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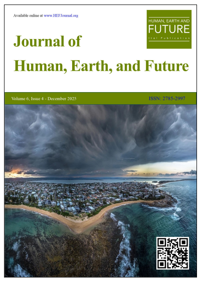
Journal of Human, Earth, and Future

ISSN: 2785-2997

Vol. 6, No. 4, December, 2025



Effect of Ozone Treatment on Walnut Storage at Different Temperatures

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Received 23 May 2025; Revised 17 October 2025; Accepted 04 November 2025; Published 01 December 2025

Abstract

Modern approaches to food storage require the use of safe and effective methods for extending shelf life without compromising quality. One such method is ozone treatment, which has pronounced antimicrobial and antioxidant properties. In the context of the increasing demand for high-quality nuts with a long shelf life, it is important to study the effect of ozonation on their stability under various temperature conditions. The purpose of this study was to examine the effect of ozone treatment and storage temperatures (10°C and 25°C) on the physicochemical, microbiological, and organoleptic characteristics of walnuts of the 'Kazakhstani Early-Maturing' variety. Attention was primarily paid to the assessment of such indicators as the acid number, iodine number, peroxide number, moisture content, and toxic elements, as well as microbiological contamination. The significance of the work was in clarifying the effect of ozone on the stability of the nut lipid complex and the development of microflora during storage. The practical value of the study was in testing the applicability of a safe pre-treatment method that would help maintain the quality and safety of nut products. The methodology included treatment in sealed containers at an ozone concentration of 0.50 mg/m³ for 30 min, followed by storage at two temperatures. The results of the chemical, organoleptic and microbiological analyses showed that ozonation significantly reduced microbial contamination, stabilized the lipid components and slowed the development of oxidative processes, especially with storage at 10°C. The organoleptic properties of the nuts were maintained at a high level, with no signs of rancidity, changes in taste or smell. In confirming the effectiveness of ozone treatment as a means of improving the quality and safety of nut products, this study contributes to the field of nut storage technologies, and our findings can be used in developing regulations for the storage of nuts and other oil-containing crops.

Keywords: Walnut; Storage; Ozone Processing; Enzymatic and Non-Enzymatic Processes; Temperature.

1. Introduction

The walnut (*Juglans regia* L.) is widely regarded as one of the most valuable plant-based food products owing to its high content of biologically active compounds and unique chemical composition. It is particularly rich in

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http://dx.doi.org/10.28991/HEF-2025-06-04-07

polyunsaturated fatty acids—notably omega-3 (α-linolenic acid) and omega-6 (linoleic acid)—which play a crucial role in maintaining a healthy lipid profile and supporting cardiovascular function. Additionally, walnuts are a source of essential fat-soluble vitamins, such as tocopherols (vitamin E), known for their strong antioxidant properties, as well as B-complex vitamins, which are vital for metabolic processes and the proper functioning of the nervous system [1, 2].

The mineral composition of walnuts includes essential elements, such as magnesium, potassium, phosphorus, zinc, selenium and iron, making them especially valuable for preventing deficiencies. Walnuts also contain phenolic compounds and phytosterols, which help neutralise free radicals, reduce inflammation and promote general strengthening of the body. The high protein content, coupled with a favourable amino acid profile, further enhances the nutritional value of the product [3, 4].

Numerous scientific studies have confirmed that regularly incorporating walnuts into the diet can help reduce the risk of cardiovascular diseases, type 2 diabetes and neurodegenerative disorders. Additionally, walnuts have been shown to enhance memory, improve concentration and support overall mental and emotional well-being [5–8]. Owing to this broad range of health benefits, walnuts are regarded as a key component of functional nutrition.

Due to their nutritional value and functional properties, walnuts are widely consumed and extensively used in various sectors of the food industry, including baked goods, confectionery and health foods. However, walnuts are highly susceptible to quality deterioration during storage. Their high lipid content, especially in unsaturated fatty acids, makes them prone to oxidative rancidity, which leads to undesirable changes in flavour, aroma and nutritional profile. Moreover, under improper or fluctuating storage conditions—particularly elevated temperatures and high humidity—walnuts are vulnerable to contamination by mould fungi, including species of *Aspergillus* and *Penicillium*, some of which are capable of producing harmful mycotoxins, such as aflatoxins [8, 9]. These factors significantly limit the shelf life and safety of walnut products, posing serious challenges for producers, distributors and retailers.

In response to these challenges, the search for effective and environmentally friendly preservation methods has intensified in recent years. One of the promising approaches is the application of ozone (O_3) in post-harvest treatment and storage. Ozone is a powerful oxidising agent with strong antimicrobial, antifungal and deodorising properties. It can effectively inactivate a wide range of microorganisms, including bacteria, yeasts and moulds, without leaving harmful chemical residues. As a result, ozone has gained recognition as a safe and sustainable technology for food preservation, and its application has been explored in the treatment of fresh produce, cereals, dried fruits and nuts [10, 11].

However, the efficacy of ozone treatment is not uniform, depending on multiple factors, including the concentration of the ozone, the duration of exposure, the humidity and the physicochemical properties of the treated product. Among these, storage temperature plays a crucial role. It not only influences the rate of lipid oxidation and microbial growth, but also affects the interaction between ozone and food matrices. For instance, low-temperature storage may enhance the stability of treated products and improve the retention of their sensory and nutritional attributes, whereas higher temperatures can accelerate quality degradation even after ozone exposure [12, 13]. Therefore, the combined effects of ozone treatment and storage temperature must be systematically investigated to optimise preservation strategies for a variety of foods, including walnuts. Understanding how these variables interact is essential for developing practical recommendations aimed at extending shelf life, maintaining product safety and minimising loss in quality during storage and distribution [14, 15].

Of particular interest in the context of sustainable agriculture and food security is the regional walnut variety 'Kazakhstani Early-Maturing'. This variety was bred to accommodate the continental climate of the southern regions of Kazakhstan and is distinguished by high frost resistance, early ripening, high productivity and good adaptability to local soil and climatic conditions. The fruits are characterised by a thin, easily separated shell, a large kernel and a harmonious taste, which makes them attractive for both fresh consumption and processing [16, 17]. Despite its agronomic advantages, this variety, like other walnut varieties, is subject to qualitative changes during storage, primarily due to its chemical composition. The high concentration of polyunsaturated fatty acids in the lipid fraction of walnuts makes them particularly vulnerable to degradation processes. Fluctuations in temperature and humidity conditions, contact with oxygen and exposure to light can trigger both enzymatic and non-enzymatic hydrolytic and oxidative reactions. As a result, the organoleptic properties of walnuts deteriorate, their nutritional value decreases, and the shelf life of the product is significantly shortened [18, 19]. Enzymatic changes are caused by the activity of lipases and lipoxygenases, which catalyse the breakdown of triglycerides and lipid peroxidation, respectively. Non-enzymatic changes—primarily the autocatalytic oxidation of unsaturated fatty acids—further accelerate the spoilage process by forming compounds that impair the taste, smell and safety of the product [20, 21].

Previous works have focused mainly on the effects of traditional storage conditions—temperature, humidity, light conditions and packaging type—on the quality of nuts. In particular, the effects of low temperatures on slowing lipid oxidation and suppressing microbiological activity have been investigated. However, as current data show, temperature control alone does not always provide sufficient stability during long-term storage, especially in the case of suboptimal transportation conditions or packaging integrity issues. In view of this, there is growing interest in combining methods for stabilising the quality of food products, including physicochemical methods that do not require the use of preservatives, such as ozone treatment. In addition to its preservative properties, ozone does not leave toxic residues and quickly decomposes into oxygen, which makes it a safe and environmentally acceptable treatment method [22, 23].

To date, there has been no practical consideration of the complex biochemical changes that occur in the lipid fraction of walnuts under the influence of ozone, or how these changes depend on the subsequent storage temperature regime. There have also been no systematic studies devoted to the evaluation of the enzymatic and non-enzymatic activity in nuts subjected to ozone treatment in combination with different storage temperatures. In addition, there are virtually no data on the varietal specificity of the response to ozonation, which is especially relevant in the context of local varieties. The benefits and methods of ozone-based food storage have, however, been discussed in detail in several previous works [24–27].

Thus, there is a clear gap in the literature regarding comprehensive data on the interaction of ozone treatment and temperature conditions in the context of the chemical and microbiological stability of stored walnuts. We set out to fill this gap by systematically analysing the dynamics of enzymatic and non-enzymatic hydrolytic and oxidative processes in kernels of the 'Kazakhstani Early-Maturing' walnut variety subjected to ozone treatment and stored under different temperature conditions. The aim was to establish the relationship between the parameters of ozone treatment and storage temperature conditions in walnuts, and their combined effect on the main quality indicators, including the level of oxidative spoilage and microbiological contamination and the organoleptic characteristics of the product. Our findings deepen our understanding of the mechanisms of preservation of lipid-containing plant products, and also lay the foundation for the development of an effective storage technology applicable to nut products under the conditions of Central Asia.

2. Material and Methods

2.1. Study Material

We investigated kernels of the 'Kazakhstani Early-Maturing' walnut variety, cultivated under the agroclimatic conditions of the Zhambyl region of Kazakhstan. This area is characterised by a continental climate with considerable daily and seasonal temperature fluctuations. 'Kazakhstani Early-Maturing' is a promising breeding cultivar, possessing several agronomically and technologically valuable characteristics, including early ripening, which allows the cultivation cycle to be completed before the onset of frost, ensuring stable yields even in a shortened growing season, and high-quality morphological characteristics of the fruits, including medium-sized kernels with a thin, easily split shell, which is beneficial for both industrial processing and the retail trade. To enhance the reliability of the results, uniform samples were analysed in terms of their mass, size, and maturity. The samples were chosen from a single batch of a crop that had undergone preliminary calibration and visual inspection.

2.2. Processing and Storage Conditions

All samples (2 control samples and 8 test samples) were placed in sealed containers made of certified food-grade polymer, which are resistant to active gases, including ozone. The nuts were treated using an OG-O10 industrial ozonator (Figure 1), designed to generate a highly concentrated ozone air flow derived from atmospheric oxygen via two ozone-generating lamp plates at a concentration of up to 10 g/h. The device allows precise control of the processing parameters, including time, flow rate and gas concentration.

Throughout the experiment, different temperature conditions were applied. The biochemical changes in the samples were analysed before and after ozonation. Special attention was paid to monitoring the dynamics of key indicators of oxidative and hydrolytic degradation to allow for a comprehensive evaluation of the effectiveness of the experimental conditions.

Figure 1. Illustration of the OG-O10 ozonator

Two experimental conditions were in operation during sample ozonation—ozone concentrations of 0.5 and 1.0 mg/m and ozone exposure times of 30 and 60 min, respectively. These conditions were chosen based on data from previous works on the ozone treatment of nut crops and other lipid-containing products [28–31], as well as on preliminary pilot experiments conducted before the main stage of the study. During the pilot tests, various ozonation modes were evaluated based on four criteria: (1) the preservation of organoleptic properties (absence of ozone odour, foreign taste); (2) the stability of the peroxide and acid numbers; (3) the absence of damage to the shell or kernel; and (4) the efficiency of microfloral suppression.

Based on an analysis of our results and the literature, the optimal ozone exposure was selected as 10 mg/l for 20 min. This mode gave the best balance between the antiseptic effect and safety for the quality of the product. It did not cause any degradation of the organoleptic properties, but at the same time, it contributed to a noticeable decrease in microbial contamination and a slowing of the oxidation processes, as confirmed by both chemical and microbiological indicators in the main phase of the study. Thus, the selected ozone treatment parameters were substantiated and found to fully correspond to the objectives of this study.

Following ozonation, all treated samples were grouped based on their processing conditions. The samples were then stored under one of two temperature conditions for a period of 45 days: (1) moderately cool conditions (approximately +10°C) to reduce the biochemical processes and microorganism activity; and (2) room temperature (approximately +25°C), corresponding to typical domestic storage conditions. At the end of this period, the samples were analysed to assess their quality and safety. A flowchart describing the experimental procedure is provided in Figure 2 and details of the experimental conditions are given in Table 1. These selected storage temperatures are typical and thus practically significant for walnut storage conditions under real conditions, the temperature of 10°C reflecting the parameters of cold storage used in industrial warehouses and 25°C being close to room temperature, typical of storage in retail and household environments. The main aim was to evaluate the effect of ozone treatment under conditions as close as possible to typical logistics and household storage situations.

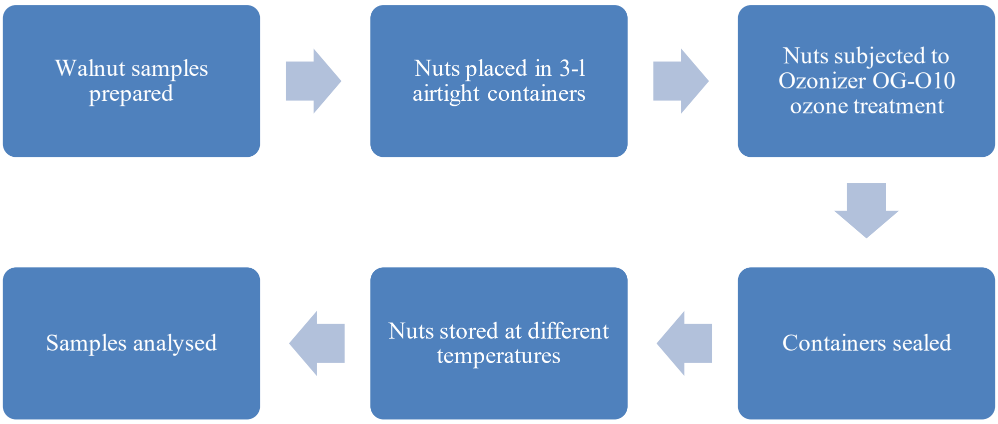

Figure 2. Flowchart of the research methodology

Table 1. Factors pertaining to the preparation of walnuts for the storage experiment

Indicator	Coding	Factors		
		x_1 – Ozone concentration (mg/m^3)	x_2 – Processing time (min)	x_3 – Storage temperature (°C)
Upper level	+	1.0	60.0	25
Zero level	0	0.75	45.0	17.5
Lower level	–	0.5	30.0	10
Variation interval		0.25	15.0	7.5

During the storage period, the quality of the walnuts was monitored at specified intervals. This evaluation included organoleptic assessments (i.e. appearance, taste, smell and texture) and laboratory tests for key chemical indicators (e.g. peroxide number, acid number and iodine number). These parameters provided an objective measure of the degree of fat oxidation, freshness and stability of the oil phase.

The quality of the samples was tested using standardised and generally accepted methods, following current regulatory documents. The organoleptic characteristics were determined in accordance with GOST 32874–2014 [32]. This included assessments of the appearance (nut integrity, shape, colour, presence of defects), smell (absence of foreign or musty smell), taste (sweetness, bitterness, taste of burned oil) and consistency (fragility, texture, nut moisture). The evaluation was carried out by a tasting committee consisting of five experts who had undergone training in the organoleptic analysis of plant-based products (in accordance with the internal regulations of the laboratory). The evaluation was performed under normal lighting conditions, at room temperature, using standardised glass containers and sample codes. The results were recorded on a scale: 5 points = fully complies with regulatory requirements and characteristic features of a fresh, high-quality product; 4 points = minor deviation with no impact on consumer appeal; 3 points = acceptable deviations that partially reduce the quality; 2 points = pronounced defects that reduce consumer value; and 1 point = the product is unfit for consumption.

The peroxide number was determined following GOST 26593–95 [33]. This is a measure of the amount of peroxide in all types of fats and oils, and serves as an indicator of their oxidative stability. To obtain this, each sample was dissolved in a mixture of acetic acid and iso-octane. Potassium iodide was added and the mixture titrated with a solution of sodium thiosulfate until the yellow colour disappeared.

The acid number was determined according to GOST 31933–2012 [34]. This reflects the quantity of free fatty acids in an oil, which is linked to its quality and shelf life. The samples were dissolved in ethanol with an indicator (phenolftalein) and titrated with a solution of alkali until a constant pink colour appeared. The results are expressed in milligrammes of potassium hydroxide (KOH) per gramme of sample.

The iodine number was determined according to the international standard GOST ISO 3961–2020 [35]. This indicates the degree of unsaturation in fats and oils. The samples were dissolved in a mixture of solvents, after which a VIIS reagent was added, and the sample was left to sit in dark conditions. Then, potassium iodide was added and the mixture titrated with a solution of sodium thiosulfate. The results are expressed in grammes of iodine per 100 grammes of sample.

Other chemical and microbiological indicators were analysed, including the mass fraction of moisture, according to GOST 32874-2014 [32], the mass fraction of fat, according to GOST 29033-91 [36], the mass fraction of ash, according to GOST 2555.4-91 [37], the heavy metal (lead and cadmium) contents, according to GOST 30178-96 [38], and the content of microbiological indicators (yeast and mould), according to GOST 10444.12-2013 [39].

All laboratory measurements were conducted in triplicate to ensure the reliability and reproducibility of the results. Statistical analyses of the experimental data were performed using Microsoft Excel (2010 version for Windows). The statistical significance level of the obtained data was 0.91, which confirms their reliability and scientific validity. To obtain a reliable assessment of the impact of individual grain-processing factors on the quality indicators, we used multifactorial design methods. The data processing and necessary calculations were carried out using the PLAN algorithm and program for consistent regression analysis, developed by Stankevich and Ostapchuk [40].

3. Results

The results indicate that the organoleptic characteristics of the walnuts remained unchanged in both the control group and the ozone-treated samples. This suggests that the selected ozonation conditions did not negatively affect the product's consumer qualities and can be considered a safe method for walnut storage. The shells remained well-formed, were free from any contamination, damage, mould or signs of pest impact. The colour remained uniform and natural, with no darkening, spots or any indications of damage. The kernels were adequately developed, with no signs of excessive external moisture or visible defects (such as cracks, spots or mould). The kernel surfaces were smooth, with the natural gloss typical of high-quality walnut kernels, and were free of mechanical damage. All samples retained the aroma and flavour typical of fresh walnuts. No off or chemical odours, nor any signs of mustiness or burnt taste, were detected. When chewing, a pronounced natural nutty aroma was present, with no unpleasant or foreign aftertaste. The kernels were easily separated from the inner shell, indicating complete physiological maturity. Additionally, the internal partition of the shell showed characteristic darkening, typical of mature walnuts. The kernels were dense and elastic, showing no signs of excessive dryness, looseness or fragility. The nuts were free of any excess moisture, and the texture was consistent throughout the entire mass of the kernel.

The relationships between the ozone-treated walnut quality indicators and the storage temperatures were also determined, as well as the chemical and microbiological indicators that would reflect the extent of any preservational or compositional changes that occurred in the walnuts during storage and the heavy metals contents, which were important for evaluating the toxicological safety of the product, and the microbial indicators (Table 2).

Table 2. Experimental results for the 'Kazakhstani Early-Maturing' walnut variety after treatment

Sample no.	Factor			Walnut quality indicator after storage									
	C_o (mg/m³)	τ (min)	T (°C)	y_1 (%)	y_2 (%)	y_3 (%)	y_4 (mmol½O/kg)	y_5 (mg KOH/kg)	y_6 (g/100 g)	y_7 (mg/kg)	y_8 (mg/kg)	y_9 (CFU/g)	y_{10} (CFU/g)
Control sample 1	—	—	10	2.82	44.53	1.18	12.39	3.65	88	0.011	0.0010	4	0
Control sample 2	—	—	25	2.81	44.55	1.19	10.87	2.71	92	0.010	0.0009	3	0
1	1.0	60	25	2.79	44.58	1.18	18.28	3.22	133	0.004	0.0009	1	0
2	0.5	60	25	2.75	44.56	1.21	21.54	2.77	124	0.006	0.0003	1	0
3	1.0	30	25	2.81	44.59	1.19	23.18	3.32	125	0.009	0.0001	1	0
4	0.5	30	25	2.80	44.55	1.17	24.56	3.07	129	0.006	0	3	0
5	1.0	60	10	2.85	44.49	1.20	21.77	2.98	131	0.005	0	1	0
6	0.5	60	10	2.83	44.51	1.19	28.44	3.73	123	0.010	0.0001	5	0
7	1.0	30	10	2.84	44.55	1.17	25.18	3.45	125	0.010	0	1	0
8	0.5	30	10	2.88	44.50	1.16	23.15	5.01	126	0.009	0.0002	3	0

Note. C_o = ozone concentration, τ = processing time and T = storage temperature. y_1 = mass fraction of moisture (walnut water content, related to microbiological damage), y_2 = mass fraction of fat (important quality parameter), y_3 = mass fraction of ash (reflecting the total mineral content), y_4 = peroxide number (indicating initial stage of fat oxidation, reflecting oil freshness and potential degradation), y_5 = acid number (amount of free fatty acids, related to degree of fat hydrolysis), y_6 = iodine number (degree of fat unsaturation, which may change during storage due to oxidation), y_7 = lead content, y_8 = cadmium content, y_9 = yeast (reflecting the level of microbial contamination) and y_{10} = mould (indicator of the sanitary condition and potential danger from possible mycotoxin formation).

The data in Table 2 show that the moisture, fat and ash remained at a stable level, indicating the absence of dehydration or destruction of the nut structure. The peroxide and acid numbers in the ozonised samples were within permissible limits, with the slight increase explained by natural oxidation processes, but not indicating rancidity or deterioration in quality. The iodine number was higher in the ozonised nuts, which may indicate the preservation of unsaturated fatty acids and a slowing of lipid degradation processes. Ozonation significantly reduced the number of yeast cells in the treated samples, from 1 to 3 CFU/g, whereas the control samples contained 3–4 CFU/g. Mould was not found in any of the samples, which is especially important to monitor in stored nuts because mould fungi are capable of producing mycotoxins and can quickly deteriorate the consumer properties of the nuts. The results highlight the fungicidal and bactericidal action of the ozone, which ensured the microbiological stability of the product during storage. After ozone treatment, the samples showed reduced lead and cadmium contents compared to the control group. It is likely that the ozone promoted the oxidation or destruction of the organic complexes of the heavy metals, improving the toxicological safety of the nuts during storage.

Regression models based on the experimental data were developed to accurately describe the relationships between the quality indicators of the nuts and the three storage factors—ozone concentration, storage time and storage temperature. The adequacy of these models was verified using Fisher's criterion, which confirmed their statistical reliability and their suitability for predicting the behaviour of the studied parameters.

To mathematically process the results and construct the regression equations, PLAN—a specialised program for consistent regression analysis, developed by the Odessa National Technological University—was used. This program employs classical experimental planning methods, including a full factorial design.

To assess the reproducibility of the results and calculate the dispersion of errors, triple parallel measurements were conducted at the central point of the experimental plan. This approach allowed for a more precise evaluation of any errors and the stability of the models in the case of data fluctuations. The regression coefficients were calculated using matrices in their natural dimension, which simplified the interpretation of the resulting equations without the need to convert variables into coded form. As a result, all regression models were obtained directly in the measurement scale of the studied factors.

The obtained regression equations for the three-factor model had the following form:

$$y_i = b_0 + b_1C_o + b_2\tau + b_3T + b_{12} C_o\tau + b_{13} C_oT + b_{23}\tau T \quad (1)$$

where y_i is the value of the *i*-th quality indicator (e.g. peroxide number, acid number), C_o is the ozone concentration ($\times10^{-9}$ mg/m³), τ is the storage duration (mins) and T is the storage temperature (°C). $b_0 \ldots b_{23}$ are the regression coefficients, which characterise the influence of individual factors and their paired interactions. A summary of all the obtained regression equations is provided in Table 3.

The comparison between the calculated and critical Fisher's values confirmed the statistical significance of all models. For all processing and storage conditions, the adequacy of the constructed regression equations was observed. This ensured that the models could be used to predict changes in the quality characteristics of the nuts as a function of the studied factors.

Table 3. Regression equations and coefficient values of the variables for the treated walnut quality indicators

Regression equations for natural variables	Medium sequaric deviation		Fisher's criterion	
	Average experimental error	Model inadequacy error	Calculated value	Critical value*
$y_1 = 2.81875$	0.080	0.0402	3.97	4.74
$y_2 = 44.54125$	1.91	0.0372	2636.05	4.74
$y_3 = 1.02875 + 0.1150C_o + 0.002583\tau + 0.00350T - 0.001667C_o\tau - 0.00200C_oT - 0.000033\tau T$	0.0360	0.0169	4.56	4.74
$y_4 = 25.5025 + 0.128794\tau - 0.127733C_o\tau - 0.004738\tau T$	0.76	2.0404	7.21	19.25
$y_5 = 8.30125 - 4.316667C_o - 0.017917\tau - 0.1970T + 0.200667C_oT$	0.17	0.3660	4.63	19.16
$y_6 = 127.000$	6.31	3.5857	3.10	4.74
$y_7 = 0.00650 + 0.009167C_o + 0.000200\tau - 0.000400T - 0.000367C_o\tau + 0.000333C_oT$	0.0003	0.0004	1.30	19.00
$y_8 = 0.001983 - 0.001867C_o - 0.000030\tau - 0.000093T + 0.000020C_o\tau + 0.000067C_oT + 0.000001\tau T$	0.0000	0.0001	200.00	18.51
$y_9 = 5.000 - 7.929825C_o + 0.090058\tau + 0.224561C_oT - 0.005146\tau T$	0.1000	0.8377	70.18	19.16
$y_{10} = 0$				

*Confidence probability of p = 0.05.

Note. See Table 2 for an explanation of the factors and variables.

Analysis of the regression equations allowed an identification of the extent to which the various factors influenced the key quality indicators of the 'Kazakhstani Early-Maturing' walnuts during storage after ozonation. Among the 10 indicators studied, only two—fat content (y_2) and cadmium content (y_8)—showed inadequate regression models. This suggests that the behaviour of these characteristics was not adequately explained by the factors considered (C_o, τ, T) or that additional parameters, not accounted for in the experimental design (e.g. environmental humidity, initial raw material quality), may need to be included.

Assessment of the significance of the factors' influence on other quality parameters revealed that four indicators—y_1, y_2, y_6 and y_{10}—were largely independent of the variable technical conditions. This might suggest these properties were stable in relation to the ozonation and storage conditions, or that other factors unaccounted for played a dominant role. By contrast, the remaining six indicators showed a strong dependence on all three factors. This highlights the high sensitivity of these characteristics to the processing conditions and underscores the need to consider the technical process in detail.

The developed regression equations are second-order mathematical models that account for both the individual effects of each factor and their pairwise interactions. These models not only describe the behaviour of the indicators over the studied range of conditions, but also enable the prediction and optimisation of walnut quality as the technical parameters are varied.

To determine the optimal conditions for ozonating and storing the nuts, the acid number (y_5) was selected as the target indicator (optimisation function). The acid number reflects the degree of hydrolytic decomposition of lipids, which in turn influences the freshness and nutritional value of the walnut. The following regression equation was used as the target function:

$$y_5 = 8.30125 - 4.316667C_o - 0.017917\tau - 0.1970T + 0.200667C_oT \rightarrow max. \quad (2)$$

This model analysis showed that all three factors affected the acid number. Ozone concentration (C_o) had the most negative effect, the acid number notably decreasing with increasing concentration. This highlights the effectiveness of the ozonation in inhibiting the oxidation and hydrolysis of the fats. Storage time (τ) had a modest negative effect, which was expected, the quality of the product gradually deteriorating with prolonged storage. Storage temperature (T) also had a negative impact, with higher temperatures accelerating enzymatic and oxidative processes. There was also a significant positive interaction between ozone concentration and storage temperature, suggesting that ozone treatment can counteract the detrimental effects of higher storage temperatures. Thus, the negative impact of elevated temperatures can be mitigated by an appropriate ozone concentration, and calculations derived from Equation (2) can be used to optimise walnut storage conditions and minimise oxidative damage through the selection of technical parameters that ensure the highest preservation of nut quality over an extended storage period.

Given the statistically significant mutual influence of ozone concentration (C_o) and storage temperature (T) in Equation (2), it is challenging to draw a definitive conclusion about the independent effect of each factor on the acid number (y_5). The paired interaction (the product term $C_o \cdot T$) suggests that the impact of one factor varies depending on the level of the other. For example, the effect of ozone concentration differs at low versus high storage temperatures.

This interaction effect is typical of complex biochemical and physicochemical processes, such as a change in the acid number of the fats in walnuts during storage. Ozone, with its strong oxidative potential, can effectively suppress microbiological activity, slow the hydrolysis of triglycerides and reduce the formation of free fatty acids. However, the effectiveness of this suppression may decrease at higher temperatures, which accelerate autocatalytic oxidation reactions. Given this, a traditional linear analysis, relying only on the interpretation of individual regression coefficients, is insufficient to elucidate completely the influences of different factors. A more effective and visually informative approach is to construct a three-dimensional response surface, which enables the observation of the spatial relationship between the y_5 response and two variables while keeping the third variable constant.

The response surface shown in Figure 3 clearly illustrates the combined effect of the ozone concentration (C_o) and storage temperature (T) factors on the acid number. From the surface shape, it is evident that, at lower storage temperatures, an increase in ozone concentration effectively reduces the acid number. However, at higher temperatures, this effect is more pronounced. This confirms the existence of a compensatory mechanism between the studied factors—to maintain a low acidity at higher temperatures, an increase in ozone concentration is required. The response surface provided a more comprehensive and accurate understanding of the factors' influence on the test indicator, highlighting the importance of an integrated approach when analysing multidimensional regression models in technical optimisation tasks.

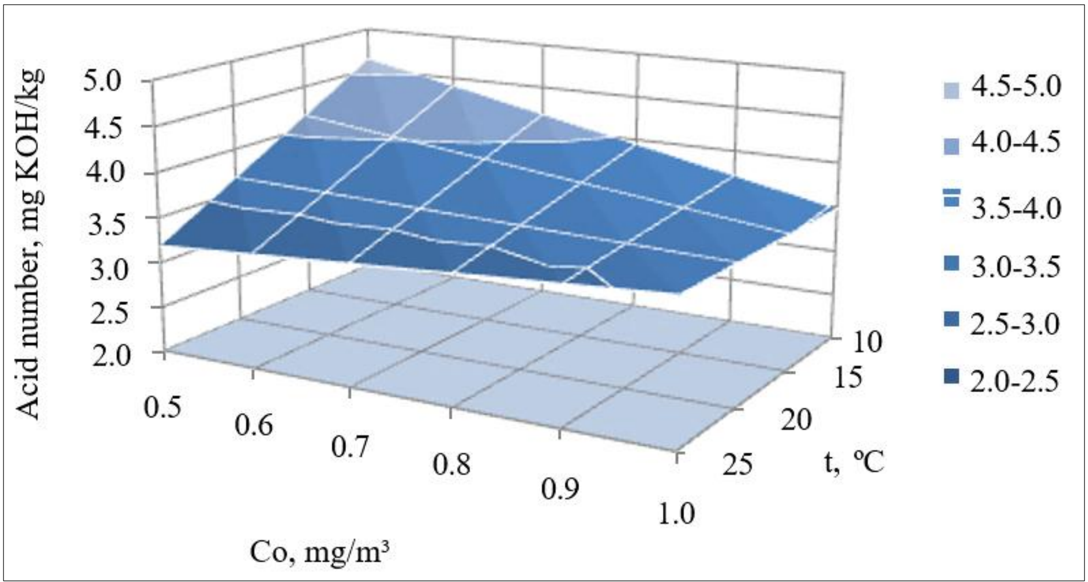

Figure 3. Surface response of the combined influence of C_o and T on the acid number (at T =10 °C)

The analysis of the response surface shown in Figure 3 provides valuable insights into how the studied technical factors influenced the acid number of the walnuts. The visualisation clearly shows that a simultaneous decrease in storage temperature (T) and ozone concentration (C_o) led to a considerable increase in the acid number, up to 4.64 mg KOH/kg. This effect is attributed to the interaction of the factors, as reflected in the regression model through the significant paired term $C_o \cdot T$. Specifically, at low ozone concentrations, the lack of a stabilising antioxidative effect combined with inadequate cooling of the product, created conditions that intensified the processes of hydrolysis and fat oxidation that led to free fatty acid accumulation.

To identify the optimal values of the technical parameters to minimise the acid number, the ozone treatments were optimised. The optimisation problem was addressed using the multidimensional dichotomy method, which involves systematically varying the factors within acceptable ranges to locate the global extreme of the target function. The regression equation for the acid number (y_5) was used as the target function, with the aim of minimising its value while ensuring the desired product quality. The optimal values were determined to be ozone concentration (C_o) = 0.50 mg/m^3, processing duration (τ) = 30 min and storage temperature (T) = 10°C, which gave a minimum acid number value of 2.61 mg KOH/kg, indicating excellent stability of the lipid phase and no signs of damage or oxidative degradation.

The values for the other walnut quality indicators were calculated using the optimal treatment conditions. The results (Table 4) show the absolute values of the indicators and their compliance with the regulatory standards. These data confirm that the optimal conditions preserve the high organoleptic and chemical qualities of the product, highlighting ozonation as a promising approach for walnut storage.

Table 4. Ozone-treated walnut quality indicator values

Indicator	Minimum		Optimum		Maximum
y_1 – mass fraction of moisture, %	2.75	≤	2.82	≤	2.88
y_2 – mass fraction of fat, %	44.49	≤	44.54	≤	44.59
y_3 – mass fraction of ash, %	1.16	≤	1.18	≤	1.21
y_4 – peroxide number, mmol ½O/kg	18.28	≤	26.03	≤	28.44
y_5 – acid number, mg KOH/kg	2.77	≤	4.64	≤	5.01
y_6 – iodine number, g/100 g	123	≤	127	≤	133
y_7 – lead, mg/kg	0.004	≤	0.0093	≤	0.010
y_8 – cadmium, mg/kg	0	≤	0.0003	≤	0.0009
y_9 – yeast, CFU/g	1	≤	3.32	≤	5
y_{10} – mould, CFU/g	0	≤	0	≤	0

Thus, treating walnuts with ozone under optimal conditions (0.50 mg/m^3 of ozone for 30 min and storage at 10°C) resulted in the maximum reduction of the acid number to 4.64 mg KOH/100 g. Notably, in addition to minimising the acid number, ozone treatment under these conditions also preserved all the other key quality parameters of the walnuts, keeping them within the regulated standards. Furthermore, optimising the ozonation process helped minimise the formation of unwanted by-products, such as acids, which could negatively influence the taste and safety of the nuts. Therefore, ozonation, when carried out under optimal processing conditions, is an effective method for extending the shelf-life of walnuts while maintaining their high quality.

4. Discussion

External environmental parameters, such as humidity and oxygen access, have a significant impact on oxidation processes in fatty and oily products, including nuts. Here, after ozone treatment, the walnuts were stored under standard laboratory conditions in hermetically sealed 3 l containers at 10–25°C so we could evaluate the walnuts' behaviour. The relative humidity in the storage room was not artificially regulated, but the hermetically sealed containers minimised its impact, and analysis showed that the humidity of the nuts during storage changed only insignificantly, from 2.75% to 2.88%, confirming the effectiveness of the selected packaging.

Our results provide a comprehensive assessment of how various storage temperatures influence the development of enzymatic and non-enzymatic processes in 'Kazakhstani Early-Maturing' walnuts. Special emphasis was given to the changes in key variables that reflect the extent of lipid hydrolysis and oxidation, including the acid, peroxide and iodine numbers. Based on our findings, we can conclude that storage temperature has a statistically significant effect on the intensity of the degradation process. At a storage temperature of 25°C, there was a rapid increase in the acid number, which reached 4.64 mg KOH/100 g—the upper permissible limit for products containing vegetable fats. This suggests that enzymatic lipolysis (triggered by lipase) and non-enzymatic lipolysis (autocatalytic breakdown of triglycerides) were actively occurring. Concurrently, there was an increase in the peroxide number, indicating the onset of lipid oxidation and the accumulation of unstable peroxide compounds. Additionally, the iodine number decreased, reflecting a reduction in the unsaturated fatty acids in oxidative reactions.

By contrast, at a storage temperature of 10°C, there was a considerable reduction in the rate of these processes. Under optimal ozonation conditions (0.5 mg/m^3 for 30 min), the acid number only reached 2.61 mg KOH/100 g and the organoleptic properties remained stable. This demonstrates that lower temperatures slow both enzymatic and oxidative reactions, helping to preserve the fat component of the nuts for longer.

Importantly, the synergistic effect of preliminary ozone treatment was highlighted. Ozone possesses both antiseptic and antioxidant properties, stabilising lipid structures by eliminating microflora and preventing mould, and inhibiting oxidative enzyme activity [41–43]. Under low-temperature conditions, the effectiveness of ozonation is further enhanced by suppressing compensatory oxidative stress reactions, making the combined use of these two factors particularly effective [44, 45]. In addition, ozone treatment improves toxicological safety by reducing the heavy metal content and preserves the taste and visual qualities of the nuts.

Thus, the data clearly demonstrate the dependence of hydrolytic and oxidative process rates on storage temperature, emphasising the importance of monitoring the thermodynamic conditions during long-term walnut storage. Our findings align with those in the literature concerning the sensitivity of nut crops to temperature fluctuations, and further support the potential of ozonation as a key component in a comprehensive technology for quality stabilisation that can be recommended for use in the agro-industrial sector for storing nuts in warehouses and processing plants.

5. Conclusion

The aim of this study was to investigate the effect of ozone treatment and storage temperatures on the dynamics of enzymatic and non-enzymatic oxidative–hydrolytic processes in walnut kernels of the 'Kazakhstani Early-Maturing' variety, with an emphasis on assessing changes in the acid number as a key indicator of product quality. We used chemical analytical methods (determination of acid, peroxide and iodine numbers), an organoleptic assessment and mathematical data-processing, including the construction of regression models and response surfaces. The samples were treated with various concentrations of ozone and then stored at two temperatures—10°C and 25°C. We found that storage temperature had a critical effect on the oxidation rate of the lipid components of the nut. At 25°C, there was an increase in the acid number, indicating the activation of spoilage processes. Contrastingly, when stored at 10°C together and with a preliminary ozone treatment of 0.5 mg/m³ for 30 min, there was a significant decrease in the intensity of the oxidation processes, the acid number reaching a minimum of 2.61 mg KOH/100 g. A decrease in microbial contamination and stabilisation of the organoleptic characteristics were also noted, confirming the effectiveness of ozone treatment as a preservation method.

Thus, we can confirm our initial assumption about the possibility of increasing the stability of walnuts during storage using preliminary ozone treatment and a low-temperature regime. This work expands our understanding of the influence of technical factors on the storage of nut crops, in particular demonstrating how ozonation can be used to slow destructive biochemical processes without compromising the nutritional value or organoleptic properties of the product. Prospects for further research include studying a wider range (including intermediate and extreme) of temperatures, as well as environmental factors, such as humidity, the gas composition of the package and light conditions. It is also advisable to study the microbiological aspects of storage in more depth, including monitoring mould fungi and bacteria. Our recommendations and data can be practically implemented in the food industry, and in the logistics and warehousing of nuts, contributing to improving the quality of storage, reducing losses and increasing the safety of nut products.

6. Declarations

6.1. Author Contributions

Conceptualization, A.I. and B.M.; methodology, A.I. and B.M.; software, A.I. and B.M.; validation, A.I. and B.M.; formal analysis, G.S. and B.M.; resources, M.S. and P.M.; data curation, M.S. and P.M.; writing—original draft preparation, M.Y. and G.S.; writing—review and editing, M.Y. and G.S.; visualization, S.T. and A.Z.; supervision, S.T. and A.Z.; project administration, M.Y. and G.S.; funding acquisition, S.T. and A.Z. All authors have read and agreed to the published version of the manuscript.

6.2. Data Availability Statement

The data presented in this study are available in the article.

6.3. Funding

This research was carried out within the framework of project no. AP19174427 'Development of technology for the safe long-term storage of walnuts', financed by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan.

6.4. Acknowledgments

The authors express their gratitude to the management of Almaty Technological University for their support in conducting the research.

6.5. Institutional Review Board Statement

Not applicable.

6.6. Informed Consent Statement

Not applicable.

6.7. Declaration of Competing Interest

The authors declare that there are no conflicts of interest concerning the publication of this manuscript. Furthermore, all ethical considerations, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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