is 9,11,13-octadeca-trienoic acid (punicic acid), a conjugated linolenic acid characteristic from pomegranate seeds, with contents between 3523 and 10586 mg 100 g^{-1} of seeds [21]-[27].

Mounting evidence suggests that the beneficial health effects of fruits and vegetables in the prevention of disease are due to the bioactive compounds such as phenolic acids, flavonoids, tannins and antioxidant properties. Antioxidants are compounds that have the ability to scavenge free radicals and protect the human body from oxidative stress, the main key factor for the occurence of cancers and heart diseases [28]-[30]. Reference [31] stated that the antioxidant level in pomegranate juice was higher than found in many fruit juices, such as blueberry, cranberry, and orange. Reference [32] and Reference [33] demonstrated that pomegranate juice and seed extracts have 2-3 fold more *in vitro* antioxidant capacity than red wine or green tea. Ellagitannins, mainly punicalagins, are reported to be responsible for over 50% of the antioxidant activity and the above health promoting features of pomegranate [34], [35].

Pomegranate can be consumed as fresh, fruit juice, fermented fruit juice, dried aril, frozen aril, minimally-processed aril, canned aril, jam, jelly, wine, vinegar, paste, fruit leather and in flavoring products. The '*pomegranate molasse*, also named as sour pomegranate pekmez, nar eksisi, pomegranate sauce, is a traditional condiment commonly used in salads and many dishes to improve the taste and aroma characteristics in Turkey. It is a concentrated product produced simply by boiling of pomegranate juice without the further addition of sugar or other additives. Traditional methods are still being used to produce pomegranate molasses, a thick, dark red liquid formed after cleaning, crushing, extraction, filtration, and evaporation in an open vessel or under vacuum (Fig. 1). Clarification step is generally omitted since customers prefer bitterness and sourness caused by phenolic substances and acidity [36]-[38].

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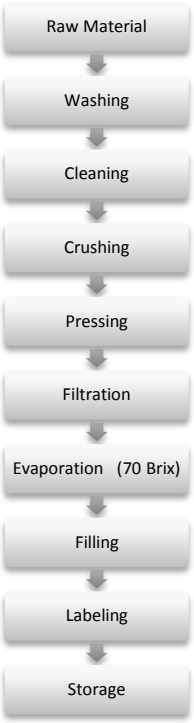

Fig. 1. The process of pomegranate molasses production.

The antioxidant activity of pomegranate fruit juice, peels, leaves, arils or seeds have been reported separately by several researchers due to their wellknown ethnomedical relevance and chemical features [33], [39]-[44], [25], [4], however, there are few reports on the chemical composition and antioxidant activity of pomegranate molasses [45], [46]. Therefore, the main objective of the present work was to determine the antioxidant activity of pomegranate molasses by the DPPH assay, as well as the total phenolics contents.

II. Materials and Methods

A. Materials and Methods

Pomegranate molasses samples were purchased from a local markets in Bursa. All chemicals were analytical-reagent grade and obtained from the following sources: 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, Na_2CO_3 (Sigma-Aldrich S.p.A., Milan, Italy); gallic acid and ethanol (Merck, Darmstadt, Germany).

B. Chemical Analysis of PM

The water soluble dry matter content (brix) of the samples was expressed as g $100g^{-1}$ by using an Abbe refractometer [47] Viscosity was measured in mPa.s using a rotary viscosimeter (NDJ-1, Shanghai Precision & Scientific Inst. Co. Ltd.). Total acidity was determined as citric acid, by titrating samples against NaOH solution of known normality [48]. pH was measured potentiometrically by a Nel-pH 890 model pH meter. Invert and total sugars were determined by the Luff-Schoorl method [49].

C. Analysis of Total Phenolic Content of PM

Samples were diluted to 14 brix and 1 mililiter of sample and 10 mL of extraction solution (Methanol for TPC, ethanol for AC) were mixed and shaken at 20 ℃ for 10 minutes. Then the suspension was centrifuged at 3 500 rpm for 10 minutes and filtered.

The total phenolic content of pomegranate molasses was determined spectrophotometrically at 725 nm according to modified Folin–Ciocalteu colorimetric method [50]. After adding 0.1 mL extract, 2.3 mL distillated water, 0.15 mL Folin-Ciocalteu reagent and vortexed for 15 seconds. After 5 minutes 0.30 mL 35% Na_2CO_3 added and content was mixed and left to stand at room temperature in dark for 120 min. Samples were measured 725 nm. A standard calibration curve was plotted using gallic acid. The results were expressed as "milligrams of gallic acid equivalents per gram of pomegranate molasses".

D. Measurement of Antioxidant Activity by DPPH

The antioxidant activity of pomegranate molasses were determined according to the method reported by Reference [51]. 0.1 mL prepared supernatant and 3.9 mL DPPH (6×10^{-5} M) solution were mixed. The mixture was shaken vigorously and was allowed to stand at room temperature for 30 min before the decrease in absorbance at 515 nm was measured. Antioxidant activity was determined as the percentage of DPPH decrease using the equation:

$$\text{Inhibition } \% = (\text{Abs}_{t=0} - \text{Abs}_{t=30\ \text{min}}) / \text{Abs}_{t=0} \times 100$$

$\text{Abs}_{t=0}$ was the absorbance of DPPH at time 0.

$\text{Abs}_{t=30\ \text{min}}$ was the absorbance of DPPH after 30 min of incubation.

Inhibition (%) was calculated according to trolox calibration curve as "µmol trolox equivalent per gram of sample".

III. Results and Discussion

Total phenolic content and antioxidant activity of pomegranate molasses were shown in Table I.

TABLE I: Functional Parameters of PM

Sample	Antioxidant Capacity	Total Phenolic Component
PMI	190,60 ±0,48	344,30 ±1,84
PMII	271,89 ±10,52	691,25 ±4,86
PMIII	471,85 ±2,33	828,15 ±6,79
PMIV	140,22 ±2,84	168,89 ±2,91
PMV	410,15 ±4,60	745,46 ±4,45
PMVI	155,34 ±0,58	325,51 ±0,64
PMVII	208,01 ±3,00	118,28 ±0,00
PMVIII	411,92 ±3,07	711,94 ±0,91
PMIX	222,57 ±0,75	631,69 ±4,08

The chemical composition of PM samples were found as: the water soluble dry matter content 62.40-75.00 g $100g^{-1}$; viscosity 176 and 2900 mPa.s.; total acidity 4.70-9.73 g $100g^{-1}$; pH of the samples changed 1.71 and 2.96; invert sugar and total sugar 23.71-56.95 g $100g^{-1}$ and 30.33-70.94 g $100g^{-1}$, respectively (data not shown).

The total polyphenol content was calculated via the gallic acid curve of the spectrophotometric measurement result of the absorbance of the blue color at 765 nm, which is the result of the redox reaction of the reduction of the Folin-Ciocalteu reagent by phenolic compounds and turning into oxidized forms in an alkali medium. Phenolic compounds give the color, astringency and bitter taste to pomegranate Since pomegranate molasses is a concentrated product, it was expected to have high phenolic content. However, due to thermal processing conditions (simply boiling) and

inclusion of saccharose syrup to give a caramelized flavour with artificial pomegranate flavour phenolic contents were low.

Reactive oxygen species (ROS) such as superoxide anion ($O2^{\bullet-}$), hydroxyl (•OH), peroxyl (ROO•), and alkoxyl radicals (RO•), hydrogen peroxide (H_2O_2), and singlet oxygen ($O_2{}^1\Delta g$) may attack biological macromolecules, giving rise to protein, lipid, and DNA damage, cell aging, oxidative stress-originated diseases (e.g., cardiovascular and neurodegenerative diseases), and cancer. Antioxidants, either exogenous or endogenous, are vital substances which possess the ability to scavenge or quench ROS and reactive nitrogen species (RNS) in order to protect the body from the potent injuries caused by these radicals. Measuring the antioxidant capacity/activity of any foodstuff is carried out for the meaningful comparison of the antioxidant content for the diagnosis and treatment of oxidative stress-associated diseases. The DPPH radical has widespread use in the free radical scavenging activity assessment because of the ease and convenience of the reaction.

In the presence of arbutus extracts and standard solutions the absorbance of DPPH solution decreased, of which were found to be between 16.11 to 75.22%, indicating that radical scavenging activity was concentration dependent.

It could be said that the antioxidant activity of pomegranate molasses depend on several factors, such as cultivar and climatic conditions during fruit maturation and ripening and the part of the fruit used.

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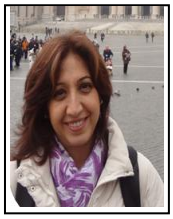

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