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EFFECT OF ARIL BROWNING ON PHYSICHO-CHEMICAL PROPERTIES OF POMEGRANATE

Darsana K.[1], Bhosale Yuvraj K.[1] and Sinija V.R.[2]
[1]M. Tech Scholar, [2]Associate Professor
IICPT Thanjavur
E-mail: sinija@iicpt.edu.in

Abstract: India is one of the major producer of pomegranate but the quantity which is been is exported is very low due to lack of quality in fruit and lack of technology for sorting internally defective fruits. In pomegranate the major internal defect is aril browning (AB). Aril browning doesn't have any external symptoms but causing some internal physicho-chemical changes, so it very difficult to sort it manually, thus posing serious problems in export trade. This present study has been analyzed the physico-chemical properties to find the difference between black and brown arils with respect to healthier one. The results shown significant changes in most of the properties. The parameters like TSS, TA, Antioxidant activity along with L* and a* values showed significant difference with mean difference of 2.412, 0.297, 38.85, 8.253 and 10.083 respectively. But the other parameters like moisture (0.363), density (0.454) and b* (0.056) values are statistically not different for the independent sample t test though it showed the mean difference of 1.574, 0.0354 and -5.451 respectively. Together the assumption can be made that the higher browning in the fruit will change overall TSS, TA, antioxidant and L* and a* values of fruit. But there won't be much changes in overall moisture, density and b* value of fruit.
Keywords: Aril Browning, Physico-chemical properties, Independent sample t test, Mean difference.

1. Introduction

The pomegranate (*Punicagranatum*) is a naturally dense, deciduous, bushy, multi- stemmed shrub that typically grows to heights of 10 to 12 feet and bears highly colored fruit with many juicy seeds inside. The edible part of the fruit is the arils which develop in ripening about four to six months of flowering and constitute 52% by weight of fruit. The arils of comprising 78% juice and 22% seed can be used as such or can be processed into canned beverages, jelly, jam, and paste. It is also used for flavoring and coloring drinks (Zaouay et al., 2012). Pomegranate is supposed to be originated from Iran and considered as a crop of the arid and semiarid regions because it withstands different soil and climate stresses. It thrives best under hot dry summer and cold winter provided with irrigation facilities. The best fruits are obtained in those areas where the period of development occurs during the time of high

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temperatures. The fruit is non-climacteric (will not ripen off the shrub) and should be picked after it has reached maturity. Most commercial orchards pick the fruit just before complete maturity as the fruit will tend to crack if left too long (Ashton, 2006).

The quality of the fruit is often severely affected by a physiological disorder called 'aril browning'. **Aril browning** in pomegranate is a physiological disorder wherein, the flat browned and soft arils are noticed when fruit is cut open. Affected arils are soft, light creamy-brown to dark blackish-brown, deformed, and undesirable for consumption. Therefore, consumption of the affected fruit has threatened the popularity of the pomegranate. The browning of aril starts with a dark dot and later on spreads to the entire aril and many of them have a streaked appearance due to fine white lines radiating from the seeds. So there is financial loss to the farmers as well as consumers. As the fruits affected by the disorder remain free from external symptoms, they cannot be separated out before being packed, thus posing serious problems in export trade. Main causes of the disorder is yet to be established. This has become a serious concern to consumers, growers and researchers in the recent times. Researchers are finding out the main causes/ effects of the disorder and to suggest solutions to overcome the disorder. Furthermore, it is a serious challenge to the quality control of the expert. Aril browning is usually reported in over-ripe fruit during harvest (Kulkarni & Arabhya, 2005) or during postharvest storage (Elyatem and Kader, 1984) but this disorder is initiated during fruit development. Aril browning (AB) in pomegranate is critically affecting fruit quality in some commercially important varieties such as cv. Ganesh and cv. Bhagwa.

2. Materials and methods

Collection of sample- Fruits

Pomegranate fruits of cv. Bhagwa were collected from a farmer's orchard located in Mettur, Salem District, Tamil Nadu and brought to laboratory. Fruits for analysis were harvested at 90% maturity. Fruits are cut open then browned arils and healthy ones are separated from each fruit and analysed.

Density of fruit and Arils

Pomegranate fruits were weighed on electronic balance (PHOENIX, India), having 0.001 precision and average weight was expressed in grams (g) For whole fruit first displaced water is measured then fruit is cut open and the brown and black arils were separated carefully from healthy arils further density is measured by water displacement method for both Fruits and Arils.

$$Density = \frac{Mass\ of\ material}{Volume\ of\ material}$$

Estimation of moisture content

The moisture of the arils was determined by using oven dry method. Five grams of the test sample were placed in stainless steel dishes and kept in a hot air oven (Ms. Everflow, Chennai) at 100^0 C and heated to 24 h. The weight of the sample before and after drying was recorded and the moisture percentage was calculated (AOAC, 1996).

$$Moisture\ (\%) = \frac{Initial\ wt - Final\ wt}{Sample\ wt} \times 100$$

Arils color

Pomegranate aril color was measured using a Hunter color lab model D25 optical sensor (Hunter Associates Laboratory, Reston, VA). Aril color was assessed according to the Hunter color lab and expressed as L*, a*, b*. In this coordinate system, the L* value is a measure of lightness, ranging from 0 (black) to +100 (white); the a* value ranges from −100 (greenness) to +100 (redness), and the b* value ranges from −100 (blueness) to +100 (yellowness) (Ester et al., 2014).

Fruit Firmness

Fruit firmness is an important indicator of fruit maturity. Fruit firmness was determined on whole and unpeeled fruit using TA-HD plus texture analyzer (Stable Micro Systems, UK). Puncture test was set to penetrate fruit surface with the P/2 cylindrical probe (2mmØ) with cross head velocity of 1mm s^{-1} and 5kg load cell. The fruit was placed on a heavy duty platform and positioned such that the cylindrical probe penetrated at the equatorial position of the fruit, to a depth of 10 mm. The fruit was penetrated with 3 different positions at the angle of 120˚. The average peak was considered as the penetration force in the force-time curve which represent fruit firmness. The mean value of the firmness was expressed as gram force required for penetrating the fruit (gf).

Aril firmness

Texture analysis is done for each aril such as healthy aril and browned aril. Puncture test was set to penetrate fruit surface with the P/2 cylindrical probe (2mmØ) with cross head velocity of 1mm s^{-1} and 5kg load cell. The arils were penetrated to the depth of 1mm.

Refractive index

Refractive index of the food is mainly dependent on the soluble solute present in it i.e. TSS. Refractive index is measured by the lab level refractometer (ATAGO, RX7000α, Japan) at temperature about 20^{o} C and the results are noted.

Total soluble solids (TSS)

TSS is an index of soluble sugar content in fruit. TSS (˚Brix) in juice samples was determined with a lab level refractometer (ATAGO, RX7000α, Japan) at temperature about 20^{o} C and results were reported in degree Brix.

Titrable acidity (TA)

Titrable acidity was estimated in terms of Citric acid by titrating the juice against 0.1 N NaOH using phenolphthalein as indicator until it gives light pink color (oeca.org)

$$\%\ Titrable\ Acidity = \frac{Titrate\ value \times Acid\ Factor \times 100}{ml\ of\ juice}$$

Antioxidant Activity

Antioxidant activity was expressed as the percentage decline in absorbance relative to the control, corresponding to the percentage of DPPH scavenged (% DPPHsc) (Shekhar and Anju, 2014), which was calculated as follows:

$$\%\ DPPHsc = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Statistical Analysis

The statistical analysis is done in the IBM SPSS software Version 20.0 with different statistical tests such as compering the means of the specific fruits for different characters. The results are expressed with P-values of different treatments.

3. Results and discussions

Density

Browning of fruits which also depend on the size and weight of the fruit (Khodade 1987), harvesting (Pawar et al., 1994) and season of the growth (Shete, 1998). When size and weight of the fruit increases the presence of browned arils is also increased (Khodade 1987). Peel thickness is positively correlated with color and browned arils that means as peel thickness increases the rate of browning was observed to be increasing. The browned arils are mostly present in the surface of the peel. There is a small density difference observed between healthy arils and browned one. The reason can be told that the moisture losses will leads to the shrinkages of the particular arils than healthy aril leading to the difference in volume

compared to browned aril. Statistically the difference in not significant as the p-value is 0.454.

Aril color

The color difference values were shown in Table 1, According to analyses L* was increased in the juice of affected fruit. This indicates that the aril color becomes brighter in affected fruit in agreement with our results, that darker juice contains higher levels of antioxidants and total phenolics. In contrast, values a* and b* were higher in the healthy pomegranate juice. The direct oxidation of phenolic compounds by polyphenol oxidase (PPO) and peroxidase (POD) enzymes is a major cause of fruit tissue browning (Barberan and Espin, 2001). These results indicate that these pomegranates have more of the red and yellow color components, respectively. These results are in agreement with the pattern of changes in anthocyanin. A decrease in a*, b*, and hue angle can be an indication of the appearance of aril browning. The red color is one of the factors that affected pomegranate consumer's behavior.

Table 1: Color difference for Healthy and Brown Arils

HEALTHY			BROWN			Color Difference
L*	a*	b*	L*	a*	b*	ΔE
33.84	20.84	34.32	41.17	16.58	18.36	18.07
34.36	27.22	13.45	38.88	13.65	15.73	14.48
30.96	29.98	18.85	35.01	12.18	14.64	18.73
34.76	27.77	23.39	43.36	18.02	17.25	14.37
37.19	27.27	27.49	43.1	11.33	17.25	19.84
29.58	34.28	15.72	30.96	17.03	16.91	17.34
39.35	15.87	21.5	54.67	10.37	19.75	16.37
22.33	28.18	29.51	41.24	23.07	20.73	21.46

Refractive index

The refractive index was shown in Fig.1. As TSS of fruit is been increasing the refractive index is also increasing. So for browned arils the refractive index is more which due to sugar content is more there will be scattering of light. Absorbance of healthy aril is more compared to the browned ones because of pigments such as anthocyanin, phenolic compounds and antioxidant is more in healthy arils.

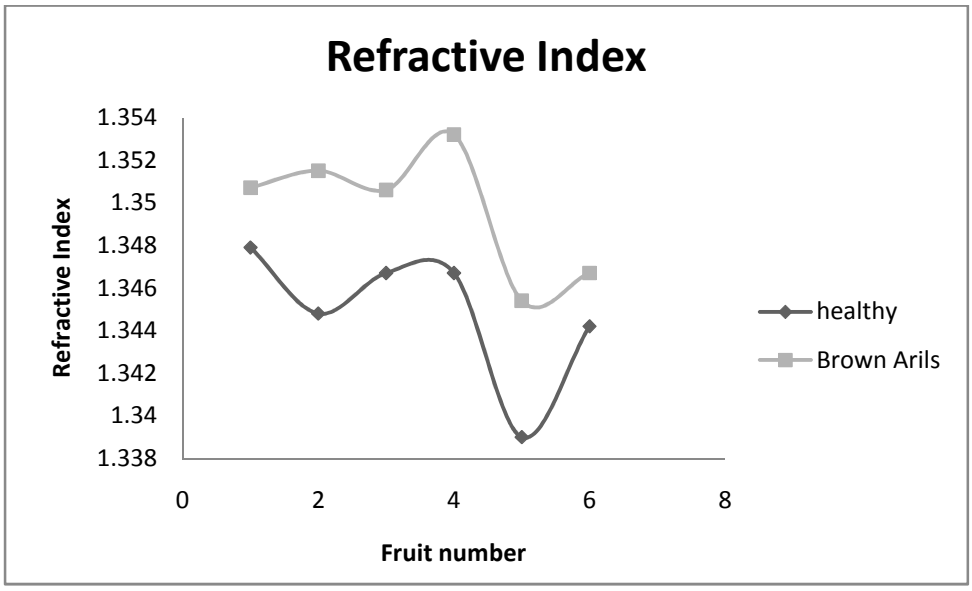

Fig 1: Refractive index for Healthy and Brown arils

Fruit Firmness

The fruit firmness was found and it is being shown in Fig.2. According to the analyses in browned and over ripened fruits the requirement of the peak force is more compared to mature and healthy ones this is due to over ripening of the fruit by which the biological catalysts the enzyme activity will increase that results in increase in rate of the respiration in the fruit. The increase in the respiration will increases the metabolic activities in the fruit which leads to higher rate of water losses and the utilization of nutrients. Similar results regarding water loss were found by Frank et al (2007). As the basic concept of fruit morphology the fruit peel is made up of the polysaccharides and the firmness of it is due to the water bound in these polymeric molecules. But as in over ripening of the pomegranate the moisture losses are more so the polymeric structures will lose the firmness and become harder. Due to getting harder with moisture losses the average force required to rupture the fruit peel increased.

Aril Firmness

The textural property for the arils noted was firmness. Here it was seen that the force required for the brown arils was less than the healthy ones. This is due to the deterioration in the quality of the fruit. Stress conditions during fruit development might promote aril browning, because enzymatic browning is a direct consequence of membrane disintegration (Franck et al, 2007). Under optimal conditions, the produced reactive oxygen species (ROS) are efficiently removed by the antioxidant system. However, stress conditions, including drought stress and desiccation, salt stress, chilling, heat shock, heavy metals, mechanical stress,

nutrient deprivation, pathogen attack, and high light stress, enhance the production of ROS (Mittler, 2002), which results in membrane degradation and possible browning reactions (Franck et al., 2007). Shivashankara et al. (2004) reported that the browning of pomegranate arils is caused by oxidative damage to membranes leading to higher enzymatic browning by PPO and POD. And the result is being shown in the fig.2.

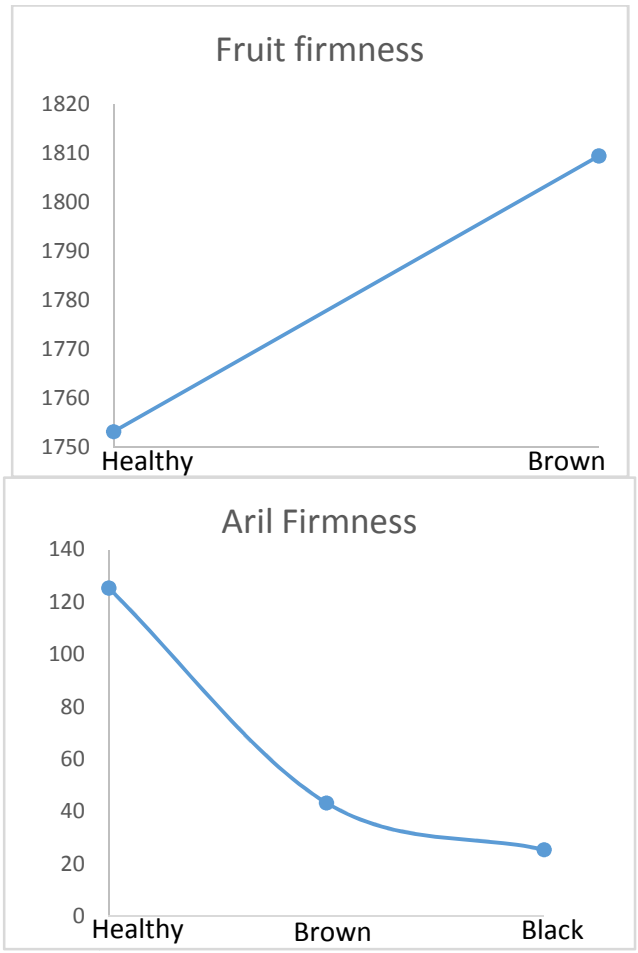

Fig 2: Fruit and Aril firmness of pomegranate.

Moisture Content

The moisture content analyzed was shown in table.2. Though there is no significant difference in moisture content of the healthy and brown arils, the data showed that the reduced moisture content of browned aril led to reduced viability of seeds compared to healthy arils. Softening of membrane will cause leathery membrane having very soft texture so along with more moisture loss there is observed loss in textural characteristics also of browned arils. In pomegranate each aril will act as individual fruit representing its own traits, characters and properties. The higher rate of respiration in brown arils (Zaouay et al, 2012) is much more which leads to more evaporation rate resulting in lower moisture content of

brown arils than healthy, the effect is enhanced by the degradation of the membrane (Franck et al., 2007).

Total soluble solids (TSS)

TSS, TA and pH in terms of quality of a fruit, are important components which provide characteristic taste and flavor to fruits and their products. As per statistical analysis for browned and healthy aril TSS, the TSS is more in brown compared to healthy ones with p-value of 0.011 at 5% significant Level. Zaouay et al.(2012) reported that from respiration rate of healthy and affected arils, The affected arils had higher respiration when compared to the healthy arils. Respiration is a basic physiological process that provides the energy for plant biochemical processes. Carbohydrates, lipids, and organic acids are substrates that are broken down in this process (Fonseca et al, 2002). The higher respiration rate in affected fruits indicates a faster overall metabolism and deterioration (Chung and Moon, 2009). In browned arils activity of amylases is more so starch quantity will decrease. Starch will be converted into sugars such as sucrose and fructose. Singh et al. (2013) reported that aril browned affected aril had higher total sugars, reducing sugar, TSS and low starch as compared to healthy aril. Jalikop et al. (2010) found that aril browning is affected by the levels of TSS in pomegranates; for every unit increase in TSS, there was an increase in the severity of aril browning.

Titrable acidity (TA)

There was a negative correlation ($p < 0.05$) between TA and TSS. TA will decrease during browning where acid is used as the substrate to break drown the starch (Acid hydrolysis). This is why the TA showed significant difference in brown and healthy arils. Affected fruit had a higher pH than healthy fruit.

Table 2: Comparison of TSS, Moisture and Acidity of healthy arils and brown arils

Constitutes	Healthy Arils	Brown Arils
Moisture	81.27±2.61	79.75±2.22
TSS	8.76±1.03	11.01±1.65
Acidity	1.44±0.08	1.14±0.133

Antioxidants

The antioxidant activity was shown in Fig.3. Antioxidant activity was lower in affected fruit than in healthy fruit and significantly different at 5% level of significance, This decrease is related to the reduced total phenolic and anthocyanin contents in affected fruit (Meighani et al., 2014). This is due to increased polyphenol oxidase activity. A positive correlation was

found between antioxidant activity and total phenolic, flavonoid, and anthocyanin contents (r = 0.94, 0.98 and 0.93, respectively) (Zaouay et al., 2012). However, ascorbic acid increased with maturity up to stage II and decreased afterwards (ICAR, 2005).

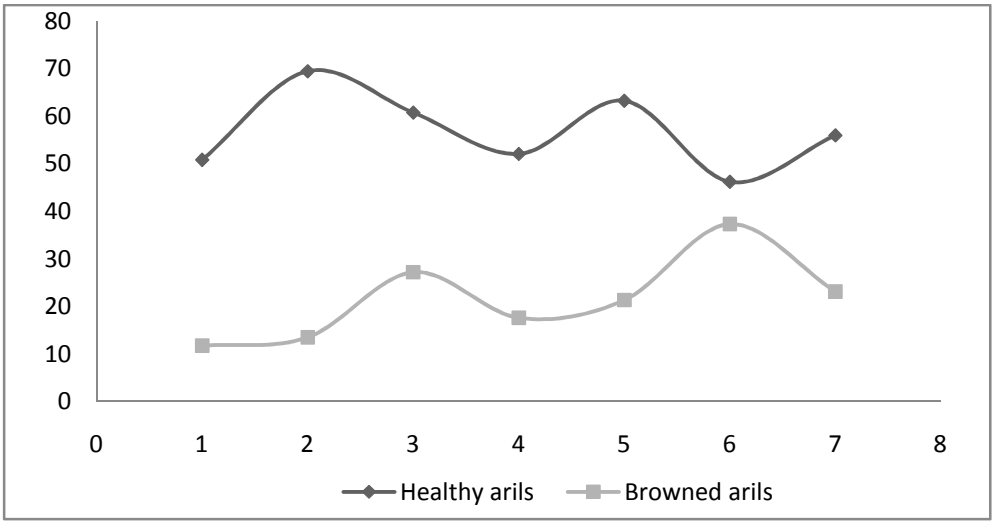

Fig 3: Antioxidant activity of healthy and brown arils

Statistical Analysis

Following table shows the statistical analysis results obtained for the different parameters for the different quality of fruits and arils.

Table 3: Statistical analysis for the different pomegranate

Parameter	Mean Difference	SE	p-Value
Density	0.0354	0.005	0.454
Moisture Content	1.574	1.652	0.363
L*	8.253	3.079	0.018
a*	10.83	2.789	0.003
b*	-5.451	2.614	0.056
Refractive Index	-0.0013	0.0006	0.019
Fruit Firmness	318.137	86.635	0.001
Aril Firmness	66.447	5.929	0.000
TSS	2.412	0.775	0.011
Total Acidity	0.297	0.065	0.001
Antioxidants	38.85	5.154	0.000

4. Conclusion

Browned aril doesn't have any microbial count but when the fruit is externally injured the microbial count in the browned fruits will be increased. Although black heart is always recognized as a postharvest quality problem, the infection begins in the orchard (Alternaria spp. or Aspergillus spp) (Zhang et al, 2012). The lack of obvious external symptoms makes Aril browning identification a challenge for sorters in the packing house or processing line.

The ability to Non –destructively detect infested produce is required to eliminate the risk of infested produce reaching consumers. The non-destructive techniques such are NIR, Acoustic Resonance technique, Soft x-ray, Hyperspectral imaging, Ultrasonic imaging can be tried

5. References

[1] Ashton, R. (2006). *The Incredible Pomegranate: Plant and Fruit*. Third Millennium Publishing.

[2] AOAC (Association of Official Analytical Chemists), Official methods of analysis, Washington D.C. 37:1-53, 1996.

[3] Chung, H.S. and Moon, K.D. 2009. Browning Characteristics of Fresh-Cut 'Sugaru' Apples as Affected by Pre-Slicing Storage Atmospheres. *Food Chemistry*. 114:1433-1437.

[4] Elyatem, S.M., and Kader, A.A. (1984). Post-harvest physiology and storage behavior of pomegranate fruits. *Scientia Horticulturae*, *24*(3), 287-298.

[5] Ester, M. S., Kavitha, A.C.V., Alagusundaram K. (2014). Modeling thermal degradation kinetics of colour in curry leaf paste. *Asian Journal of Science and Technology*, 5(3):272-275.

[6] Fonseca, S.C., F.A.R. Oliveira, and J.K. Brecht. 2002. Modelling Respiration Rate of Fresh Fruits and Vegetables for Modified Atmosphere Packages: a *Review. Journal of Food Engineering.* 52:99-119.

[7] Franck, C., Lammertyn, J., Ho, Q.T., Verboven, P., Verlinden, B., and Nicolaï, B.M. (2007). Browning disorders in pear fruit. *Postharvest Biology and Technology*, *43*(1), 1-13.

[8] http://www.oecd.org/dataoecd/32/47/19515719.pdf

[9] Jalikop, S.H., Venugopalan, R., and Kumar, R. (2010). Association of fruit traits and aril browning in pomegranate (Punicagranatum L.). *Euphytica*,*174*(1), 137-141.

[10] Khodade, M. S. (1987). Studies on Physico-chemical Changes During Growth and Development of Pomegranate Fruit (*PunicaGranatum L.*).

[11] Kulkarni, A.P., and Aradhya, S.M. (2005). Chemical changes and antioxidant activity in pomegranate arils during fruit development. *Food Chemistry*, *93*(2), 319-324.

[12] Meighani, H., Ghasemnezhad, M., and Bakshi, D. (2014). Evaluation of Biochemical Composition and Enzyme Activities in Browned Arils of Pomegranate Fruits. *International Journal of Horticultural Science and Technology*, *1*(1), 53-65.

[13] Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in plant science*, *7*(9), 405-410.

[14] Pawar, S.K., Desai, U.T., and Choudhari, S.M. (1994). Effect of pruning and thinning on growth, yield and quality of pomegranate. *Annals of Arid Zone*,*33*(1), 45-47.

[15] Shekhar, T.C., and Anju, G. (2014). Antioxidant Activity by DPPH Radical Scavenging Method of Ageratum conyzoides Linn. Leaves. *American Journal of Ethnomedicine*, *1*(4), 244-249.

[16] Shivashankar, K.S., M.S. Chander, R.H. Laxman, G.P. Vijayalaxmi, and C.S. Bujjibabu. 2004. Physiological and Biochemical Changes Associated with Aril Browning of Pomegranate (Punicagranatum Cv. 'Ganesh'). *Plant Physiol. Biochem*. 31:149-152.

[17] Singh, H., Singh, N., Marathe, A., and Ugalat, J. (2013). Influence of aril browning on biochemical properties of pomegranate (Punicagranatum L.). *Journal of Crop and Weed*, *9*(1), 184-187.

[18] Zaouay, F., Mena, P., Garcia-Viguera, C., and Mars, M. (2012). Antioxidant activity and physico-chemical properties of Tunisian grown pomegranate (Punicagranatum L.) cultivars. *Industrial Crops and Products*, 40, 81-89.

[19] Zhang, L., and McCarthy, M.J. (2012). Black heart characterization and detection in pomegranate using NMR relaxometry and MR imaging. *Postharvest biology and technology*, 67, 96-101.