Ozone Treated Acorns: Chemical Pathways, Regulatory Context, and Implications for Food Use

Author: Gabriel Duncan

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1. Abstract

Use of ozone as an antimicrobial agent in food processing is authorized in the United States when applied within defined good manufacturing practices. Ozone is a strong oxidant that reacts with unsaturated lipids through known mechanisms that generate ozonides, peroxides, aldehydes and other oxidation products. Acorns are lipid containing seeds that include linoleic acid and other unsaturated fatty acids and are increasingly processed into acorn flour for human consumption. This paper reviews the chemistry of ozone induced lipid transformation, summarizes relevant toxicological and food science findings, situates these within the framework of the Federal Food, Drug, and Cosmetic Act and 21 CFR 173.368, and outlines ethical considerations that arise when such treatments are applied to culturally significant Indigenous foods. The available evidence does not establish that ozone induced ozonides and associated oxidation products in acorn lipids are safe for routine dietary exposure at uncharacterized levels. In the absence of targeted analytical data, ozone treated acorns should be regarded as chemically modified ingredients whose safety and regulatory status require explicit evaluation.

2. Introduction

Ozone is used in the food industry as an antimicrobial agent for water, surfaces and some commodities. Its use is supported by its high oxidizing potential, its rapid decomposition to oxygen and its regulatory recognition as a processing aid under specific conditions. At the same time, ozone is documented to react with unsaturated lipids and to generate oxygenated products that are mechanistically linked to ozone toxicity in biological systems.

Acorns used for traditional and commercial foods contain triglycerides rich in unsaturated fatty acids, particularly linoleic acid (C18:2, n-6). When dried acorns are exposed to ozone in storage or processing, the conditions approach those used to produce ozonated oils rather than a brief aqueous sanitation step. This raises technical questions about the formation and persistence of ozonides and secondary oxidation products within acorn lipids, their potential biological activity and the applicability of existing regulatory allowances for ozone use in food.

This paper synthesizes relevant chemistry, toxicology, food science and regulatory sources to clarify the expected consequences of ozone exposure in acorn lipids, the documented behavior of ozonated vegetable oils, how the Federal Food, Drug, and Cosmetic Act and 21 CFR 173.368 apply, and why a cautious, analytically supported approach is warranted when such treatments intersect with Indigenous food systems.

3. Ozone Induced Reactions in Unsaturated Lipids

3.1 Mechanistic Overview

The interaction of ozone with unsaturated fatty acids follows the Criegee ozonolysis mechanism and is distinct from spontaneous autoxidation of lipids. Ozone adds across a carbon-carbon double bond to form a primary ozonide, or molozonide, which rapidly fragments to a carbonyl compound and a carbonyl oxide, also called a Criegee intermediate. These intermediates can recombine or rearrange to form more stable secondary ozonides, also known as 1,2,4 trioxolanes, along with a range of carbonyl and peroxidic species. In contrast, non ozone autoxidation of lipids proceeds by radical chain reactions that primarily yield linear hydroperoxides along the fatty acyl chain before breaking down to secondary products. Ozone treatment therefore introduces cyclic peroxide structures that are not the predominant products of ordinary oxidative rancidity.

In systems that contain linoleic acid, each double bond is a potential site for ozonide formation and subsequent cleavage to shorter chain aldehydes and related compounds. Extended ozonation can also yield oligomeric peroxidic structures that contribute to increased viscosity and to properties that differ from those of the parent oil.

3.2 Evidence From Mechanistic and Toxicological Studies

Hempenius, in “Ozonides: Intermediates in Ozone Induced Toxicity,” synthesized ozonides of methyl linoleate as model compounds and evaluated their behavior. The work shows that secondary ozonides are stable enough in lipid phases to persist and that they exhibit cytotoxic effects in vitro and ozone-like tissue injury in vivo, supporting their role as intermediates in ozone induced toxicity.

Soo, in “Mechanisms of Ozone Toxicity,” describes how ozone reacts with unsaturated lipids in respiratory tract lining fluid to produce aldehydes, hydroxyhydroperoxides and oxidized phospholipids, which act as secondary toxic mediators rather than inert end products. Studies on cultured bronchial epithelial cells support the contribution of lipid oxidation products to ozone related injury. This is consistent with the classical work of Pryor and colleagues, who identified aldehydes and hydrogen peroxide as major products of unsaturated fatty acid ozonation and proposed them as mediators of ozone related toxicity. These products include low molecular weight aldehydes such as formaldehyde and acetaldehyde, which are of toxicological interest when present at elevated levels in air or food matrices. Both formaldehyde and acetaldehyde are recognized in toxicological and regulatory assessments as reactive carbonyl compounds with carcinogenic or genotoxic potential at sufficient exposures, reinforcing the need to characterize their presence rather than assume it is negligible. Their identification as potential products in ozone treated lipid systems supports targeted analysis, but this paper does not quantify expected concentrations in acorn specific applications.

Taken together with classical ozonolysis studies, these data indicate that ozone exposure in unsaturated lipid systems yields a spectrum of reactive and bioactive compounds, including cyclic peroxides, aldehydes and other secondary products that can persist and interact with biological systems.

4. Ozone Treatment in Food Lipid Systems

4.1 Ozonated Oils

Ozonated vegetable oils are produced by passing ozone through unsaturated oils for controlled periods. They are used primarily in pharmaceutical and veterinary contexts.

Sadowska and colleagues characterized ozonated vegetable oils using spectroscopic and chromatographic methods. They reported significant increases in peroxide value, loss of unsaturation, formation of secondary ozonides and the appearance of higher molecular weight ozonide species at higher ozone doses. In standard lipid chemistry, such increases in lipid hydroperoxides are interpreted as primary oxidation or primary rancidity. Subsequent formation of aldehydes and ketones from hydroperoxide breakdown is interpreted as secondary oxidation and secondary rancidity. These findings confirm that ozonation converts edible oils into oxidatively modified systems that are chemically distinct from the parent oils and that must be analytically characterized. The presence of cyclic ozonides and higher molecular weight peroxidic structures, in addition to linear hydroperoxides, differentiates ozone specific lipid modification from background autoxidation and supports the need for targeted analytical methods to resolve these species. In these studies, characterization of peroxidic species, including ozonides, has relied on combined chromatographic and mass spectrometric methods, such as GC-MS or LC-MS, together with NMR and infrared spectroscopy, which are likewise appropriate tools for identifying ozone specific lipid products in acorn samples.

Ugazio and coauthors described ozonated oils as antimicrobial systems for topical applications and emphasized that ozonated oils are modified products whose composition and stability require control. Rizzi and collaborators further discussed technical considerations for medical uses, again treating ozonated oils as specialized materials rather than simple food fats.

Studies on storage stability, including work on the influence of storage conditions on ozonized vegetable oils, show that these products retain high peroxide values and ozonide related structures over time, with degradation rates influenced by temperature, light and packaging. This supports the conclusion that ozone induced peroxidic products are not transient at typical storage conditions.

4.2 Ozone Effects in High Fat Foods

Work on food grade lipids confirms that ozone treatment can lead to measurable and sometimes substantial oxidation.

Perna and colleagues, in a study on cream, reported that increasing ozone exposure raised peroxide values and secondary oxidation markers and negatively affected sensory attributes, demonstrating that ozone can accelerate lipid oxidation in dairy fat systems.

Dominguez Lacueva and coauthors examined ozonation of virgin and pomace olive oils. They observed large increases in peroxide index at higher doses, substantial loss of unsaturated fatty acids and development of antimicrobial activity associated with the modified oils. The authors concluded that ozonated oils show potential as antimicrobial agents, but also noted that specific legislation and defined thresholds are needed to regulate their use and prevent mislabeling in food contexts.

4.3 Relevance to Acorn Lipids

Acorns contain unsaturated fatty acids, especially linoleic acid, in triglyceride form. Applying ozone to dried, lipid rich acorns creates conditions similar to partial ozonation of an embedded oil phase.

Based on the above studies, ozone treatment of acorns is expected to:

1. Form secondary ozonides and other cyclic peroxides in situ.
2. Increase peroxide values during early stages of oxidation.

3. Produce aldehydes and other secondary oxidation products as peroxides decompose.
4. Potentially generate higher molecular weight peroxidic species under stronger or prolonged exposure.
5. Alter shelf life and sensory quality in ways consistent with oxidative degradation.

From a chemical perspective, this treatment is expected to form cyclic peroxides in situ, generate hydroperoxides and carbonyl compounds and potentially shorten shelf life through accelerated oxidative degradation. In this framework, elevated peroxide values indicate primary rancidity and elevated aldehydes and ketones indicate secondary rancidity. If analytical measurements of peroxide value, aldehydes or related markers in ozone treated acorns exceed established quality limits or reference thresholds used for acceptable edible oils and nuts, the material would be consistent with oxidatively degraded lipids rather than a purely sanitized, unmodified ingredient. For example, many commercial and compendial standards for refined edible oils treat peroxide values on the order of about 10 to 20 milliequivalents of active oxygen per kilogram as upper limits for acceptable quality, with higher values associated with oxidative deterioration.

These outcomes would indicate that ozone treated acorns differ chemically from traditionally processed acorns and should not be assumed equivalent without targeted analysis.

5. Regulatory Framework

5.1 Ozone Under 21 CFR 173.368

Title 21, Code of Federal Regulations, section 173.368, authorizes the use of ozone in the treatment, storage and processing of foods as an antimicrobial agent, in gaseous or aqueous phase, provided that the ozone used meets the specifications of the Food Chemicals Codex and that use conforms to current good manufacturing practice.

The allowance is based on the properties of ozone as an unstable gas that decomposes to oxygen, with the assumption that proper use does not result in harmful residues in food.

The regulation does not explicitly address scenarios where ozone is applied in a way that deliberately or incidentally generates stable ozonides, elevated peroxides and other oxidation products in high fat foods.

5.2 FDCA Provisions on Adulteration and Food Additives

Under the Federal Food, Drug, and Cosmetic Act, a food is adulterated if it bears or contains any added poisonous or deleterious substance that may render it injurious to health, or if it consists in whole or in part of any decomposed substance or is otherwise unfit for food. A substance that becomes a component of food through processing may be considered a food additive if it is not generally recognized as safe under the conditions of its intended use. The presence of an unsafe food additive renders the food adulterated. Industry specifications and pharmacopoeial or compendial standards routinely treat markedly oxidized fats and oils as out of specification or unsuitable for use in finished foods, so the use of such materials in products represented as wholesome and of normal quality may raise concerns under the statutory definition of adulteration.

Ozone itself, when used as described in 21 CFR 173.368, functions as an antimicrobial processing aid. In contrast, ozone induced lipid oxidation products such as ozonides, oligomeric peroxides and specific aldehydes in a lipid rich matrix are not individually listed as approved food additives, and their safety at elevated levels is not established.

Where ozone use in acorns results in measurable accumulation of such products and material changes in stability or composition, the resulting ingredient falls outside the simple processing aid model and may require evaluation under adulteration and food additive provisions, rather than being presumed compliant solely due to the permissive status of ozone gas.

6. Ethical and Contextual Considerations

Although the primary focus of this paper is chemical and regulatory, ethical considerations are relevant when novel processing technologies are applied to culturally important foods.

Key considerations include the expectation of accurate representation of processing methods and material changes to ingredients supplied to communities, institutions and cultural programs; the principle that uncharacterized chemical modifications to staple or traditional foods warrant a precautionary approach, especially in communities with lived histories of environmental exposure and dietary harm; and the recognition that Indigenous foods and foodways hold cultural, historical and health significance that justify heightened scrutiny when nontraditional technologies are introduced.

These points support transparent characterization of ozone treated acorns and open communication of results with affected communities and partner organizations before such materials are incorporated into food products.

7. Discussion

The combined literature on ozonolysis of unsaturated fatty acids, ozonated vegetable oils and ozone treated high fat foods leads to several consistent observations.

First, ozone reacts predictably with double bonds in fatty acids to produce secondary ozonides, peroxides, aldehydes and related compounds. These products are mechanistically associated with ozone toxicity in biological systems and are not transient at typical storage conditions.

Second, ozonated oils are repeatedly shown to be chemically distinct from their parent oils, featuring elevated peroxide values, altered fatty acid profiles, formation of oligomeric peroxidic species and modified sensory and functional properties. They are treated in the literature and in applied settings as specialized materials that require specification, stability assessment and in some cases toxicological evaluation.

Third, studies on ozone in high fat foods, such as cream and olive oil, confirm that ozone exposure can drive substantial oxidation and that relatively high doses produce conditions outside standard edible oil norms. Authors who explore potential food uses of ozonated oils often call for explicit regulatory frameworks and clear labeling.

Applying ozone to dried acorns intended for human consumption is therefore chemically significant. Treating such acorns as equivalent to conventionally processed acorns, without targeted analysis of ozonides, peroxides, aldehydes and related products, is not supported by current evidence. Existing regulations allow ozone as an antimicrobial processing aid, but do not explicitly endorse the presence of ozonated lipid matrices that are chemically distinct from their source fats and that have not been characterized for safety within food applications. Current good manufacturing practice regulations require that food which has become contaminated or otherwise adulterated, including through decomposition, be rejected or effectively reconditioned before use in finished products, reinforcing that oxidatively degraded ingredients are not acceptable substitutes for sound raw materials.

In food lipid chemistry, formation of lipid hydroperoxides is recognized as a marker of primary oxidation or primary rancidity, while subsequent formation of aldehydes and ketones is recognized as a marker of secondary oxidation and secondary rancidity. Materials that exhibit these changes to a significant extent are considered oxidatively degraded and unsuitable replacements for fresh, unmodified fats in applications that presume intact lipid quality.

8. Conclusion

The reaction of ozone with unsaturated acorn lipids is expected, based on established ozone chemistry and experimental work on vegetable oils and high fat foods, to form secondary ozonides, peroxides, aldehydes and higher molecular weight oxidized products. These compounds are chemically distinct from the native lipids and, in several cases, have recognized or plausible biological activity.

Under the Federal Food, Drug, and Cosmetic Act and 21 CFR 173.368, ozone is permitted as an antimicrobial processing aid when used in accordance with good manufacturing practice and when it does not result in unsafe residues. When ozone treatment of acorns is applied in ways that significantly alter lipid chemistry, increase oxidation markers or reduce shelf life, the resulting material should be considered a modified ingredient whose safety and regulatory status are not assumed and require explicit assessment.

Further work is warranted to quantify ozone induced oxidation products in ozone treated acorns, including secondary ozonides and aldehydes; to evaluate their toxicological profiles at plausible intake levels; to clarify regulatory treatment of ozone modified nut ingredients; and to develop process conditions and limits if ozone treatment is to be used while preserving safety, quality and cultural integrity.

In practice, verification of process impact would involve measurement of peroxide value and aldehyde related indices, such as p-anisidine value or other carbonyl assays, against established quality criteria for edible fats and nuts and, where indicated, the use of chromatographic and mass spectrometric methods to detect ozone specific ozonides and related products. Pending such data, it is reasonable, on scientific and regulatory grounds, to avoid treating ozone treated acorns as interchangeable with traditionally processed acorns in food applications.

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