Chemical Methodologies 6(2022) 783-789

Chemical Methodologies

Journal homepage: http://chemmethod.com

*Original Research Article*

# The Impact of Ozone Treatment on the Level of Free Fatty Acids, the Number of Peroxide, and the Flavor Compound 2-Acetyl-1-pyrroline in Local Rice Storage

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**ARTICLE INFO**

**Article history**

Submitted: 2022-05-09
Revised: 2022-06-17
Accepted: 2022-07-26
Manuscript ID: CHEMM-2207-1573
Checked for Plagiarism: **Yes**
Language Editor:
Dr. Fatimah Ramezani
Editor who approved publication:
Dr. Zinattosadat Hossaini
**DOI**:10.22034/CHEMM.2022.350942.1573

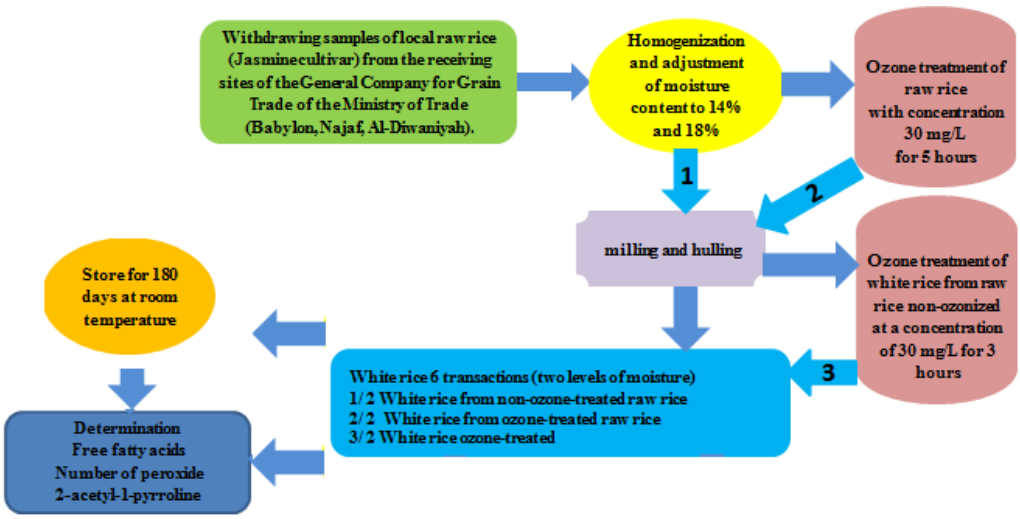
**KEYWORDS**

Raw rice
White rice
Moisture
Jasmine
2-Acetyl-1-pyrroline

**ABSTRACT**

The aim of this study was to examine the effect of ozone treatment on the concentration of the flavor compound 2-AP, the percentage of fatty acids, and the peroxide number in local jasmine rice with reducing pollution rate to microorganisms during storage and preserving the flavor quality compounds. The rice treatment was done with air mixed ozone at a concentration of 30 mg/L. Thus, the percentage of moisture for rice was 14% and 18%. The treatment was carried out for 5 hours for raw rice and 3 hours for white rice and identified the severity of treatment effect before and after milling, and the sample weight was 2 kg. The ozone treatment time was determined depending on the efficiency of eliminating most microorganisms. White rice from raw rice 14% moisture content was treated and ozonized. The percentage of free fatty acids increased from 2.55 to 2.65, the value of the peroxide number from 1.825 to 2.568 milliequivalents per kilogram, and the value of 2-AP extracted from 327 to 339 ng/g. Raw rice and ozone-white rice were treated at both humidity levels, with an insignificant increase in the percentage of free fatty acids and peroxide number after ozone treatment and significant increase after storage, while the flavor compound 2-AP reduced after storage.

**GRAPHICAL ABSTRACT**

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## Introduction

Rice feeds more than half of the world's population and it plays a critical role in satisfying the growing need for grain; as the population of the world increases, the amount of rice produced in Iraq is 574,705 tons [1]. The postharvest treatment of crops is one of the most important applications of ozone in agriculture [2]. Ozone is a powerful oxidant that has a wide range of uses in the food industry. It has been used to decontaminate foods such as fruits, vegetables, spices, herbs, drinks, meat, and fish in both gaseous and aqueous forms. In the food processing industry, ozone processing is one of the most promising non-thermal and bio-friendly procedures. Because of its antibacterial characteristics and potential to change the functional aspects of foods, ozone technology is being more widely used in the food business (including longer shelf life) [3]. The advantage of utilizing ozone is that it decomposes quickly into oxygen in the air, leaving fewer residues in the product and eliminating the need to remove gas. Ozone is the most promising green technology to improve food safety and quality since it has non-residual qualities and is an environmentally beneficial solution [4]. It is also been labeled as "Generally Recognized as Safe" (GRAS) for food matrix disinfection [5]. In ozonated rice, the pH decreased from 5.43 to 5.23, a concentration of 0.4 ppm [6]. Peroxide values of rice milled variety KDML 105 Jasmine (1.87-3.28) mg eq/kg, according to CODEX standard, the maximum PV level should be 10 mg eq/kg oil and less [7].

2-Acetyl pyrroline level decreases with increasing storage period, higher storage temperature, and storage package type. Biosynthesis of 2-AP before the starch structure is formed and it plays a major role in the aroma quality of aromatic rice. Post-harvest processes such as drying, milling, and storage are especially important for aroma preservation. The flavor is aromatic rice, so storage and handling at a low temperature reduce 2-AP volatilization. High-fat acidity is a useful indicator to estimate the decrease in 2-AP content in rice during early storage and extraction temperature affects starch gelatinization, the speed of starch breakdown, and disengagement with the 2-AP compound. It was reported that the concentration of 2-AP extracted compound was 300-450 ng before storage for milled rice variety Khao Dawk Mali 105 cultivar. After seven weeks of storage, it was 0-100 ng according to storage conditions [8]. When treating the grains of milled rice with ozone at a concentration of 500 ppm for 450 minutes, it was more acceptable in terms of cooking characteristics [9]. The metabolomes of jasmine rice grains differ greatly, which is congruent with known variances in sensory qualities. These differential qualities can now be specified. Clearly, rice aroma is not as simple as the presence or lack of 2AP and is not the result of a single factor [10]. Discovered that the 2-AP concentration in Jasmine (Millagrosa MRR) was 598 ng/g, 810 ng/g in Jasmine (ITC) and 251 ng/g in the extract (equivalent to the amount in the bran), according to the manner and efficiency of extraction, the extraction rate ranges from 10-80 percent. It was discovered that increasing the milling time (lower fat percentage) leads to a larger extraction rate [11].

Jasmine rice is one of the widely cultivated varieties in Iraq. It is an aromatic variety with a distinctive flavor due to the presence of the flavor compound 2-AP. Processing and storing rice leads to contamination, a rise in the number of microorganisms, grain spoilage, and a decrease in rice flavor.

## Materials and Methods

Random samples were taken from raw rice, Iraqi jasmine variety, harvested in the 2020 season, as stated in AACC 64-70.02 from the receiving sites of the general company for grain trade of the Ministry of Trade/sites (Babylon, Najaf, and Diwaniyah) drawn by the employees of the sites by 14% and 18% moisture.

Satake rice machine husker (Type THU,35A) was used for crushing 250 g of refined raw rice, which was placed in the upper feeding slot, which was closed. The device was turned on and the feed slot was gradually opened for the passage of the grains through the crushing rolls after adjusting them according to the type of grains (it is

necessary that the grains pass smoothly). The brown rice is gathered in one of the two lower boxes and crushed again to acquire the most amount of brown rice possible.

The satake is a grain testing mill [TM05C]. 200 g of brown rice was weighted and put in the top tank, and then the tank door was closed. Brown rice grains were separated into white rice and bran after passing through the disc stone and sieve. A watch was included with the gadget. According to Iraq standard, the timing was set at 45 seconds to achieve the white rice grain's required whiteness to 32 degrees for the control sample T1. IQS 1343/2019 for rice by the Central Organization for Standardization for Quality Control.

The researcher created an ozone treatment system that included an ozone device from Chinese Laisen Electronic Devices Company, powered by an Electric Charging method, with a pumping capacity of 10 g/h and a flow rate of 5-7 L/min and an ozone treatment system that included an ozone device from Chinese Laisen Electronic Devices Company powered by an Electric Charging method, with a pumping capacity of the gadget operates by pushing air (Input) through an aluminum-based purification processor. An air stream is formed in a glass insulator between two parallel electrodes and the oxygen molecule is broken, and the atoms are re-formed in a triple ($O_3$) form as a result of the high voltages of the device. A stream of generated gas is pumped through an output tube (Output). It goes to a cylindrical container of acrylonitrile butadiene styrene (ABS), 10 cm in diameter, and 50 cm in height, with airflow and polyvinylidene difluoride (PVDF) plastic connection tubes, sample weight for all treatments. A 2 kg is placed in the above-mentioned cylindrical container. The flows velocity is set to 5.5 L/min by using an airflow meter, and the utilized concentration is 30 mg/L.

Raw rice samples (moisture content 14% and 18%) were exposed to ozone gas at a concentration of 30 mg/L for 5 hours. The treatments after grinding and polishing the raw rice samples were as follow:

T1: White rice (milled rice) from raw rice 14% moisture content of untreated with ozonation. T2: White rice from raw rice with 14% moisture content treated ozonized at a concentration of 30 mg/L for 5 hours. T3: White rice from raw rice with 18% moisture content of untreated ozonation. T4: White rice from raw rice with 18% moisture content treated ozonized at a concentration of 30 mg/L for 5 hours. Then, the treatments (T1 and T3) (white rice from untreated with ozonation raw rice) were exposed to ozone gas at a concentration of 30 mg/L for 3 hours. T5: white rice with 14% moisture content treated ozonized at a concentration of 30 mg/L for 3 hours. T6: white rice with 18% moisture content treated ozonized at a concentration of 30 mg/L for 3 hours.

Each sample was examined for three replicates. The ozone treatment time was calculated for raw and white rice based on the elimination of most microorganisms to prolong storage time. Under the specified storage conditions at room temperature, white rice samples were packaged in 1.5-liter glass bottles with a metal screw lid. The storage was for 180 days. A laboratory mill (BUHLER) was used to grind white rice grains to obtain rice flour.

The oil was extracted by the cold method. 12 g of the white rice flour was mixed with 175 mL of solvent. Hexane was placed in a 250 mL volumetric flask and mixed for two hours by a magnetic stirrer, and then filtered through No. 4 Whatman filter paper. Next, the hexane was evaporated with a rotary evaporator. Free fatty acids were estimated as stated in AOAC method 940.28. 10 mL of 95% ethanol is taken and boiled for several minutes to remove dissolved gases, and then crushed with 0.1 N potassium hydroxide in the presence of phenolphthalein drops, reaching a pale pink color that settles for several minutes (plank). 0.6 g was taken from the cold extracted oil in an erlenmeyer flask, and 10 mL of distilled water was added. 10 mL of 95% ethanol was added and heated in a 60 °C water bath for several minutes. The hot mixture with 0.1 N potassium hydroxide was rubbed until the color is pale pink. Blanc was removed from the sample.

The value of free fatty acids (as oleic acid) is calculated as follow:

$AV_0$=(mL) Potassium Hydroxide×(N)0.1×56.1 / Oil Weight (g)

56.1 = molecular weight (KOH)

AV= (mL) Potassium Hydroxide×(N)0.1×282 / Oil Weight (g)

282 = molecular weight (oleic acid)

FFA = 1- AV/ $AV_0$

The peroxide number was estimated as stated in (AOCS Cd 8-53). 1 g of oil was added and dissolved in 6 mL of chloroform: acetic acid in a of 2:3 ratio, and then 0.1 mL of saturated potassium iodide (KI) solution was added with shaking for a minute. Next, 6 mL of distilled water was added, and immediately after that 0.4 mL of starch solution was added. It was wiped with 0.1% sodium thiosulfate solution until the color turned blue and it returned to yellow. The value of the peroxide is calculated according to the following equation:

Peroxide value (mEq/kg oil) = (mL of thiosulfate saturation of the sample - mL of plank thiosulfate)×Standard×1000 / Oil weight (g)

mEq / kg = milliequivalents per kilogram

1 g of each sample and 2 mL of methyl chloride-containingone were added to 2,4,6-trimethylpyridine (Sigma co.) in a 5 mL glass tube with a metal lid. It was tightly closed and placed in an 80 ºC water bath for 2.5 hours. After it is cooled for half an hour, the extracted sample's filtered solution is clarified in small, opaque tubes of 2 mL and kept refrigerated until examination. The extract is examined by GC-MS [11].

2-AP compound concentrations (200, 400, 600, 800, and 1000) ng/mL were created to draw a standard solution between the concentration and the curve area for computing extracted taste values.

0.1 g of 2-AP (Sigma co.) was added with the solution with dichloromethane as a supplement in a 100 mL volumetric flask. 0.1 mL of the solution is taken into a 100 mL beaker and supplemented the solution with dichloromethane, and then (2, 4, 6, 8, and 10 mL of solution) 1 µg/mL was taken in five flasks, respectively, and the volume was supplemented to 10 mL with dichloromethane [8, 10].

Ultra-gas chromatography was conducted by using HP-5MS (5%-Phenyl)-methylpolysiloxane column (30 m length, 0.25 mM diameter). The Advanced Pressure controller (APC) and Flame Thermionic Detector (FTD) were utilized by helium gas, with total flow velocity 34 mL/sec, pressure 47.6 kPa, linear velocity 36 cm/sec, and column flow speed 1.0 mL/sec. Volume 2 microliters were injected, temperature was 35-250 °C at the first stage and 35 °C for two minutes. The second stage is 9 ºC each 0.5 minute until reaching 120 °C. The third stage is 25 °C per 5 minutes until reaching 250 °C. Injection mode split, detector 0.9 kV was at 200 °C, scanning speed was 588, (start time) was 3 min, and expiry time was 22 min.

The statistical analysis system-SAS statistical program was used for data analysis to investigate the effect of various treatments on the studied traits by using a Complete Random Design (CRD). Significant differences between the means were compared by using the Least Significant Difference-LSD test [12].

## Results and Discussions

Table 1 indicates the (FFA) proportion for white rice and all treatments before and after 180 days of storage for white rice and all treatments. The FFA values before storage were (2.55, 2.65, 2.60, 2.57, 2.69, and 2.67) percent for the treatments (T1, T2, T5, T3, T4, and T6), respectively. We notice an increase in the FFA content between treatments (T1, T2, and T5) treatments (T3, T4, and T6) with no significant differences at the level of probability (P ≤0.05*).

Table 1 demonstrates that after storage, free fatty acids for 180 days were (3.05, 3.15, 3.65, 3.35, 3.55, and 3.85%) for the treatments (T1, T2, T5,

T3, T4, and T6), respectively. We notice an increase in the FFA content between the treatments (T1, T2, and T5) treatments (T3, T4, and T6) with no significant differences at the level of probability (P≤0.05*) due to the ozone treatment.

Table 2 depicts the (PV) values for white rice and all treatments before storage and after storage for 180 days. The (PV) number before storage was (1.825, 2.568, 2.087, 2.576, 2.854, and 2.995) mEq/kg for treatments (T1, T2, T5, T3, T4, and T6) respectively, we notice an increase in the values of peroxide number between treatments (T1 and T2) with significant differences due to ozone treatment of raw rice and between treatments (T1 and T5) due to treatment of white rice with ozone and between treatments (T3, T4, and T6) with non-significant differences at the level of probability (P≤0.05*) due to ozone treatment.

As indicated in Table 2, the (PV) values after 180 days of storage were (3.255, 4.736, 5.174, 5.565, 5.754, and 5.990) mEq/kg for the treatments (T1, T2, T5, T3, T4, and T6) respectively. We saw a rise in the (PV) value after storage. The ozone therapy caused significant variations in the level of probability (P≤0.05) across treatments (T1, T2, and T5) treatments (T3, T4, and T6).

**Table 1:** Percentage of FFA fatty acids for white rice samples before and after storage for 180 days

| Treatment | Before storage | After storage |
|---|---|---|
| T1 | 2.55 | 3.05b |
| T2 | 2.65 | 3.15ab |
| T5 | 2.60 | 3.65ab |
| T3 | 2.57 | 3.35ab |
| T4 | 2.69 | 3.55ab |
| T6 | 2.67 | 3.85a |
| LSD value | 0.351NS | 0.696* |

* Values bearing similar letters have no significant differences, and those carrying dissimilar letters have significant differences at the probability level (P≤0.05)

**Table 2:** Peroxide value (mEq/kg) for white rice before storage and after storage for 180 days

| Treatment | Before storage | After storage |
|---|---|---|
| T1 | 1.825c | 3.255c |
| T2 | 2.568abc | 4.736b |
| T5 | 2.087bc | 5.174ab |
| T3 | 2.576ab | 5.565ab |
| T4 | 2.854ab | 5.754ab |
| T6 | 2.995a | 5.990a |
| LSD value | 0.883* | 1.209* |

* Values bearing similar letters have no significant differences, and those carrying dissimilar letters have significant differences at the probability level (P≤0.05)

Table 3 represents the values of flavor compound 2-AP extracted before and after storage for 180 days. It was before storage (327, 339, 352, 294, 320, and 328) ng/g for the treatments (T1, T2, T5, T3, T4, and T6) respectively, we note an increase in the flavor compound values between treatments (T1, T2, and T5) with insignificant differences, between treatment T3 and T4 with significant differences, and between T3 and treatment T6 with insignificant differences on the probability level (P≤0.05*) due to the ozone treatment.

As indicated in Table 3, after storage, the taste compound values for the treatments (T1, T2, T5, T3, T4, and T6) were (211, 244, 249, 125, 253, and 258) ng/g, respectively. Because of the ozone treatment, we see an increase in the flavor compound values between the treatments (T1, T2, and T5) with insignificant differences between treatment T3 and treatment T4 with significant differences, and between T3 and treatment T6 with insignificant differences in the level of probability (P≤0.05*).

**Table 3:** Flavor compound 2-AP values extracted ng/g from samples of white rice before storage and after storage for 180 days

| Treatment | Before storage | After storage |
|---|---|---|
| T1 | 327a | 211b |
| T2 | 339a | 244ab |
| T5 | 352a | 249ab |
| T3 | 294b | 125c |
| T4 | 320ab | 253ab |
| T6 | 328a | 258a |
| LSD value | 32.08* | 45.91* |

*Values bearing similar letters have no significant differences, and those carrying dissimilar letters have significant differences at the probability level (P≤0.05)

## Conclusion

At both humidity levels, there is a clear difference in the treatment of raw rice and ozone-white rice, with an insignificant rise percentage of free fatty acids and peroxide number after ozone treatment, a significant rise after storage and high values of the flavor compound 2-AP after ozone treatment, and their decrease after storage. Where worldwide aims are focused on both yield stability and grain quality, the rice quality is becoming increasingly essential, not only in terms of nutritional content, but also in terms of its fragrance and flavor.

## Acknowledgements

The authors would like to convey their gratitude to Employees of the Quality Control Laboratories of the General Company for grain trade in the Ministry of Commerce who contributed to complete this study.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

## Conflict of Interest

There are no conflicts of interest in this study.

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**HOW TO CITE THIS ARTICLE**
Emad Shaker Whaib, Makarim Ali Mousa. The Impact of Ozone Treatment on the Level of Free Fatty Acids, the Number of Peroxide, and the Flavor Compound 2-Acetyl-1-pyrroline in Local Rice Storage. *Chem. Methodol.*, 2022, 6(10) 783-789
https://doi.org/10.22034/CHEMM.2022.350942.1573
URL: http://www.chemmethod.com/article_154162.html