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Original article

# Fruit quality and biochemical characterization of recently developed seedless lemon cultivars

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## Summary

*Introduction* – **Seedlessness is an important characteristic demanded by consumers of citrus fruit and gamma irradiation is a common technique used to obtain seedless citrus fruits. The present study was conducted to investigate the fruit quality and nutritional bio-components of seedless lemons ('Alata', 'Uzun' and 'Gulsen') recently developed by using gamma irradiation in comparison with the original seedy cultivar.** *Materials and methods* – **High-performance liquid chromatographic methods together with UV and refractive index detectors were employed to identify and quantify these compounds.** *Results and discussion* – **Fruit quality and bio-chemical properties were affected differently; some cultivars presented no changes compared to the control, while others exhibited significant differences. The seedless lemon fruits under study were characterized by high ascorbic acid contents (262.48 to 410.74 mg $L^{-1}$), high total soluble solids, and high fructose and glucose contents.** *Conclusion* – **Budwood irradiation is considered as a suitable technique to improve citrus cultivars, produce seedless cultivars and raise the content of health-promoting compounds. The present results revealed that irradiation technique affected the bioactive properties of lemon.**

**Keywords**
Turkey, lemon, *Citrus* spp., gamma irradiation mutagenesis, sugar composition, ascorbic acid, rind color

## Significance of this study

*What is already known on this subject?*

- Three seedless lemon cultivars (Alata, Uzun and Gulsen) were developed by using gamma irradiation. The same research team gave information about fruit and cultivation conditions but did not discuss the bio-chemical properties of the fruits.

*What are the new findings?*

- This study revealed the quality characteristics of seedless lemon fruits derived from the seedy most popular variety in Turkey. These new varieties expressed different characteristics useful in production: for example 'Gulsen' had high fruit size, well colored rind, and high total soluble solid and sugar contents.

*What is the expected impact on horticulture?*

- Turkey is among the top ten countries in citrus production. Lemon is the third citrus species produced in the country. The market is in high demand for seedless citrus fruits as it is the case in mandarin, orange and lime. The production of seedless citrus varieties is crucial for Turkey to address the market needs. This study revealed the additional quality characteristics obtained in seedless lemon such as a higher rind thickness which is considered as a significant attribute for storage.

## Résumé

Caractérisation biochimique et qualité des fruits de cultivars de citron sans pépins récemment développés.

*Introduction* – **L'absence de pépins est une caractéristique essentielle pour les consommateurs d'agrumes, et l'irradiation gamma est une technique couramment utilisée pour obtenir des agrumes sans pépins. La présente étude a caractérisé la qualité et les composés nutritionnels des citrons sans pépins ('Alata', 'Uzun' et 'Gulsen') récemment développés en utilisant l'irradiation gamma.** *Matériel et méthodes* – **Des procédés de chromatographie liquide à haute performance à détecteurs UV ou d'indice de réfraction ont été employés pour identifier et quantifier ces composés.** *Résultats et discussion* – **La qualité des fruits et les propriétés biochimiques ont été affectées différemment par l'irradiation. Certains cultivars n'ont présenté aucun changement par rapport au témoin, tandis que d'autres ont présenté des différences significatives. Les fruits étudiés ont présenté des teneurs élevées en acide ascorbique (entre 262,48 et 410,74 mg $L^{-1}$), en matières solubles, en fructose et en glucose.** *Conclusion* – **L'irradiation de bourgeons est considérée comme une technique appropriée pour améliorer les agrumes, pour produire des cultivars sans pépins et augmenter la teneur en composés bénéfiques pour la santé. Les résultats obtenus montrent que la technique d'irradiation affecte les propriétés bioactives du citronnier.**

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**Mots-clés**
Turquie, citron, *Citrus* spp., mutagenèse par irradiation gamma, composition en sucres, acide ascorbique, couleur du fruit

## Introduction

Citrus fruit is reported to be a very rich source of health promoting substances (Benavente-Garcia *et al*., 1997). Antioxidant activity of citrus fruit mainly comes from ascorbic acid, polyphenols and carotenoids, and citrus fruits are highly rich in these compounds (Marlett and Vollendorf, 1994). As well as nutritional properties, citrus fruits may prevent various health problems with their antioxidant compounds including vitamin C, phenolic compounds and carotenoids. Effects of genetics and ecological conditions on composition of bioactive compounds were studied and contributions were provided to plant taxonomy by the previous researchers (Bermejo and Cano, 2012).

Seedlessness is a major goal of fruit breeders around the world and many seedless fruits have been developed. Some common seedless cultivars exist in citrus fruits such as Satsuma mandarin (*Citrus unshiu* Marc.), Washington Navel orange (*Citrus sinensis*), Oroblanco (*Citrus grandis* × *C. paradisi*) and Tahiti lime (*Citrus latifolia* (Yu. Tanaka) Tanaka) (Deng *et al*., 1996). Seedless fruits can be stimulated in many ways, including through male and female sterility, self-incompatibility, defective ovules and embryo sac abortion, abnormal climate and application of growth regulators (Zhang *et al*., 2012). On the other hand, bud sport and chance seedling selections are still the predominant source of seedless citrus cultivars. The seedless cultivars that originated from a bud sport mainly belong to two types: lemon (Xiangshui' *Citrus limon* (L.) Burm. F.) (Zhang *et al*., 2012), and Murcott mandarin (Bermejo *et al*., 2012).

Turkey is among the leading citrus producing countries of the world with an annual production of 3.55 Mt (FAO, 2014). Citrus production of Turkey is dominantly made in the Mediterranean region. Lemon is the most significant commercial and exported citrus species of Turkey. Recently, wide range of citrus breeding programs has been generated by using various methods in Turkey. Three completely seedless lemon cultivars were developed by budwood irradiation in the Alata Horticultural Research Institute, Mersin, Turkey (Uzun *et al*., 2008). The genotypes were named 'Alata', 'Gulsen', and 'Uzun'. All seedless cultivars were developed by budwood irradiation from 'Kutdiken' cultivar as described by Gulsen *et al.* (2007). 'Kutdiken' is one of the most important lemon cultivars of Turkey for high yield, quality and storage performance.

In this study, some fruit quality and biochemical composition characteristics of new seedless lemon cultivars were evaluated and compared with their original seedy-cultivar 'Kutdiken'.

## Materials and methods

### Plant materials

Three seedless cultivars, 'Alata', 'Gulsen', and 'Uzun', previously developed with gamma budwood irradiation of 'Kutdiken' lemon (Uzun *et al*., 2008), were used as the material of study. The original 'Kutdiken' lemon was also used as control. All lemon cultivars were planted to field with ten replicates in the Alata Horticultural Research Institute of Mersin, Turkey in 2005. Environmental, cultural and soil conditions were same for all cultivars, thus the differences among cultivars were not influenced by climatic factors or cropping techniques.

### Fruit sampling

A total of 25 fruits were collected from the trees for each analysis at the end of December, commercially harvest time of cultivars, for two seasons (2011–2012 and 2012–2013). The fruits were pressed in a manual press in each replicate to extract the juice. The soluble solids content was obtained by an ATAGO 1T table refractometer (Atago Co. Ltd., Japan) and expressed as °Brix. Acidity was determined by using an automatic titration apparatus (Digitrat, Isolab, UK) with 0.1 mol $L^{-1}$ NaOH up to pH 8.1; results were expressed as g citric acid $L^{-1}$, the predominant organic acid in lemons. Measurements were conducted in three replicates and the results were expressed as means ± standard deviations.

### Biometric and color analysis

The biometric parameters (fruit height, diameter and rind thickness) were measured with a digital caliper (Mitutoyo CD-15CPX, Japan). Twenty-five fruits were randomly collected for measurements in each treatment. The average weight of fruits was determined by using CAS Computing Scale. Fruit rind color was measured with a Konica Minolta colorimeter CR-400 (Minolta Corp., Osaka, Japan) according to CIELab color system in 2° observation angle. The parameters measured were chromaticity ($C^*$), saturation or intensity of the color and L, a* and b* values, where the L (brightness) axis ranges from black (0) to white (100); a* axis from green ($-a$) to red ($+a$) and the b* axis from blue ($-b$) to yellow ($+b$). The Hue angle ($H°$) indicating the observed color was calculated by $H_{ab} = \tan^{-1}(b^* / a^*)$ and expressed in degrees. As suggested by McGuire (1992), Hue angle ($H°$) and chroma ($C^*$) are accepted as more intuitively understandable color coordinates. The colorimeter was standardized by using the Minolta calibration plate D65 before each measurement. All measurements were performed in three replications.

### Determination of ascorbic acid and soluble sugars

Ascorbic acid was determined by HPLC-UV-Vis (Shimadzu, LC-20AD, Kyoto, Japan, 2010). A similar procedure was described by Lee and Coates (1999) with some modifications. A reverse-phase ODS3 (GL Sciences, 5 µM, 4.6X250) column was used with an isocratic mobile phase of 2% $KH_2PO_4$ (pH 2.4). The total run time was 15 min at 0.6 mL $min^{-1}$ and injection volume was 10 µL. Quantification of ascorbic acid was performed at 244 nm by external standard calibration. L-ascorbic acid was obtained from Merck (Merck, Darmstadt, Germany). All solvents used were of HPLC-grade and ultrapure water (Milli-Q) was used.

Sugar separation was performed with a NH2 bound silica column (GL Sciences, 5 µM, 4.6X250) at 30 °C. Elution was carried out isocratically with a mobile phase made of acetonitrile/water (80:20,v/v) at a flow rate of 1.3 mL $min^{-1}$. Detection and sugar quantification were performed with a refractometer index detector (Shimadzu LC-20AD-RID). Sugars and ascorbic acids were determined again in three replicates.

### Statistical analysis

Effects of cultivars on the nutritional properties of lemon cultivars were assessed by analysis of variance (ANOVA).

**Table 1.** Biometric parameters of seedless lemon cultivars derived from the seedy one ('Kutdiken'). Data are expressed as mean ± standard deviation ($n = 3$).

| Cultivars | Weight (g) | Diameter (mm) | Height (mm) | Rind thickness (mm) | Seed number | Pulp yield (%) |
|---|---|---|---|---|---|---|
| 'Kutdiken' | 139.67±0.57$^{ns}$ | 60.53±0.16$^{ns}$ | 80.13±0.8$^{ns}$ | 5.30±0.48$^{b}$ | 10.36$^{a}$ | 33.01±0.71$^{a}$ |
| 'Gulsen' | 135.47±0.94 | 60.47±0.61 | 85.13±0.33 | 5.76±0.32$^{b}$ | 0$^{b}$ | 29.96±0.76$^{a}$ |
| 'Uzun' | 125.73±0.89 | 59.43±0.50 | 79.03±0.91 | 6.69±0.42$^{a}$ | 0$^{b}$ | 26.36±0.65$^{b}$ |
| 'Alata' | 128.20±0.48 | 59.46±0.67 | 83.73±0.34 | 5.42±0.37$^{b}$ | 0$^{b}$ | 30.97±0.81$^{a}$ |

$^{a–b}$ Cultivars with significantly different values compared to the seedy control ('Kutdiken'); $^{ns}$: not significant.

**Table 2.** Rind color parameters ($L^*$, lightness; $C^*$, chroma; $H^*$, hue angle) of seedless lemon cultivars derived from the seedy one ('Kutdiken'). Data are expressed as mean ± standard deviation ($n = 3$).

| Cultivars | $L^*$ | $H^*$ | $C^*$ |
|---|---|---|---|
| 'Kutdiken' | 76.31±0.55$^{a}$ | 89.63±0.44$^{b}$ | 64.97±0.91$^{a}$ |
| 'Gulsen' | 78.85±0.94$^{a}$ | 93.17±0.43$^{a}$ | 60.24±0.37$^{a}$ |
| 'Uzun' | 74.11±0.89$^{b}$ | 91.61±0.47$^{b}$ | 58.12±0.64$^{b}$ |
| 'Alata' | 74.17±0.48$^{b}$ | 92.96±0.88$^{ab}$ | 57.65±0.82$^{b}$ |

$^{a–b}$ Cultivars with significantly different values compared to the seedy control ('Kutdiken'); $^{ns}$: not significant.

When a significant difference was detected, means were compared by using the least significant difference (LSD) test ($P < 0.05$). Statistical analyses were conducted by using SPSS software (SPSS, version 16.0, SPSS Inc., USA).

## Results and discussion

### Biometric and color characteristics

High yield, large fruit size, high juice and acid content and thin rind are desirable characteristics in commercial lemon production. The physical aspects of fruits, such as, weight and dimensions varied widely among the studied samples (Table 1). Fruit weight of cultivars ranged from 125.73 ('Uzun') to 139.67 g ('Kutdiken'). In a previous study, fruit weights of eight 'Kutdiken' clones were reported as between 101 and 116 g (Uzun *et al*., 2009). Fruit diameter varied between 60.53 ('Gulsen') and 59.43 mm ('Alata'), while fruit height was between 85.13 ('Gulsen') and 79.03 mm ('Kutdiken'). Among the evaluated fruits, 'Kutdiken' and 'Gulsen' yielded fruits with higher weights than others but weight, height and diameter of the others were also similar to 'Kutdiken' (control). The fruits with smaller dimensions were observed in 'Uzun' with also the least pulp yield (26.36%). These parameters showed variability within the same samples and that could be related to nutrition and irrigation programs, rainfall distribution, pruning, fruit load and the rootstock/scion combination (Al-Jaleel *et al*., 2005).

Seedlessness is an important economic trait of lemon. No seed were determined in fruits of 'Alata', 'Gulsen' and 'Uzun' whereas 10.36 seeds per fruit were observed in control cultivar 'Kutdiken'. Rind thickness was the highest in 'Uzun' (6.69 mm) and the lowest in 'Kutdiken' (5.30 mm). Higher rind thickness may be an advantage for long-term fruit storage that is commonly applied in Turkey. Fruits with thick rind are usually low in juice, whereas those with thin rinds are prone to splitting, and are sensitive to peel disorders, which can occur during shipping and storage. Rind thickness was also found to be affected by the rootstock (Zekri and Al-Jaleel, 2004).

The color parameters varied among samples (Table 2). The sample 'Gulsen' with color values of $L^* = +78.85$, $C^* = 60.24$ and $H° = 93.17$ yielded rinds with light yellow and the sample 'Kutdiken' with values of $L^* = +76.31$, $C^* = 64.97$ and $H° = 89.63$ yielded dark yellow color. These two samples, 'Gulsen' and 'Kutdiken' respectively, had the highest and the lowest Hue angles. It is important to point out that $H°$ values closer to 90 indicate fruits with a greater tendency to yellow.

### Fruit biochemical quality

The soluble solid content is usually affected by the rootstock (Al-Jaleel *et al*., 2005). The soluble solid content of the lemons of the present study ranged from 6.33 ('Kutdiken') to 7.22 °Brix ('Gulsen') that could be directly related to the amount of sugars present in samples (Table 3).

The acidity values of the lemon cultivars varied between 6.96–6.41%. 'Kutdiken' and 'Alata' were the most acidic

**Table 3.** Biochemical fruit quality characteristics of seedless lemon cultivars derived from the seedy one ('Kutdiken'). Data are expressed as mean ± standard deviation ($n$=3).

| Cultivars | Soluble solids (°Brix) | Acidity (%) | Fructose (g 100 g$^{-1}$) | Glucose (g 100 g$^{-1}$) | Sucrose (g 100 g$^{-1}$) | Ascorbic acid (mg L$^{-1}$) |
|---|---|---|---|---|---|---|
| 'Kutdiken' | 6.33±0.40$^{b}$ | 6.96±0.27$^{ns}$ | 1.81±0.95$^{d}$ | 2.04±0.16$^{d}$ | 1.99±0.10$^{a}$ | 449.91±0.51$^{a}$ |
| 'Gulsen' | 7.22±0.94$^{a}$ | 6.41±0.30 | 3.17±0.78$^{a}$ | 2.32±0.49$^{c}$ | 1.61±0.49$^{b}$ | 262.48±0.92$^{d}$ |
| 'Uzun' | 6.46±0.89$^{b}$ | 6.43±0.21 | 2.59±0.97$^{b}$ | 3.31±0.58$^{b}$ | 0.32±0.53$^{c}$ | 335.32±0.80$^{c}$ |
| 'Alata' | 7.06±0.48$^{a}$ | 6.79±0.23 | 1.98±0.11$^{c}$ | 3.01±0.69$^{b}$ | 1.98±0.74$^{a}$ | 410.08±0.53$^{b}$ |

$^{a–d}$ Cultivars with significantly different values compared to the seedy control ('Kutdiken'); $^{ns}$: not significant.

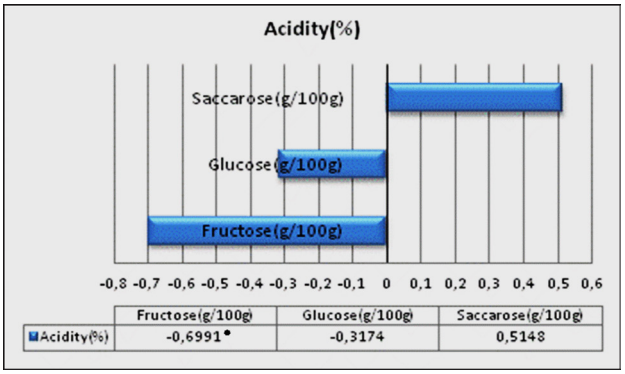


**Figure 1.** Correlation between acidity and sugar concentration of lemon cultivars.

lemon cultivars. There were no significant differences among the cultivars for acidity and acidity levels of cultivars were consistent with 'Kutdiken' clones reported previously by Uzun (2009). Sugars are the major components of citrus juice soluble solids and sweetness of citrus juice is intrinsic to its sugar composition. Sucrose, fructose and glucose are the main sugars in citrus fruits (Bermejo and Cano, 2012). The present results showed significant differences in sucrose, fructose and glucose contents of the cultivars. Compared to other sugars, glucose was dominant in cultivars except 'Gulsen'. Contrarily, sucrose was reported as the most abundant sugar in the orange juice (Kelebek *et al*., 2009). While the cultivar Uzun had both the highest glucose concentration (3.305 g 100 $g^{-1}$) and the lowest sucrose concentration (0.318 g 100 $g^{-1}$), the cultivars Kutdiken and Alata had the lowest fructose concentrations (1.813 and 1.977 mg 100 $g^{-1}$, respectively). These results showed significant correlations between fructose and acidity (Figure 1). It can be implied that the most acidic fruits ('Kutdiken' and 'Alata') were characterized by the lowest concentration of fructose. Similar results were also reported by other researchers (Albertini *et al*., 2006). A high level of citric acid accumulated in vacuoles could explain this phenomenon. This accumulation is accompanied by a large influx of protons that is mediated by the vacuolar H+-ATPase (VATPase) (Muller *et al*., 1997).

The organic acid contents of fruits and vegetables are influenced by various factors such as genotypic differences, climatic conditions and cultural practices (Albertini *et al*., 2006). Current lemon cultivars exhibited significant differences in ascorbic acid contents. Seedless cultivars had lower ascorbic acid contents (262.48–410.74 mg $L^{-1}$) than seedy control cultivar Kutdiken (441.06 mg $L^{-1}$). Such results indicated that the number of seeds in citrus fruit might play a role for high ascorbic acid levels. On the other hand, higher ascorbic acid levels were also reported in 'Fino' lemon at different ripening stages in Spain (Bermejo and Cano, 2012). The present findings are consistent with the role of genetics and the different ecological conditions on the ascorbic acid levels.

## Conclusion

Seedlessness is a desirable attribute in lemon cultivars. The present results revealed that fruit quality parameters and nutritional bio-components were affected differently; some cultivars presented no changes compared to the control 'Kutdiken' lemon, while some seedless cultivars showed significant differences. The seedless cv. Gulsen demonstrated high fruit size, well rind color, high total soluble solid and sugar contents. The cultivar Uzun had the highest rind thickness which is considered as a significant attribute for storage. Seedy control cv. Kutdiken and seedless cv. Alata had high acidity and high ascorbic acid contents. It was concluded herein that budwood irradiation was a suitable technique to improve cultivars, produce seedless cultivars, adjust ripening times or raise the content of health-promoting compounds of the fruits.

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