Marmara Pharmaceutical Journal 21/2: 298-304, 2017
DOI:

RESEARCH PAPER

Processing and storage influence on scavenging activity of fruit juices

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ABSTRACT

The food composition tables, which are necessary tools for nutritional and epidemiological studies, are representative of food stuffs consumed in only raw state. These studies don't take into consideration the facts that, the concentration of nutrients and their biological activity may be changed by processing methods. The aim of this study is to investigate the influence of processing/storing conditions on the free radical scavenging activity of different fruit juices. Free radical scavenging activity of fruit juices was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. Different types of fruit juices showed different values of scavenging activity. The free radical scavenging activity was quite stable when fruit juices were incubated at 4 °C for 4 days. When the freshly squeezed fruit juices were incubated under the sun light for 4 days, orange juice retains its scavenging activity. Lemon juices, mandarin and grapefruits lost more than 70% of their scavenging activity, while tomato juices lost more than 50% of its scavenging activity. Incubation of juices in boiling water for 60 minutes did not cause a significant loss of scavenging activity. In conclusion, sunlight may degrade many nutrients including the antioxidant. However, neither boiling nor freezing conditions affected the scavenging activity of different juices.

Keywords: Juices, Antioxidants, Sunlight, Heat, Processing, Cooling.

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Submitted / Gönderilme: 22.11.2016 **Revised / Düzeltme:** 27.12.2016
Accepted / Kabul: 28.12.2016

Introduction

A diet rich in fruits and vegetables is considered to be a good source of natural antioxidants. Antioxidants supplied with food may play an important role in scavenging free radicals, which are responsible for aging and many civilization diseases (1). Patients suffering from many inflammatory diseases often present with low levels of antioxidants either due to low dietary intake or, and even more likely, due to high demand in situations of overwhelming reactive oxygen species (ROS) production by stimulated immune effector cells like macrophages (2). Juices can have health benefits since their consumption significantly affects the plasma concentration of antioxidant agents and decreases the effect of ROS (3).

Guidelines for disease prevention and health promotion around the world include recommendations to consume a variety of fruits and vegetables daily, since they contain significant quantities of nutrients, especially sugars, vitamins, fiber, and minerals (4, 5). An alternative way to consume proper amounts of nutrients is to consume beverages such as juices (3). During the last few years, the demand for these beverages has been rising all over the world (6). This may be attributed to change in lifestyle, taste preferences and dietary habits (7).

Nutrition specialists consistently advocate a "food first" approach to achieving nutritional satisfaction, but some including the Nutrition and Dietetics Academy in USA, also recognized that dietary supplements have an important role in improving nutrient intake to support wellness and health (8). However, many clinical trials with antioxidants as dietary supplements have failed to show clear beneficial effects (9). According to these data, the population studies showed that effective health protection only can be achieved through the consumption of those foods that are naturally rich in antioxidant compounds. Thus, the health-promoting effects of dietary fruits and vegetables probably reflects a complex interaction among many naturally occurring compounds, which has not been duplicated by consumption of isolated antioxidant compounds.

The food composition tables, which are necessary tools for nutritional and epidemiological studies, are not representative of food stuffs consumed in only raw state (10). These studies don't take into consideration the facts that, the concentration of nutrients and their biological activity may be changed by processing method, as well as by environmental conditions (11). These aspects are of great value considering that only a little amount of fruit and vegetables are consumed in their raw state, whilst most of them need to be processed, before being served, for quality, safety and economic reasons (10).

There is substantial genetic variation in the content of each of antioxidant type among fruit and vegetable varieties (12).The levels of phenolic antioxidants appear to be more sensitive to environmental conditions compared with vitamin C and carotenoids. Although the content of certain carotenoids and phenols can actually increase during suitable conditions of fresh storage, vitamin C can be readily lost during the fresh storage (11). The combination of cultivar variation and responsiveness to certain environmental conditions can create opportunities for the production and processing of fruits and vegetables with enhanced antioxidant properties.

Domestic and commercial food storage typically has drastic effects on the structural integrity of fruits and vegetables (11). The health-promoting capacity of fruit strictly depends on their processing history and storage conditions (10). These aspects has been generally neglected or scarcely considered in present nutritional and epidemiological studies. The aim of the current study was to investigate the influence of storage conditions, duration, and temperature on the antioxidant properties of different fruit juices. It is believed that the implications of this challenging and rapidly advancing area may improve the consumer health and enhance industrial competitiveness.

Materials and Methods

Chemical reagents

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) reagent was purchased from Sigma-Aldrich (Denmark); fully matured fresh fruits were purchased from the local market. All other chemicals used were of the highest purity grade available commercially.

Fruit juice preparation

Fully matured and high-quality fruits were used. These fruits include orange (*Citrus sinensis* L. Osbeck*)*, lemon (*Citrus limon* L. Osbeck), mandarin (*Citrus reticulate* Blanco*)*, grapefruits (*Citrus paradise* Macfad*)*, tomato *(Solanum lycopersicum* L.*)*, and guava *(Psidium guajava* L.*)*. All fruits were identified by pharmacognosist, Nidal Jaradat (the second author) at the faculty of pharmacy, and they were given the following voucher specimen codes, orange (Pharm-PCT-2775), lemon (Pharm-PCT-2741), mandarin (Pharm-PCT-2794), grapefruits (Pharm-PCT-2795), tomato (Pharm-PCT-2793) and guava (Pharm-PCT-2720). Fresh fruits were thoroughly washed, peeled, cut into small pieces (tomato and guava were not peeled). The freshly squeezed juice was obtained by careful hand-squeezing of fruits. The collected juices were filtered through 5-fold muslin cloth and stored in clean dark containers.

Scavenging activity of fruit juices by DPPH assay

The radical scavenging ability of fruit juices was tested on the basis of the radical scavenging effect on the DPPH free radical (13, 14). The fruit juices (25 μL to 200 μL/mL) were incubated under different experimental conditions. All tubes were tightly closed with rubber stoppers during different incubation conditions to avoid any evaporation. In clean and labeled test tubes, 4 mL of DPPH solution (0.002% in methanol) was mixed with 4 mL of different concentrations of fruit juices separately. The optical density was measured at 517 nm using spectrophotometer. The scavenging activity of the juices was calculated using the formula (15):

Scavenging activity (%) = [(A – B) / A] x 100

Where A is absorbance of DPPH and B is absorbance of the mixture (DPPH and fruit juice). All statistical analyses were done with SPSS 18 statistical software (SPSS Inc, Chicago, Illinois, USA).

Results:

Free radical scavenging activity of fresh fruit juices:

Free radical scavenging activity of fruit juices was determined by DPPH scavenging assay (13, 14). In this assay, the antioxidants reduce the DPPH radial (purple color) to a yellow colored compound, diphenylpicrylhydrazine. Different fruit juices showed different values of scavenging activity (Fig 1). Lemon, orange, mandarin and grapefruit exhibited almost similar scavenging activity, while, tomato and guava possessed low scavenging activity.

Free radical scavenging activity of fruit juices after 60 minutes of boiling:

The effect of temperature on the antioxidant activity of different juices was evaluated. The freshly squeezed fruit juices were incubated in boiling water for different time intervals (Fig 2). It was shown that even after 60 minutes of incubation in boiling water (90 ^{o}C) all juices retain their antioxidant activity.

Free radical scavenging activity of fruit juices incubated at 4ºC:

When these juices were incubated at 4 ^{o}C for 4 days and the scavenging activity was determined, it was found that the highest scavenging activity was shown by lemon juices, followed by orange juices, grapefruits, and mandarin, tomato and guava (Fig 3). These results showed almost a stable scavenging activity of different juices even after 4 days of incubation in a refrigerator.

Free radical scavenging activity of fruit juices incubated under the sun:

The freshly squeezed fruit juices were incubated under the sun light. After 4 days of incubation, the free radical scavenging activity was measured using the DPPH method. The scavenging activity of different fruit juices have been differently affected by sunlight (Fig 4). Orange juice retains its

Figure 1: Free radical scavenging activity of fresh fruit juices:

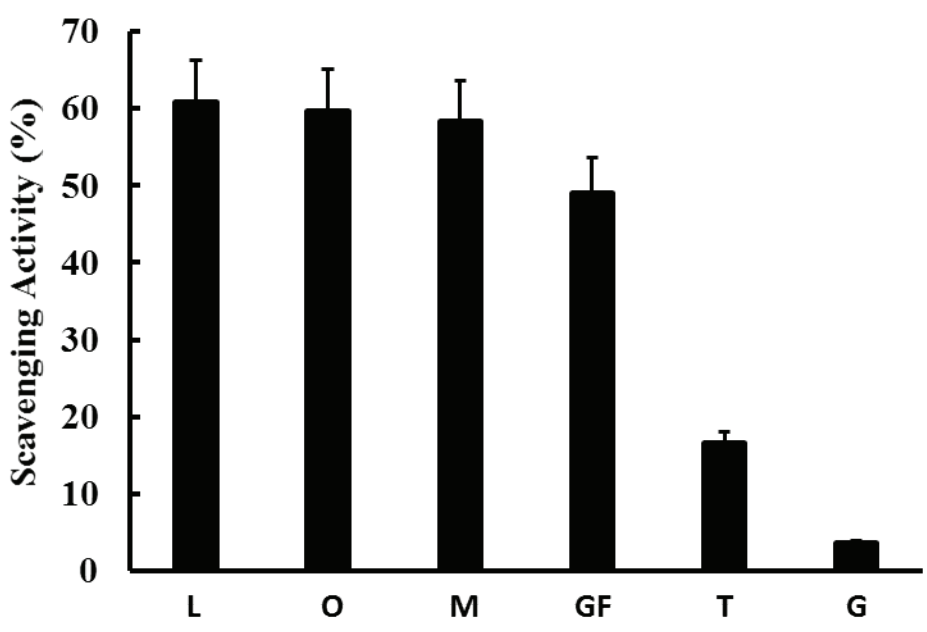

Figure 1: Free radical scavenging activity of fresh fruit juices. The fruits used were Lemon (L), Orange (O), Mandarin (M), and Grapefruits (GF), Tomato (T) and Guava (G).

Figure 2: Free radical scavenging activity of fruit juices after 60 minutes of boiling

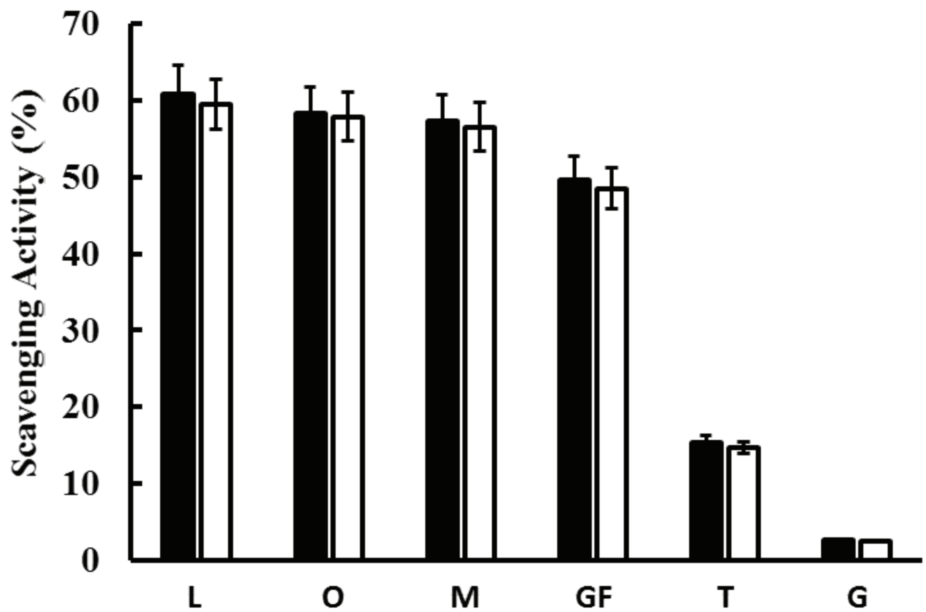

Figure 2: Free radical scavenging activity of fruit juices after 60 minutes of boiling. Scavenging activity of fruit juices before (▪) and after (□) 60 minutes of boiling.

Figure 3: Free radical scavenging activity of fruit juices incubated at 4°C

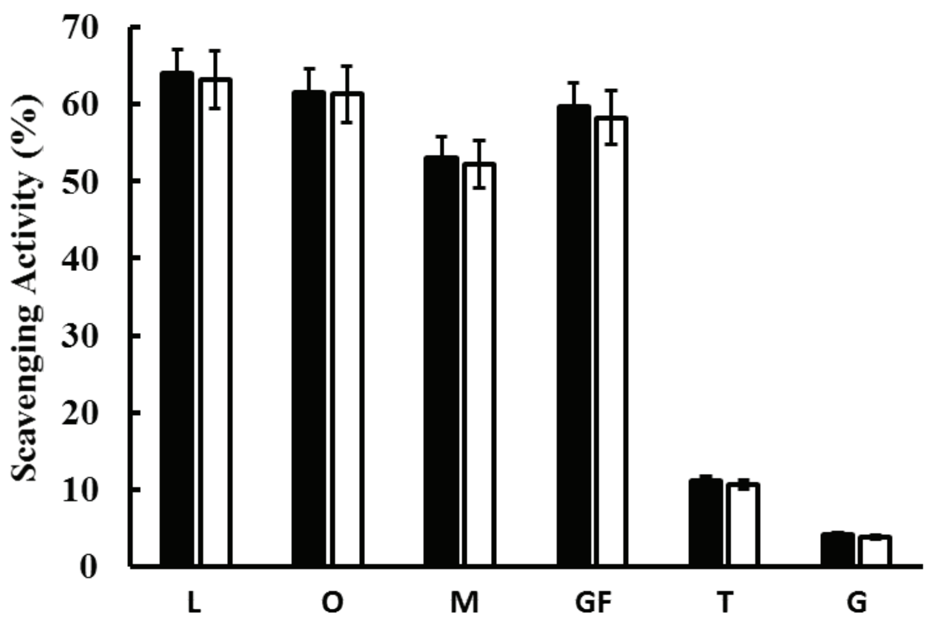

Figure 3: Free radical scavenging activity of fruit juices incubated at 4°C for 4 days. Scavenging activity of fruit juices before (▪) and after (□) incubation at 4°C.

Figure 4: Free radical scavenging activity of fruit juices incubated under the sun

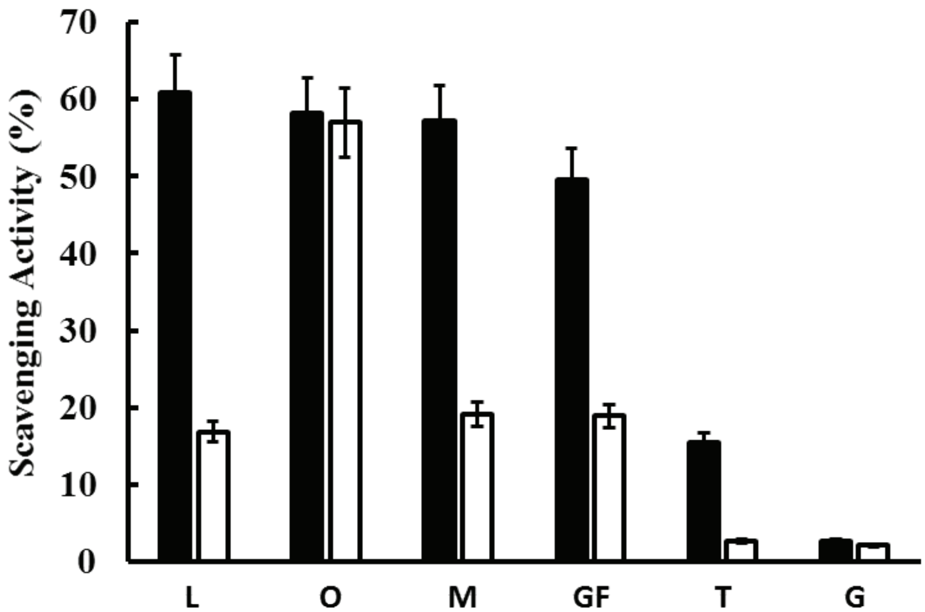

Figure 4: Free radical scavenging activity of fruit juices incubated under the sun for 4 days. Scavenging activity of fruit juices before (▪) and after (□) incubation under the sun.

scavenging activity after 4 days of incubation under sunlight. Lemon juices, mandarin and grapefruits lost more than 70% of their antioxidant activity. Tomato juices lost more than 50% of its antioxidant activity, while the guava retained its poor antioxidant activity.

Discussion:

There are many beneficial nutrients contained in freshly made juices. Because plant foods contain many different classes and types of antioxidants, knowledge of their total antioxidant capacity, which is the cumulative capacity of food components to scavenge ROS, would be useful for nutritional purposes (16). This study supports the common perception that fresh is often best for optimal antioxidant activity, although the storage of juices at low temperature or after processing at high temperature induces, if any, minor changes in the antioxidant activity. These results showed that different fruit juices possess different antioxidant activities and the variation was very significant (Fig 1). Lemon, orange, mandarin, and grapefruits possessed the highest antioxidant capacities, and are considered important dietary sources of natural antioxidants for prevention of diseases caused by oxidative stress.

Many of the plant nutrients are very sensitive to air, heat and light. In the light of this consideration, the changes in the overall properties of foods can be attributed to processing and storage conditions. However, no change in antioxidant activity was observed in heat-treated fruit juices, indicating that the high temperature did not induce significant changes in the scavenging property of these juices (Fig 2). Interestingly, no change in the scavenging property was observed in any type of fruit juices after heat treatment. The stability of scavenging capacity in the case of heat-treated juices during processing or storage could protect the juices against ROS generated during senescence or pathogen attack.

The prevention of spoilage by bacteria is a current challenge for fruit juice and beverage industries worldwide due to the bacterium's growth capability, heat resistance, and spoilage potential (17). Moreover, the general idea of storing juice at a cool and a dark place as possible, will help juices to lose nutrients at a much slower pace. The best way to preserve juices was proved to keep it in a cold place with capping. When all juices were stored in a cold place for 4 days, the following values of the antioxidant activity were reported (lemon 63%, orange 61%, grapefruits 58%, mandarin 52%, tomato 10% and guava 3%) (Fig 3). No significant change was observed in comparison to fresh juices. This suggests that the low temperature can be used to increase the juice shelf-life without affecting their scavenging activity.

Sunlight can degrade the juice over time and different juices are highly sensitive to sunlight exposure (18). Oxygen and light react with many nutrients like vitamin C, antioxidant, and causing their degradation. Sunlight can degrade the juice over time and may make the juice a breeding ground for organisms from bacteria to parasites. As the figure below (Fig 4) shows, the loss of juices scavenging activity is accelerated when the juices are incubated under sunlight. Orange juice showed the highest scavenging activity suggesting its special content that enables it to resist the damaging effect to of sunlight. The effect of sunlight on other juices, lemon, mandarin and grapefruits was very significant. All these 3 juices lost more than 50% of their scavenging activity. Although the orange juice and the other three juices (lemon, mandarin and grapefruit) belong to the same family, *Citrus*, the orange juice behaved differently suggesting something different in its composition which needs to be clarified extensively. If this is found to be true, this will be a great finding and can be used extensively in medical and cosmetic formulation.

In conclusion, loss of scavenging activity under different conditions depends on fruits type. These results showed that scavenging activity levels were maintained substantially under different temperature. The scavenging activity for all juices, except orange juice, was partially lost during incubation under sunlight. Sunlight is found to be the most destructive agent for the scavenging property of different juices. Storing juices at the backyard of big stores uncovered and under sunlight should be prohibited in order to maintain the nutrient content of juices including the scavenging activity. The optimum conditions for storing/storing juices can increase the juice shelf-life and maintain most of their nutrients.

Conflict of interest:

The authors declare no conflict of interests.

İşleme ve saklama koşullarının meyve sularının radikal süpürücü özelliklerine olan etkileri

ÖZ

Beslenme ve epidemiyolojik çalışmalar için gerekli olan besin tüketim tabloları besinlerin işlenmemiş şekilde tüketildiğini göz önüne alan örneklerdir. Bu çalışmalar, işlenme yöntemlerine göre besin içeriklerinin ya da biyolojik etkilerinin değişebileceği durumları dikkate almamaktadır. Bu çalışmanın amacı; işlenme ve saklanma koşullarının, meyve sularının serbest radikal süpürücü özellikleri üzerindeki etkisini araştırmaktır. Meyve sularının serbest radikal süpürücü etkileri 2, 2-difenil-1-pikrilhidrazil (DPPH) yöntemi kullanılarak belirlenmiştir. Farklı meyvelere ait suların değişen değerlerde radikal süpürücü etki gösterdiği tespit edilmiştir. Meyve sularının 4 gün boyunca, 4^{o}C'de tutulduğunda serbest radikal süpürücü etkilerinin oldukça stabil olduğu bulunmuştur. Taze sıkılmış meyve suları 4 gün boyunca, güneş ışığına maruz bırakıldığında ise portakal suyunun radikal süpürücü etkisini koruduğu bulunmuştur. Limon, mandalina ve greyfurt suları radikal süpürücü etkilerinin %70'den fazlasını kaybetmiş, domates suyunda ise bu kaybın %50'den fazla olduğu bulunmuştur. Meyve sularının kaynar su içerisinde 60 dakika tutulması radikal süpürücü etkilerinde dikkat çekici bir kayıp oluşturmamıştır. Sonuç olarak, güneş ışığının antioksidan özellik gösteren birçok besin bileşenini degredasyona uğratabileceği düşünülmektedir. Buna rağmen, kaynatma ve dondurma işlemlerinin meyve sularının radikal süpürücü özelliklerini etkilemediği tespit edilmiştir.

Anahtar kelimeler: Meyve suları, Antioksidanlar, Güneş ışığı, Isı, Gıdaların işlenmesi, Soğutma.

References

1. Huang WY, Zhang HC, Liu WX, Li CY. Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing. J Zhejiang Univ Sci B 2012;13:94-102.
2. Mangge H, Becker K, Fuchs D, Gostner JM. Antioxidants, inflammation and cardiovascular disease. World J Cardiol 2014;6:462-77.
3. Tonin FS, Steimbach LM, Wiens A, Perlin CM, Pontarolo R. Impact of natural juice consumption on plasma antioxidant status: A Systematic review and meta-analysis. Molecules 2015;20:22146-56.
4. Slavin JL, Lloyd B. Health benefits of fruits and vegetables. Adv Nutr 2012;3:506-16.
5. Hyson DA. A review and critical analysis of the scientific literature related to 100% fruit juice and human health. Adv Nutr 2015;6:37-51.
6. Singh GM, Micha R, Khatibzadeh S, Shi P, Lim S, Andrews KG, Engell RE, Ezzati M, Mozaffarian D, Global Burden of Diseases Nutrition and Chronic Diseases Expert Group (NutriCoDE). Global, regional, and national consumption of sugar-sweetened beverages, fruit juices, and milk: A systematic assessment of beverage intake in 187 countries. PLoS One. 2015;10:e0124845.
7. Wang YC, Bleich SN, Gortmaker SL. Increasing caloric contribution from sugar-sweetened beverages and 100% fruit juices among US children and adolescents, 1988-2004. Pediatrics 2008;121:e1604-14.
8. Dickinson A, Bonci L, Boyon N, Franco JC. Dietitians use and recommend dietary supplements: Report of a survey. Nutr J 2012;11:14.
9. Kris-Etherton PM, Lichtenstein AH, Howard BV, Steinberg D, Witztum JL. Antioxidant vitamin supplements and cardiovascular disease. Circulation 2004;110:637-41.
10. Nicoli M, Anese M, Parpinel M. Influence of processing on the antioxidant properties of fruit and vegetables. Trends Food Sci Techn 1999;10:94-100.
11. Kalt W. Effects of production and processing factors on major fruit and vegetable antioxidants. J Food Sci 2005;70:R11-9.
12. Walsh RP, Bartlett H, Eperjesi F. Variation in carotenoid content of kale and other vegetables: A review of pre- and post-harvest effects. J Agric Food Chem 2015;63:9677-82.
13. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;181:1199-200.
14. Rekha C, Poornima G, Manasa M, Abhipsa V, Devi JP, Kumar V, Kekuda TRP. Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. Chem Sci Trans 2012;1:303-10.
15. Kekuda TP, Shobha K, Onkarappa R. Studies on antioxidant and anthelmintic activity of two *Streptomyces* species isolated from Western Ghat soils of Agumbe, Karnataka. J Pharm Res 2010;3:26-9.
16. Rop O, Jurikova T, Mlcek J, Kramarova D, Sengee Z. Antioxidant activity and selected nutritional values of plums (*Prunus domestica* L.) typical of the White Carpathian Mountains. Sci Hort 2009;122:545-9.
17. Spinelli AC, Sant'ana AS, Rodrigues-Junior S, Massaguer PR. Influence of different filling, cooling, and storage conditions on the growth of *Alicyclobacillus acidoterrestris* CRA7152 in orange juice. Appl Environ Microbiol 2009;75:7409-16.
18. Wang Z, Zhang M, Wu Q. Effects of temperature, pH, and sunlight exposure on the color stability of strawberry juice during processing and storage. LWT-Food Sci Tech 2015;60:1174-8.