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ORIGINAL ARTICLE

Effect of UV Radiation as an alternative to thermal Pasteurization on microbiological Characteristics, sensory and shelf life of tomato juice

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ABSTRACT
Most foods are usually pasteurized by using thermal processes. Pasteurization can reduce organoleptic and nutritional characteristics of the products. Radiation is a new technique for reduction of food poisoning due to food consumption and increased shelf life of the product. In the present study, the effect of UV radiation as a cold pasteurization was investigated on the quality of tomato juice. The aim was to improve sensory, chemical and microbial properties of tomato juice. Treated samples were designated as T1, T2, T3, and T4. Respectively, T1 was the sample undergone heat treatment (treatment temperature 60 °C for 2 min). This was considered the control treatment. T2 was the pasteurized samples (treatments 72 °C for 2 min). T3 was the irradiated samples (from 15 cm for 30 min), and finally, T4 was the irradiated sample (15 cm for 60 min). The prepared samples were stored at a refrigerator for 90 days. Every 30 days, microbial tests (total count of bacteria, mold and yeast) and sensory characteristics (odor, color, taste, in terms of consumer acceptance) were performed. The results of total microbiological counting showed that T1 was the most contaminated sample and T2 did not have any contamination. In addition, it was found out that UV radiation was effective only for 15 days. However, after 15 days, the radiated samples showed contamination as well; even though the degree of contamination was less than the control sample. This was also true about the mold and yeast content of the samples. Organoleptic assessment showed that T1 was consumable only on production day but after that it was not consumable and had the lowest acceptability by test panel because of microbial contamination. Finally, the radiated samples had the highest organoleptic scores. Therefore, it could be concluded that UV radiation can be an effective method for preserving the quality of tomato juice and extend its shelf life.
Key words: tomato juice, pasteurization, ultraviolet radiation, quality and shelf life properties.

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INTRODUCTION

Changes in the pattern of production and consumption can play an essential role in improving the country's new economy. Improvement of production and maintenance techniques and creation of a variety in products are a few of the improvements. Nowadays, various fruit and vegetable product are produced in countries around the world, tomato juice drink being one of them. Like other agricultural products, most vegetables, which are rich sources of vitamins, minerals and carbohydrates, are seasonal. Often, due to excess production, if proper storage methods are not used, most of the product will suffer various types of microbial, chemical and physical contamination and has to be thrown away. One of these methods of protection and spoilage prevention is production of beverages from that product, packaging it properly and applying some hot or cold pasteurization processes [11]. Nowadays, demand of the

consumers for minimally processed products with high quality has increased remarkably. Foods and drinks with new, healthy and enriched flavors are preferred [9]. Typically, most food products are processed by being exposed to temperatures ranging from 60-100°C. The exposure time varies from just a few seconds to a few minutes. Use of thermal processes such as pasteurization, a hot thermal treatment (92°C-2 minutes), cold thermal treatment 60°c - 2minutes), which is used on tomato juice, can lead to reduced organoleptic properties and qualitative – nutritional characteristics of the product. For this reason, heating is gradually losing its application as a method for sterilization [3]. According to the Unanimous opinion of the Food and Agriculture Organization and the World Health Organization in 1983, radiation, as safe and effective method of food preservation, was accepted and a special code was assigned to it by the General standards division of Codex Assembly (Vashtani *et al*., 2011). Therefore, sing radiation on food products to lower the risk of food poisoning has become a common new method used by food product producers. Nowadays, a number of countries in the world are using this method systematically in their food industry. Therefore, this method increases the shelf life and storage time of the products. In addition, research shows that99.9%of disease causing organisms are destroyed by Ultraviolet radiation[12].UV rays' wavelength are between 0/0144 micrometers 0/39 micrometers. UV rays are divided into three zones [12].

1. The long-wavelength ultraviolet or ultraviolet A (300 - 400 nm).
2. Intermediate wavelength ultraviolet or ultraviolet B (320 - 280 nm).
3. The short-wavelength ultraviolet or C wave length shorter than 280 nm).

In this study, the effect of ultraviolet radiation (UV-C) with wavelength shorter than 280nm was used on tomato juice was investigated. It was selected due to the strong germicidal effects on sensory characteristics, microbial and shelf-life.

MATERIALS AND METHODS

Raw material:

Super-chief tomatoes with the scientific name of *Lycopersicum esculantum* were used. This plant produces tomatoes which are roundel, ripe and red in color. The tomatoes were grown in the farms in Outskirts of Tabriz, Iran, but were purchased from the produce market. The Chemicals used in this study were of analytical grade and were manufactured by Merck of Germany.

Tomato juice produced

In this stage, thirty kg. Of tomatoes was washed using tap water, and then, to make juicing easier, tomatoes were sliced into small pieces and passed through a filter. Then the filtered material was Cold breaked (T = 60°C - t=2 min), [10]. The resulting tomato juice was divided into the four following samples: T_1, control sample, T_2, pasteurized by heating, T_3, treated tomato juice using ultraviolet radiation for 30 minutes and T_4, under ultraviolet radiation for 60 minutes.

Producing pasteurized tomato juice

Tomato juice was poured in the stainless steel containers and then placed in a hot water bath. When the temperature reached 72°C in its center, it was pasteurized at this temperature for 2 minutes. The pasteurized tomato juice was quickly placed in an ice water bath, until the temperature reached 5°C. Then, it was held in a refrigerator (4°C) for experimentation [10].

UV-treated tomato juice production

The Cold beaked tomato juice was poured into sterile containers (100mm × 15mm with depth 10mm) and then, it was exposed to UV (fluorescent light emission peak at 254 nm) for 30 and 60 minutes from the distance of 15 cm. On average, the samples received UV rays at a dose of (2.158 j/m^2) at room temperature [13]. Then, various microbial tests such as yeast and mold, total bacterial count and sensory tests with three repetitions were carried out.

Total bacterial count

According to the Iranian National Standard of 1381 with No.2965 and references to the 5272 standard, dilutions of 10^{-5} were used to determine the total bacterial count about. To obtain the total count, 0.1mL of each dilution was poured into the plates with plate-count-agriculture. Then, superficial cultivation was carried out.

Mold and yeast count

Potato dextrose agar medium was used for determination of mold and yeast count. All the samples were incubated for 5 days at 27°C [7, 8].

Gram staining

After the smears were prepared and colored, they were observed under a microscope (100 - magnification)[1].

Identification of bacterial spores

Using staining by Whirts conkin, bacteria's spores were tested and studied using a microscope with 100x magnification microscope (Roland, 1997).

Sensory evaluation

For Sensory evaluation, in an initial test, a few people with strong sense of taste were selected. Then, using different codes, which were unknown to the testers, the samples were given to them. The people who were capable of identifying similar and different samples from one another were selected to test the main samples. For every feature, a score of 1representing the lowest intensity of the characteristics being tested and a score of 5 representing the highest was assigned. The panelists were, first, familiarized with the concepts of the features being tested, and then, each sample with a hypothetical code was given to them. To each panelist, a glass of water was given so that they could use it to clean their pallets. The same procedure was used for assessing the flavor, aroma and appearance of the samples.

Statistical analysis

In this investigation, the effects of treatments on characteristics of tomato juice samples, T_1, T_2, T_3 and T_4, were measured during the 90 days of storage in refrigerator (4°C) in the form of repeated measurements (Repeated measurement and 3 replicates were performed). The data obtained from these measurements was analyzed using two-way ANOVA at $P< 0.05$ and using least squares mean($P<0.05$). To carry out these statistical operations, SAS 9.1 (SPSS statistical analysis software) was used (SPSS Inc. USA).

RESULTS

Total count of microorganisms

The results of total count of microorganisms in the culture (PCA), culture incubated for 5 days at 30°C, are displayed in Table 1.

Table 1. Results of total count of microorganism (log cfu / g),for samples, within 90days

Treatments				(day) time	Measurement Parameters
T_4	T_3	T_2	T_1		
$0/00\pm0/00^{Db}$	$0/00\pm0/00^{Cb}$	$0/00\pm0/00^{Ab}$	$4/13\pm0/02^{Ba}$	1	total count of microorganisms (log cfu/g)
$0/00\pm0/00^{Db}$	$0/00\pm0/00^{Cb}$	$0/00\pm0/00^{Ab}$	$4/14\pm0/02^{Ba}$	15	
$2/95\pm0/05^{Cc}$	$3/35\pm0/03^{Bb}$	$0/00\pm0/00^{Ab}$	$4/18\pm0/05^{Ba}$	30	
$3/61\pm0/04^{Bc}$	$3/90\pm0/03^{Ab}$	$0/00\pm0/00^{Ad}$	$4/51\pm0/04^{Aa}$	60	
$3/76\pm0/03^{Ac}$	$3/95\pm0/02^{Ab}$	$0/00\pm0/00^{Ad}$	$4/59\pm0/04^{Aa}$	90	

Dissimilar Small Latin letters in each row indicate significant differences ($P < 0.05$) between treatments at a given time. Dissimilar capital Latin letters in each column indicate significant differences ($P < 0.05$) at different times are a given treatment. T_1: control (under heat at 60 ° C for 2 min) T_2: pasteurized by heating to 72 °C for 2 minutes, T_3: irradiated for 30 minutes and T_4: in irradiated for 60 minutes.

Samples which were cultivated on days 30, 60, 90 contained many tiny bacteria. After performing specific tests such as Gram staining and determination presence of spore, it was found out that the samples contained spores. The effect of shelf life on the amount microorganisms was significant ($P< 0.05$). In all the samples except sample T_2, with passage of time the number of microorganisms increased.

Determination of mold and yeast

The results of determination of mold and yeast count which were cultivated PDA culture and incubated for 5 days at 27°Careshownin Table 2.

Table2.Results of mold and yeast of count (log cfu / g), for samples, during 90 days storage

Treatments				Time (day)	Measurements Parameter
T_4	T_3	T_2	T_1		
$0/00\pm0/00^{Db}$	$0/00\pm0/00^{Db}$	$0/00\pm0/00^{Bb}$	$2/19\pm0/04^{Da*}$	1	The amount of mold and yeast (log cfu/g)
$0/00\pm0/00^{Db}$	$0/00\pm0/00^{Db}$	$0/00\pm0/00^{Bb}$	$3/19\pm0/04^{Ca}$	15	
$2/78\pm0/12^{Cc}$	$2/95\pm0/03^{Cb}$	$0/00\pm0/00^{Bd}$	$3/42\pm0/03^{Ba}$	30	
$4/19\pm0/03^{Ba}$	$3/77\pm0/03^{Ab}$	$0/00\pm0/00^{Bd}$	$3/47\pm0/01^{Bc}$	60	
$4/47\pm0/02^{Aa}$	$3/52\pm0/02^{Bc}$	$2/80\pm0/03^{Ad}$	$3/97\pm0/03^{Ab}$	90	

Dissimilar Small Latin letters in each row indicate significant differences ($P < 0.05$) between treatments at a given time. Dissimilar capital Latin letters in each column indicate significant differences ($P < 0.05$)at different times is a given treatment. T_1: control (under heat at 60 ° C for 2 min) T_2: pasteurized by heating to 72 ° C for 2 minutes, T_3: irradiated for 30 minutes and T_4: in irradiated for 60 minutes.

The result of microorganism count was reported zero on day first and 15th in the samples which were radiated by UV-C. The effect of storage time and radiation on the number of microorganisms was found out to be significant (P< 0.05). Radiation with UV-C had more effect on the mold spores than on yeasts as in all samples, the amount of mold during the storage time was reported to be zero except in sample designated as T_2 at 10^{-1} dilution on day 90 which was 7×10^2 . This condition existed despite the fact that all the samples had undergone radiation.

Sensory evaluation

Sensory evaluation of the product is the last stage of evaluating the quality and the success of the treatments [6]. The results of the sensory evaluations are displayed in table 3.

Table 3. Results of color and appearance changes from the panelists' perspectives, for samples after 90 days of storage

Treatments				time (day)	Measurement Parameter
T_4	T_3	T_2	T_1		
$5/00\pm0/00^{Aa}$	$5/00\pm0/00^{Aa}$	$2/00\pm0/20^{Ab}$	$3/20\pm0/13^{Ab*}$	1	color and appearance
$5/00\pm0/00^{Aa}$	$5/00\pm0/00^{Aa}$	$1/00\pm0/10^{Bb}$	$1/00\pm0/10^{Bb}$	30	
$5/00\pm0/00^{Aa}$	$5/00\pm0/00^{Aa}$	$1/00\pm0/05^{Bb}$	$1/00\pm0/10^{Bb}$	60	
$4/40\pm0/16^{Bb}$	$5/00\pm0/00^{Aa}$	$1/00\pm0/06^{Bc}$	$1/00\pm0/13^{Bc}$	90	

Dissimilar Small Latin letters in each row indicate significant differences (P < 0.05) between treatments at a given time. Dissimilar capital Latin letters in each column indicate significant differences (P < 0.05)at different times is a given treatment. T_1: control (under heat at 60 ° C for 2 min) T_2: pasteurized by heating to 72 ° C for 2 minutes, T_3: irradiated for 30 minutes and T_4: in irradiated for 60 minutes.

Table 4. The results of smell and aroma changes from the perspective of pane lists in the product, for samples during 90days.

Treatments				time (day)	Measurement Parameter
T_4	T_3	T_2	T_1		
$5/00\pm0/04^{Aa}$	$5/00\pm0/04^{Aa}$	$2/20\pm0/13^{Ab}$	$5/00\pm0/00^{Aa}$	1	Odor and aroma
$5/00\pm0/05^{Aa}$	$5/00\pm0/04^{Aa}$	$2/00\pm0/09^{Bb}$	$1/00\pm0/02^{Bc}$	30	
$4/00\pm0/06^{Bb}$	$4/20\pm0/13^{Ba}$	$2/00\pm0/07^{Bc}$	$1/00\pm0/04^{Bd}$	60	
$4/00\pm0/06^{Ba}$	$4/00\pm0/06^{Ca}$	$2/00\pm0/03^{Bb}$	$1/00\pm0/04^{Bc}$	90	

Dissimilar Small Latin letters in each row indicate significant differences (P < 0.05) between treatments at a given time. Dissimilar capital Latin letters in each column indicate significant differences(P < 0.05)at different times is a given treatment. T_1: control (under heat at 60 ° C for 2 min) T_2: pasteurized by heating to 72 ° C for 2 minutes, T_3: irradiated for 30 minutes and T_4: in irradiated for 60 minutes.

Table5. Results for taste changes from the perspective of panelists, for samples, during the 90-day maintenance.

Treatments				time (day)	Measurement Parameter
T_4	T_3	T_2	T_1		
$5/00\pm0/00^{Aa}$	$5/00\pm0/00^{Aa}$	$3/00\pm0/08^{Ab}$	$5/00\pm0/00^{Aa}$	1	Taste
$1/00\pm0/05^{Bb}$	$1/00\pm0/04^{Bb}$	$2/20\pm0/13^{Ba}$	$1/00\pm0/05^{Bb}$	30	
$1/00\pm0/05^{Bb}$	$1/00\pm0/06^{Bb}$	$1/40\pm0/16^{Ca}$	$1/00\pm0/05^{Bb}$	60	
$1/00\pm0/04^{Ba}$	$1/00\pm0/04^{Ba}$	$1/00\pm0/05^{Da}$	$1/00\pm0/04^{Ba}$	90	

Dissimilar Small Latin letters in each row indicate significant differences(P < 0.05)between treatments at a given time. Dissimilar capital Latin letters in each column indicate significant differences(P < 0.05)at different times is a given treatment. T_1: control (under heat at 60 ° C for 2 min) T_2: pasteurized by heating to 72 ° C for 2 minutes, T_3: irradiated for 30 minutes and T_4: in irradiated for 60 minutes.

Table 6.Results for general acceptance changes from the perspective of panelists, for samples during the 90-day.

Treatments				time (day)	Measurement Parameter
T_4	**T_3**	**T_2**	**T_1**		
$5/00\pm0/00^{Aa}$	$5/00\pm0/00^{Aa}$	$2/00\pm0/06^{Ac}$	$3/80\pm0/13^{Ab}$	1	General acceptance
$4/00\pm0/08^{Bb}$	$5/00\pm0/00^{Aa}$	$1/00\pm0/03^{Bc}$	$1/00\pm0/06^{Bc}$	30	
$4/00\pm0/07^{Ba}$	$4/00\pm0/08^{Ba}$	$1/00\pm0/07^{Bb}$	$1/00\pm0/05^{Bb}$	60	
$4/00\pm0/06^{Ba}$	$4/00\pm0/04^{Ba}$	$1/00\pm0/05^{Bb}$	$1/00\pm0/04^{Bb}$	90	

Dissimilar Small Latin letters in each row indicate significant differences (P < 0.05) between treatments at a given time. Dissimilar capital Latin letters in each column indicate significant differences(P < 0.05)at different times is a

given treatment. T_1: control (under heat at 60 ° C for 2 min) T_2: pasteurized by heating to 72 ° C for 2 minutes, T_3: irradiated for 30 minutes and T_4: in irradiated for 60 minutes.

DISCUSSION AND CONCLUSION

Even though the absorption of UV rays by air is insignificant, maximum intensity of the UV lights is on their surface. However, after the distance from the UV rays' source passes 5 cm. a sharp drop is observed in its effectiveness [2]. Maximum effectiveness of UV rays is observed at wave length of 2600 Angstrom. UV rays at this wavelength are not ionizing and are absorbed by proteins and nucleic acids. The photochemical changes caused by UV rays lead to the death of a cell. The low penetrability of these rays makes it a good choice only for surface usages [17]. The UV-C treatment plays an effective role in lowering the amount of bacteria on the surface of fruits [18]. Considering this fact, the surface area of the tomato juice exposed to UV rays, in this experiment, was increased by pouring it in large but not deep containers. Radiation led to total annihilation of the bacteria on the first day of production and no bacteria was found in these samples for the first 15 day of storage. Therefore, by increasing the duration of radiation, a totally safe product with respect to presence of microorganisms was produced. Radiation by UV-C rays resulted in complete eradication of mold but had lesser effect on the bacterial count and the yeasts. Despite the fact that it usually takes five days after cultivation for mold colonies to appear on the sample, it took seven days for them to appear on the samples kept in incubator. These mutated colonies (spores) were very tiny and nearly invisible to the naked eye. This made counting the number of colonies en extremely tough task. The sample undergone radiation showed no microorganisms until the day 15. However, after day 15, the mutated bacteria started to grow in the samples. In other words, radiation was incapable of totally destroying the mold spores. Treatment with UV-C had more effect on the molds than the yeasts as it resulted in complete eradication of the molds during the 90 days storage period. The pasteurized samples were also microorganism free for the 90 days holding period, too. Considering the fact that the storage conditions were the same for all the samples, it could be stated that the pasteurization would be a better choice to adopt at home because it is safer and easy to carry out. However, the downside is that it leads to lowered organoleptic quality and destruction of vitamins and useful ingredients in the stored product.

The results of the study are in line with findings of other scientific research. In a study conducted in 2011, microbial studies on a sample of star fruit showed that there was a significant decrease in APC[2] and bacteria content due to the radiation effect. Moreover there was a noticeable improvement on the physical and qualitative conditions of the product, which is beneficial both for the consumer and the producer [13].

Furthermore, in another research, the effect of UV-C was investigated on lowering the microbial content of orange juice. The DNA of the microorganism prevents bacterial growth by absorbing UV rays and forming Pyrimidine and Thymine base. The viscosity and clearness are two of the most important factors in making UV-C rays ineffective, because the fruit juices which are not clear and contain a lot of pulp do not absorb the rays as much as the clear and pulp-free juices [16]. In, yet, another research, processes which do not involve heat treatment such as Gama and UV radiation were tested on fresh carrot juice. The carrot juice sample was radiated using UV with a dose of 3.67. the radiation kept the total bacteria count at an acceptable rate of 105 CFU/ml while it had kept the bacterial growth at zero on the first day. However, the bacteria count increased after seven day of storage. When the dosage of radiation increases, the length of the time which the product stays bacteria free increases as well. It worth mentioning that radiation with Gama rays showed to be more effective [5]. In a research, the effect of UV-C was tested on deactivation of Alicyclobacillus Acidoterrestris present in white grape juice and pasteurized apple juice using a commercial method.

The results of the study indicated that when dealing with grape juice, a combination of UV-C with pasteurization is much more effective [4].

Another study showed that UV results in significant reduction in bacteria content; especially mold and yeast content, of pineapple juice. The total count of bacteria in the treated sample was reduced from 0/1 6/06 ±CFU/mlto 4/15 ± 0/07CFU/ml An important point in radiation to notice is that to obtain the best result the juice has to be clear and pulp-free or a higher dosage of UV should be used Shamsudin [15] .The DNA of the microorganisms absorbs UV photons and leads to creation of a bond between neighboring Thymine and Cytosine in the DNA helix. This in turn, creates a Pyrimidine dimer, which prevents DNA replication and transcription leading to deactivation of microorganisms in the product [14].

One of the most important features of food products to have is likeability and sensory evaluation of the product by consumers. In this study, application of radiation for 30 minutes had a suitable and acceptable

effect on the sensory characteristics of tomato juice. Radiation leads to a homogeneous distribution of particles in the juice and bestowed upon the product a nice color and beautiful appearance to the product which was liked by the customers. The radiation treated tomato juice samples resembled fresh tomato juice more than the pasteurized samples. From the panelists' point of view, flavor of pasteurized samples resembled crushed tomato. In addition, the flavor of the control sample was like that of rotten tomato during the last few days of storage period. The results of this study were in line with the findings of the current study [13]. The T_1 sample was only useable on the first day of production and scored higher, but due to the fact that it was neither pasteurized nor was it undergone UV radiation, it faced high bacteria growth and scored the lowest among the samples. Overall, the results of this study indicate that UV-C radiation could replace and/or supplement pasteurization of tomato juice as a method which is safe and keeps the quality of the product intact. However, there is a need for a much more comprehensive study in commercial scale production of tomato juice in order to be able to achieve the laboratory-obtained results in factories.

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