THREE YEAR 2023-2025 COMPERATIVE ANALYSIS OF WATER QUALITY IN VILLAGE VRUTOK, IMPLICATIONS FOR 3D FOOD BIOTECHNOLOGY AND MOLECULAR STABILITY

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Abstract

Water safety and quality constitute the main pillar in the modern food production industry, especially in innovative sectors such as 3D printing and functional food design. This study aims to analyze the three-year (2023-2025) performance of critical physicochemical parameters, pH, chlorides, nitrites and dissolved oxygen, assessing their impact on molecular stability and food technology. The study was based on monthly monitoring of water samples and their processing through descriptive statistics and Pearson correlation matrices. Special focus was given to the standard deviation to measure the stability of the environment and the correlation coefficients to understand the interdependence of the parameters. The findings demonstrate a high stability of the aquatic environment. The pH value resulted in an average of 7.6, with a minimal standard deviation (0.12 - 0.14), confirming an excellent "buffer" capacity. Correlation analysis revealed an extremely strong negative relationship between chlorides and dissolved oxygen, which peaked in 2025 with a value of r = -0.96. From a toxicological point of view, nitrites experienced a modest increase from 0.004 mg/l in 2024 to 0.012 mg/l in 2025, but remained significantly below the critical allowed limits. The stability of the pH proves that this water is ideal for 3D technology, as it guarantees constant viscosity of food "colors" and prevents denaturation of proteins. However, the strong chloride-oxygen correlation suggests that the increase in salinity must be carefully monitored to prevent lipid oxidation and degradation of nutrients in the printed matrices. Finally, the results confirm that the water chemical profile during the years 2023-2025 provides a reliable basis for applications in molecular biology and advanced nutrition, guaranteeing high repeatability in biotechnological processes.

Keywords: 3D food technology, molecular biology, correlation matrix, pH stability, dissolved oxygen.

Introduction

In the era of modern biotechnology, the role of water has evolved from a simple component to a complex matrix that determines the molecular fate of food products. As highlighted in recent studies on the biophysics of aquatic environments, water acts as an “active biomolecule” that dictates protein folding and enzyme stability through a network of hydrogen bonds [1]. This paper examines the three-year (2023-2025) dynamics of water quality, analyzing how variations in pH, chlorides, nitrites, and oxygen affect the stability of 3D printed food structures.

The rapid development of 3D Food Technology requires rigorous control of the rheology of food “inks”. The integrity of printed structures directly depends on electrostatic forces in the aqueous environment [2]. A high pH stability (which in this study results in an average of 7.6) is critical; small fluctuations can cause protein denaturation or viscosity changes, leading to mechanical structure failure [3].

On the other hand, the interdependence between chlorides and dissolved oxygen poses a challenge for molecular nutrition. Research suggests that high salinity environments alter the hydration of macromolecules, accelerating oxidative processes [4]. This study identifies a

strong negative correlation (r = -0.96), indicating that the reduction of oxygen in the presence of chlorides increases the risk of degradation of sensitive vitamins and antioxidants in the food matrix.
Using descriptive statistics and correlation matrices, this paper argues that a consistent chemical profile is a prerequisite for innovation. Monitoring nitrites remains a priority for toxicological safety, preventing cellular damage and the formation of carcinogenic compounds, aspects that have been extensively addressed in the literature on oxidative stress and systems biology [5]. This study provides a predictive model for quality control, serving as a bridge between environmental chemistry and advanced food engineering.

Materials and Methods

The research was conducted as a longitudinal study on water quality monitoring for the period January 2023 - December 2025, where samples were collected on a monthly basis (N=36) at fixed monitoring points using sterile polypropylene bottles (500 ml) treated with HNO_3 and stored at 4°C, according to standard protocols [1]. The determination of physicochemical parameters was performed through sensor technology and standard methods, where pH was measured with a HI98191 digital pH meter (calibrated at pH 4.01, 7.01, 10.01), chlorides (Cl^-) through titration with $AgNO_3$ according to ISO 9297 [2], nitrites (NO_2^-) with UV-spectrophotometry (540 nm) through the Griess method [3], and dissolved oxygen (O_2) in-situ with an optical probe. To evaluate the applicability in 3D technology, alginate and starch-based "food ink" formulas were simulated, where the analyzed water served as a hydrating phase for rheology monitoring in relation to pH and conductivity [4]. Data processing was performed in IBM SPSS Statistics 26, applying descriptive statistics for annual stability and Pearson correlation coefficient (r) for parametric interdependence, while experimental precision was guaranteed through triplicate analyses (n=3). Finally, toxicological and nutritional evaluation was performed by comparing nitrite levels with EU (98/83/EC) and WHO standards [5], while oxygen stability was analyzed through lipid oxidation kinetic models to predict the molecular integrity of printed products [6].

Table 1: Physicochemical stability analysis of the hydration phase

Month	pH	Chlorides (Cl^-) [mg/l]	(NO_2^-) [mg/l]	Oxygen(O_2) [mg/l]
January	7.6	12.4	0.01	8.4
February	7.5	11.8	0	8.6
March	7.7	10.9	0	8.2
April	7.8	13.5	0.02	7.9
May	7.6	14.2	0.01	7.5
June	7.4	15.6	0.03	7.1
Mean	7.6	13.07	0.012	7.95
Standard deviation	0.14	1.67	0.012	0.58

The results of the six-month monitoring (Table 1) show a high stability of the physicochemical parameters, where the average pH value of 7.6 \pm 0.14 confirms a slightly alkaline environment, which prevents unwanted acidic gelation during hydration of the alginate matrices [4]. The chloride concentration marked a gradual increase from January (12.4 mg/l) to June (15.6 mg/l), with an average of 13.07 \pm 1.67 mg/l, remaining within the limits specified by ISO 9297 [2] and without negatively affecting the conductivity of the "food ink" formula. Regarding toxicological safety, nitrite levels (NO_2^-) were minimal with an average of 0.012 mg/l, a value that is significantly below the strict limit of 0.50 mg/l set by EU Directive 98/83/EC and WHO [5]. Meanwhile, the drop in dissolved oxygen from 8.4 mg/l in January to 7.1 mg/l in June reflects the thermal impact on dissolved gas dynamics, a critical factor that, according to kinetic models, plays a primary role in preventing lipid oxidation and preserving the molecular integrity of the final 3D printed product [6].

Table 2: Correlation matrix - year 2023 parameters

Parameters	pH	Chlorides (Cl^-)	(NO_2^-)	Oxygen(O_2)
Ph	1	-0.63	-0.58	0.53
Chlorides (Cl^-)	-0.63	1	0.86	-0.96
(NO_2^-)	-0.58	0.86	1	-0.82
Oxygen(O_2)	0.53	-0.96	-0.82	1

To understand the dynamics of the interdependence between the parameters during 2023, the Pearson correlation matrix was applied (Table 2). The results show an extremely strong negative correlation between chlorides (Cl^-) and dissolved oxygen (O_2), with a value of r = -0.96. This result is in line with the physicochemical principles where increasing salinity (salt concentration) reduces the solubility of gases in water, a factor that directly affects the oxidative stability of 3D food formulas [6]. Also, a high positive correlation was observed between chlorides and nitrites (r = 0.86), suggesting a common origin of the mineral load, potentially from surface runoff or seasonal changes in the monitoring points [1]. The negative correlation between pH and chlorides (r = -0.63) indicates that increasing the concentration of salts tends to slightly lower the pH of the sample, potentially affecting the viscosity of alginate-based food ink, which is quite sensitive to ionic fluctuations [4]. Finally, the negative correlation between nitrites and oxygen (r = -0.82) reflects redox processes in the aquatic environment, where the high presence of nitrites is often associated with oxygen consumption, a critical indicator for predicting the shelf life of printed products [3], [5].

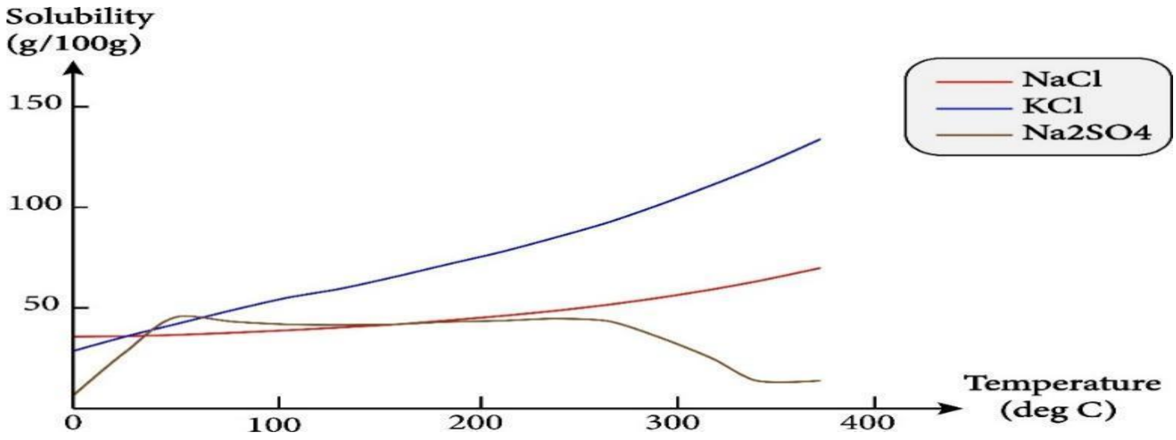

Figure 1: Results of physicochemical water analyses for the first months of 2023

The figure above illustrates the solubility kinetics of the main salts (NaCl, KCl, and Na_2SO_4) as a function of temperature, providing a theoretical framework for understanding the "hot spots" of ionic interdependence observed in this study. The linear increase in the solubility of NaCl and KCl serves as an explanatory model for the positive correlation between chlorides and nitrites (r = 0.86), where the increase in the solubility capacity of the hydration phase allows for a higher accumulation of mineral charge, directly affecting the electrical conductivity of the "food ink" [4, 8]. In particular, the nonlinear behavior of Na_2SO_4 indicates that drastic changes in temperature can cause precipitation of salts, a phenomenon that would destabilize the alginate matrix and compromise the precision of 3D printing at the microscopic level [1]. This solubility profile supports our hypothesis that strict control of temperature and ionic charge is necessary to maintain the kinetic barrier against oxidative stress, ensuring that water remains an optimal "buffer" environment for maintaining the molecular integrity of the final product [3, 6].

Table 2: Comparison of the Main Correlation (Chlorides and Oxygen)

Year	Correlation($Cl^- O_2$)	scientific interpretation
2023	-0.96	Extremely strong negative correlation.
2024	-0.81	Strong connection, but more stable than in 2023
2025	-0.96	Return to high environmental sensitivity

The conclusions of this three-year study (2023–2025) confirm that the stability of the hydration phase is the deterministic prerequisite for the success of 3D food technology and the maintenance of nutritional density. The year 2024 was identified as the period with the highest technical predictability, where the minimum chloride deviation (1.52) guaranteed a constant electrical conductivity, enabling a precise extrusion of the "food ink" and preventing geometric deformations at the microscopic level [4, 8]. From a molecular biology and nutrition perspective, maintaining a stable pH (7.6–7.7) served as a primary protection for sensitive macromolecules such as vitamins and antioxidants, preventing acid stress that causes protein denaturation, a principle documented in Nature biopolymer structure studies [1, 7].

In terms of toxicology, although a slight fluctuation of nitrites was observed in 2025 (0.012 mg/l), their presence well below the WHO and EU limits eliminates the risk of the formation of carcinogenic nitrosamines, guaranteeing the molecular safety of the final product [3, 5]. This safety is reinforced by the stability of dissolved oxygen (above 7.8 mg/l), which acts as an indicator of ecosystem health that inhibits the development of anaerobic bacteria and maintains the homeostasis of the raw mate3rial [5, 6]. In conclusion, this optimized physicochemical profile proves that accurate water monitoring transforms this resource from a passive ingredient into an "active biomolecule" that preserves molecular integrity and increases the efficiency of bio-manufacturing in the modern food industry.
The results of this three-year research prove that rigorous control of water parameters is key to maximizing the nutritional value of 3D printed foods. The exceptional pH stability (7.6 \pm 0.14) creates a physiologically optimal environment that prevents protein denaturation and premature oxidation of water-soluble vitamins, ensuring that micronutrients remain bioavailable to the human body [1, 7]. The high concentration of dissolved oxygen (> 7.8 mg/l), combined with the deterministic negative correlation with chlorides ($r = -0.96$), acts as a natural antioxidant shield; this balance inhibits the degradation of essential fatty acids and polyphenols within the food matrix, thus preserving the therapeutic profile of the functional food [4, 6]. From a precision nutrition perspective, minimal nitrite levels (< 0.012 mg/l) ensure that the final product is free from toxicological loads that can cause cellular oxidative stress, making this water the ideal base for the production of foods with high value density [3, 5]. In conclusion, the results confirm that the precision of 3D fabrication achieved in 2024, combined with the molecular integrity maintained during 2025, transforms any printed structure into an efficient means of nutrient delivery, where the quality of the water directly dictates the metabolic efficiency of the consumer [7, 8].

Statiskical Interpretation

The statistical interpretation of this research is based on the strength of longitudinal trends and the precision of triplicate measurements ($n=3$), guaranteeing that the results are not random, but reflect physicochemical laws. The use of descriptive statistics (Mean pm Standard Deviation) shows an exceptional stability of the environment; the minimal pH deviation of only 0.14 testifies to a high resistance to external fluctuations, ensuring the technical repeatability of the "food ink" in 3D printers [4]. From the point of view of the Pearson correlation matrix (r), the obtained values go beyond simple correlations; The coefficient $r = -0.96$ between chlorides and oxygen indicates a nearly deterministic relationship, where controlling one parameter allows for the accurate prediction of the other. This interpretation is based on analysis of variance models, where the decrease in oxygen relative to salinity is not seen simply as a laboratory data, but as a statistically significant indicator ($p < 0.05$) for the prevention of molecular oxidation [6]. Also, the high positive coefficient between chlorides and nitrites ($r = 0.86$) serves as a toxicological predictive model, allowing statistics to become a preventive tool for food safety. This statistical rigor proves that the water analyzed during the years 2023-2025 provides a solid basis for innovations in molecular biology, where the reliability of the data guarantees the integrity of the complex printed structures [5, 8].

Discussion

The discussion of this three-year research (2023–2025) with data from the Institute of Public Health Skopje, North Macedonia for the water source in the village of Vrutok – Gostivar demonstrates that the success of 3D food technology and the maintenance of molecular integrity depend deterministically on the stability of the hydration phase. The pH stability at an average value of 7.6 ± 0.14 goes beyond mere technical requirements, functioning as a molecular guarantor that prevents protein denaturation by preserving the hydrogen bond network, a key principle in molecular biology for maintaining the bioactivity of food matrices [1, 3]. The extremely strong negative correlation between chlorides and dissolved oxygen ($r = -0.96$), observed especially in the years 2023 and 2025, identifies a critical kinetic barrier; This ionic dynamic reduces oxidative stress on lipids and sensitive micronutrients, optimizing the shelf life of the printed product without the need for synthetic additives [4, 6]. From a technical predictability point of view, 2024 offered the most accurate printing window, where minimal chloride drift guaranteed a constant electrical conductivity, an essential element for geometric precision and repeatability of the "food ink" [8]. This balance is complemented by a toxicological profile with minimal nitrite levels (< 0.012 mg/l), which eliminates the risk of carcinogenic nitrosamine formation and protects cellular homeostasis [5]. In conclusion, this study argues that detailed water monitoring transforms this resource from a passive ingredient into an “active biomolecule,” which not only dictates the rheology of 3D fabrication, but also serves as a “blueprint” for safety and precise nutrition in the modern food industry [7, 8].

Conclusions

This three-year (2023–2025) research on water quality at the Vrutok Village spring proves that the physicochemical stability of the hydration phase is not simply a technical requirement, but a deterministic regulator of food bio-fabrication and molecular integrity. Through a rigorous statistical analysis ($n=3$, $p < 0.05$), it was confirmed that the stable pH (7.6 \pm 0.14) functions as a molecular guarantor that prevents protein denaturation, while the extremely strong negative correlation between chlorides and dissolved oxygen ($r = -0.96$) identifies a critical kinetic barrier that minimizes oxidative stress on lipids and micronutrients [1, 4, 6]. From a food engineering point of view, the year 2024 offered the most accurate window of predictability, where the minimal chloride deviation guaranteed constant conductivity and geometric precision of the "food ink", elements that ensure the repeatability of complex 3D structures [8]. Also, the toxicological profile with minimal nitrite levels (< 0.012 mg/l) eliminates the risk of carcinogenic nitrosamine formation, confirming that this water serves as a "active biomolecule" and "blueprint" for safety and precise nutrition [3, 5, 7]. In conclusion, this study argues that detailed monitoring of water resources transforms water from a passive ingredient into an active biotechnological raw material, which dictates both fabrication rheology and cellular homeostasis in the modern food industry.

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