The Effects of Pasteurization, Ultraviolet Radiation and Chemical Preservatives on Microbial Spoilage and Scent Composition of Rose Water

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Abstract: Damask Rose (Rosa damascena Mill.) is the major rose species used for rose oil production. Rose water is the major subsidiary product obtained during the distillation process. Rose water has also commercial importance because of its usage as an ingredient in some processed foods and aromatherapy. Microorganisms cause souring and therapeutic effect losses of rose water during its storage. Various preservation techniques were applied in rose water for the prevention of microbial spoilage. The physical methods used for the preservation of rose water were pasteurization, and ultra violet treatment. Phenoxyethanol and sodium benzoate were used as chemical preservatives. After methods were applied, rose water samples were stored at room temperature and spoilage microorganisms such as bacteria, yeasts and molds were enumerated. The changes in the composition of essential oils of rose water samples were also determined. Any of the applications caused a significant change in aroma composition, while inhibiting the microbial spoilage.

Key words: Damask rose, Rosa damascena Mill., rose water, microbial flora, essential oil composition

Introduction

Aromatic waters (Aqua aromaticae) are generally produced by distillation of aromatic plants containing low amounts of water-soluble constituents of essential oil. Water obtained by condensation of vapor during steam distillation of rose flowers contains low amounts of essential oil. This by-product is called hydrosol in general or “rose water” in rose distillation and contains aromatic compounds either in the form of solution or suspended particles. The amount of essential oil components in hydrosols depends on the solubility and specific gravity of aromatic compounds 22. In conventional production, water-soluble components of rose oil are separated by second distillation of rose water. Both the rose water and the second distillation water have a pleasant odor of rose flower. At the end of second distillation, the water remaining inside the distillation still is called “residual water”. The usage of this low quality end-product is limited 16.

Rose water is one of the first aromatic waters which has been produced since ancient times. There are mainly four species of rose for essential oil production. These are Rosa damascena Mill., Rosa gallica L., Rosa moschata Herrm. and Rosa
*R. damascena* (Damask rose) is the common rose species used in Turkey which is the major rose oil producer in the world. Recently rose oil and rose water are produced from this species widely in Isparta Province, Turkey and Kazanlak Province, Bulgaria. Rose oil and rose water are produced by water distillation of fresh flowers of Damask rose. Raw rose oil called “concrete” and it is produced by *n*-hexane extraction of rose flowers and “absolute” the last product is produced by ethanol extraction of the concrete. While Turkey and Bulgaria are the main producers of rose oil and rose water, rose water is also produced in Iran, Saudi Arabia, Syria, India and some other countries. Among all aromatic waters produced in Turkey, a national standard was established only for rose water by Turkish Standards Institution (TSE, “Rose Water Monograph” TS 5555, 1988).

Rose water is widely used in food flavoring, soaps, toiletry and cosmetic products. It is also used as an antiseptic facial tonic, fever reducer, cooling aid, pain reliever and used for gut problems in traditional medicine. Another important usage of rose water is aroma and taste additive in the food industry. In water distillation method rose water contains very low amounts of (below 0.1 %) essential oil and its main component is phenylethyl alcohol. Although rose oil shows a strong antioxidant and antimicrobial effect, similar effects of rose water is rather low. However some herbal hydrosols like oregano is known to have a strong antimicrobial effect. For this reason, rose water provides an ideal growth environment for bacteria, yeasts and fungi.

Distillation technique plays the key role on the quality of herbal hydrosols. In conventional process rose water is obtained in the last step of rose oil production which is held in high-capacity copper or stainless steel distillation apparatus by double distillation method. Since distillation is applied in a air-tight apparatus at high temperatures (100°C), a partial pasteurization process is also achieved. Rose water produced in this process can be used safely for at least one year without any microbial defect in markets. However the excess product is not directly marketed and stored in non-sterile barrels, exposes to light, air, and kept at moderate temperatures for a long time and sometimes diluted in non-sterile water before packaging. Inappropriate storage conditions causes loss of quality. In addition to storage conditions, microorganisms such as yeasts, molds, and spore-formers can metabolize aromatic compounds in rose water along with some other compounds and cause souring. Spoiled rose water looses its desired fragrance and its other beneficial effects. Although some producers use antimicrobial food additives like methylparaben, such chemicals protect rose water only until the package is opened. Then the rose becomes sensitive to spoilage. Because of spoilage during storage in high amounts, a significant amount of rose water is discarded every year, causing economic loss. Rose water producers either are not aware of the reasons for the spoilage or do not have enough technical knowledge about solution to the problem.

The purpose of this study is the elimination of microbial contamination causing undesired odor during 12 month shelf life of rose water. To accomplish a satisfactory elimination of microorganisms with four different methods of preservation were applied. The methods were pasteurization, UV treatment, addition of sodium benzoate and phenoxyethanol. The effect of applications on microorganisms and essential oil composition of rose water were determined during storage. Yet, to our knowledge there is no research published about either spoilage of rose water or the solution for the problem.

**Materials and methods**

**Rose water samples**

Rose water samples used in the study were produced by Sebat Rose Oil Company (Senir, County Keçiören-Isparta/Turkey) from fresh rose petals (*Rosa damascena* Mill.) harvested between May to June 2008. Rose water samples were taken from the same production batch and produced by conventional water distillation method. The pH of the rose water batch was 6.9. Rose water samples were collected during
distillation from the condensed water in Florentine flask during cohobation process. After collection, the samples were treated with proposed methods.

**Sample preparation**

Pasteurization process was applied in a stainless steel heat exchanger having a capacity of 25 liters per hour. Samples were treated with high temperature short time (HTST) at 90°C for 15 seconds. After pasteurization rose water was cooled down to room temperature in another heat exchanger and stored in sterile containers.

In UV application, rose water forced to pass through a UV source. The flow rate was 60 liters per hour at 8 bar pressure and the UV intensity was 30,000 μJoule. The UV source was a 30 Watts, TUV type at 254 nm constant bulb (980 x 120 x 125 mm). Antimicrobials, sodium benzoate (NaC₆H₅CO₂, Sigma-Aldrich, B-3375) and 2-phenoxethanol (C₆H₁₀O₂, Sigma, P-1126) were added in rose water samples at doses of 0.1 % (w/v) and 0.01 % (w/v) respectively. According to EU regulations, maximum allowed doses for sodium benzoate is 0.1% and %1 for 2-phenoxethanol in cosmetic products.

After the application of preservation methods, rose water samples were bottled in market size 500 ml plastic (PP) bottles and capped. All bottles were protected from direct sunlight, stored at room temperature for 12 months and samples were analyzed in every 3 months. Rose water samples were taken under aseptic conditions, and taken to the microbial and chemical analysis.

**GC-MS analysis**

Gas Chromatography-Mass Spectrometry analysis of the rose water samples was performed on Shimadzu GC-MS QP5050 (Kyoto, Japan) equipped with a Quadrupole detector. Separations were carried out by a CP-Wax 52 CB capillary column (50 m x 0.32 mm; film thickness 0.25 μm) purchased from Varian. Helium was used as carrier gas at a constant head pressure of 10 psi. The injection volume was 1 μl. The GC oven was programmed as follows: the initial column temperature was 60°C, the column was heated to 220°C at a rate of 2°C min⁻¹ and held at 220°C for 20 min. The GC-MS interface and injector were kept at 250°C and 240°C, respectively. The mass spectrometer was run in the electron impact mode at 70 eV. Liquid-liquid extraction of rose water was done with n-hexane. Rose water (50 mL) was diluted with 2 ml of n-hexane overnight, and upper phase including rose oil was injected into the GC-MS system. The oil was also hydrodistilled from 0.5 kg of fresh rose flowers using a Clevenger-type apparatus for 3 h for comparing rose water and rose oil volatiles.

Identification of the rose water and rose oil constituents was carried out with the help of retention times of standard substances by composition of mass spectra with the data given in the NIST library.

**Microbial Analysis**

To determine the microbial changes in rose water treated with different preservation methods, mesophilic aerobic bacteria, molds and yeasts were enumerated. Untreated rose water was used as control. The number of aerobic bacteria, yeasts and molds were determined according to the official methods of TSE (Turkish Standards Institute) and official methods of FDA (Food and Drug Administration). According to the methods 10 ml of rose water sample placed in a sterile test tube. Serial dilutions were made when necessary. All dilutions were plated on PDA (Potato Dextrose Agar, pH 3.5, containing 50 mg/l chloramphenicol) for enumeration of yeasts and molds. Aerobic mesophilic bacteria were enumerated on PCA plates (Plate Count Agar, Merck, Germany). After plating, PDA plates were incubated at 25°C for 4 days and PCA plates were incubated at 30°C for 2 days. All platings were done in triplicate.

**Results and discussion**

When compared to some other essential oil crops, Damask rose flowers generally contain low amounts of essential oil. In this study, oil yield of the fresh rose flowers hydro distilled by Clevenger was only about 0.03 % (not tabulated). Meaning that one kg of rose oil can be obtained from about 3000 kg of rose flowers. Because of the low oil content and lackness of natural and
synthetic substitutes, rose oil is one of the most expensive essential oils in the world market. The oil yields of rose water prepared from fresh flowers were between 0.03-0.06 % (v/w), and rose waters obtained from air-dried flowers contain 20-50 % less oil. The yield of rose water by liquid-liquid extraction with n-hexane was about 0.09 % (not tabulated). Similar results were reported by Kýrýmer et al., regarding the extraction yield with n-hexane extraction of rose flowers. The compositions of essential oils obtained by GS-MS were presented in Table 1. Rose water oil was extracted from fresh rose flowers by n-hexane liquid-liquid extraction and rose oil was extracted from fresh flowers by using Clevenger hydrodistillation for 3 h.

In this study 14 different essential oil components which were higher than 0.1 % were determined in rose oil sample. Those 14 components represent nearly 97.3 % of all rose oil constituent. Rose oil was characterized by high percentage of non-cyclic monoterpene alcohols, represented particularly by citronellol (32.5 %), geraniol (25.2 %) and nerol (11.8 %), and long-chain hydrocarbons represented particularly such as nonadecane (9.3 %), 9-nonadecene (3.1 %) and heneicosane (4.5 %) (Table 1). Previous studies done for the determination of rose oil composition showed that those compounds mentioned above are the major components found in rose oil.

The amount and number of components in rose water oil obtained by n-hexane extraction were found different from that of regular hydrodistilled rose oil. In rose water oil only 9 different components higher than 0.1 % were detected. While regular rose oil contains high amounts of long chain hydrocarbons, those hydrocarbons were found in rose water oil in trace amounts, showing that almost all hydrocarbons transferred to the rose oil during water distillation. While the amount of phenylethyl alcohol was determined only 1.4 % in rose oil, it was found much higher (17.2 %) in rose water oil (Table 1). Unlike hydrocarbons higher amounts of watersoluble phenylethyl alcohol was found in rose water.

Table 1. Essential oil composition of rose oil and rose water oil distilled from fresh rose flowers

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Rt*(min)</th>
<th>Rose oilb (hydrodistilled by Clevenger from freshrose flowers)</th>
<th>Rose water oilb (extracted by n-hexane from rose water)</th>
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<tbody>
<tr>
<td>Ethanol</td>
<td>8.1</td>
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<td>Geranyl acetate</td>
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* Retention times (Rt),

b relative percentage (%) obtained on CP-Wax 52 CB capillary column,

c t (trace) = < 0.1%
water oil \(^{24}\). In a study conducted by Kirimer et al., \(^{22}\), in the essential oil obtained by \(n\)-hexane extraction 14 main components were determined. Determined components were phenylethyl alcohol (34.2 %), citronellol (22.8 %), geraniol (16.3 %), nerol (7.6 %) and methyl eugenol (7.6 %). Ulusoy et al., \(^{32}\) determined only four components citronellol (29.4 %), nerol (16.2 %), geraniol (30.7 %) and phenylethyl alcohol (23.7 %) in rose water. Agarwal et al., \(^{1}\) reported that rose water extracts contained phenylethyl alcohol in between 30.8 % to 72.5 % depending on the type of solvent used.

While rose oil did not contain ethanol, rose water oil had considerably high amounts (1.7 %) of ethanol (Table 1). In addition, while rose oil contained 2.4 % methyl eugenol and 0.8 % eugenol, rose water had 4.4 % and 5.0 % respectively (Table 1). Although methyl eugenol is a high value aroma compound used in cosmetic products and flavoring agents, the amount of methyl eugenol in rose oil should be either zero or under certain limits due to negative side effects on human health \(^{19}\). However in sometimes the amount of methyl eugenol in Turkish rose oil samples can be higher than 2 % or even higher (4 %). Delayed harvest and delayed or prolonged distillation process may cause high methyl eugenol amounts in rose oils \(^{13}\). Since the total amount of oil in rose water is lower than 0.1 %, the amount of methyl eugenol is below the limits due to dilution factor.

In Table 2, the effect of various methods applied for the preservation on the essential oil composition of rose water is presented. According to the results of GC-MS analysis there is no negative effect of physical or chemical preservation methods on the components of rose water, while there is a positive effect on its shelf life.

In rose water samples during one year storage while there is a slight decrease in the amounts of phenylethyl acetate, methyl eugenol and eugenol,
The amount of phenylethyl alcohol increased (Table 2). Decreasing amounts of methyl eugenol and increasing amounts of phenylethyl alcohol is a desired change for the odor quality of rose oil.

The rate of ethanol increased until month 6 and there was a drastic decrease after that point. No ethanol was found in all the applications eventually. The rate of linalool also showed an increase until month 9 and a minor decrease was determined at month 12. The highest amounts of phenylethyl alcohol and linalool were determined in phenoxyethanol applied samples. Although a relative change in the amounts of citronellol which is one of the main components of rose essential oil was determined in some of the applications, the change was insignificant between applications. It was also determined that 12 month storage and different applications had no effect on the amounts of geraniol and nerol in rose water. The amount of these compounds was highly stable in all of the samples (Table 2).

The effect of applications on the number of microorganisms is presented in figures 1. and 2. Apparently sodium benzoate and phenoxyethanol inhibited the growth of bacteria, yeasts and molds. While there was a high rate of decrease in the number of mesophilic bacteria in all applications and control, some survived after pasteurization and UV treatment. It can be said that bacterial spores were relatively resistant to pasteurization and UV treatment but not to the chemical preservatives. Pasteurization and UV treatment are widely applied methods for the inhibition of pathogenic and spoilage microorganisms in the manufacture of dairy products and fruit juices 15,20. Studies about activation of bacterial spores also showed that 17,26 thermal processes between 80° to 100°C may increase the germination rate of bacterial spores in the presence of appropriate conditions. Since the number of yeasts and molds decreased immediately in all of the applications but not in control group, it can be said that most of the compositional changes in processed rose water was driven either by bacteria or by chemical interactions among various chemical compounds.

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<th>Compounds</th>
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<th>PEa</th>
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* %, relative percentage obtained on CP-Wax 52 CB capillary column

* t (trace) = < 0.1%
Figure 1. TMAB Counts of rose water during 12 months of storage. Note: (◆) Control; (□) Pasteurization; (▲) UV; (x) Phenoxyethanol; (■) Sodium Benzoate

Figure 2. Yeast and Mold Counts of rose water during 12 months of storage. Note: (◆) Control; (□) Pasteurization; (▲) UV; (x) Phenoxyethanol; (■) Sodium Benzoate
Yeasts and fungi were present only in control samples showing that any means of preservation is enough for their total inhibition. Decreasing rate of ethanol and some other compounds in processed rose waters during storage also supports the presence of bacterial activity.

The number of fungi was rather high in control samples even after 12 months of storage. In control samples, yeasts were thought to be major responsible group of microorganism among fungi to cause changes in chemical status of rose water during storage. Microscopic observations also proved that, all the colonies grown in PDA plates of control sample were determined as yeasts. No difference was determined among different applications regarding inhibition of yeasts and molds. Finally all applications decreased the number of microorganisms to satisfactory levels.

Rose water itself has weak inhibitory activity on microorganisms, thus it is subject to microbial spoilage unless a proper preservation method is applied. In this study it was determined that spoilage of rose water is mostly caused by microorganisms. It is known that some yeast species can metabolize aromatic compounds and metabolizes them into new products that may cause undesired odors. Chemical preservatives used in the study were certified compounds in various foods and cosmetics. The usage of sodium benzoate in cosmetic products as an antibacterial or antifungal agent is allowed by Food and Drug Administration (FDA) at a maximum dose of %0.1. Phenoxylethanol which is a non-toxic glycol ether derivative is also used for its bactericidal effects in cosmetics.

As a conclusion, various methods were applied to ensure the compositional quality and microbial safety of rose oil during shelf life. Methods used in the study were P, UV, PE and SB application. Although there were minor compositional differences among methods, it was shown that all methods provided satisfactory prevention of microbial spoilage. As the storage time extends changes in the concentrations of some major compounds of rose water were increased. It is significant that important odour components like citronellol, geraniol and nerol remained unchanged during 12 month storage. It is also determined that decreasing amounts of methyl eugenol and increasing amounts of phenylethyl alcohol are good indicators for the enhancement of desired rose water scent.

It can be suggested that application of physical preservation methods such as pasteurization, and UV treatment or addition of approved chemicals after production of rose water is necessary to assure consumers health and quality of rose water. In addition to preservation techniques, it can be recommended that rose water should be stored in air-tight stainless steel tanks or barrels. Application of nitrogen gas to the containers will also protect rose water from oxidation. It is also helpful to keep barrels or consumer size containers under refrigerated conditions and away from sun light in order to protect the quality of rose water during its shelf life.

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