



TECHNICAL UNIVERSITY OF MOLDOVA

JOURNAL OF ENGINEERING SCIENCE

Technical and applied scientific publication founded on 9 February 1995
Alternative title: Meridian ingineresc

2025
Vol. XXXII (4)

ISSN 2587-3474
eISSN 2587-3482

TECHNICAL UNIVERSITY OF MOLDOVA (PUBLISHING HOUSE)
„TEHNICA UTM” (PRINTING HOUSE)

According to the Decision of the NAQAER No. 12 from 23.02.2024, JES is classified as B+ journal

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[https://doi.org/10.52326/jes.utm.2025.32\(4\).01](https://doi.org/10.52326/jes.utm.2025.32(4).01)

UDC 537.32:538.9:539.21



TTT₂I₃ ORGANIC CRYSTALS – A PROMISING PLATFORM FOR LOW-DIMENSIONAL THERMOELECTRIC DEVICES

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Received: 12. 02. 2025

Accepted: 12. 28. 2025

Abstract. In this paper, we provide an analysis of quasi-one-dimensional organic crystals of TTT₂I₃ (tetrathiotetracene-iodide) with respect to their thermoelectric properties. An advanced physical model was employed. The main Hamiltonian of the model includes the electronic and phonon contributions, electron-phonon interactions, and an impurity-scattering term. To capture charge transport between molecular chains, the model was extended to a three-dimensional framework. Numerical simulations were conducted to evaluate electrical conductivity, Seebeck coefficient, thermal conductivity, thermoelectric power factor, and figure of merit as functions of carrier concentration, temperature, and impurity content. Additionally, the Peierls structural transition in the TTT chains was analyzed, allowing determination of the critical transition temperature. The dispersion of renormalized phonons was examined in the random phase approximation for different temperatures.

Keywords: *carrier concentration, electron-phonon interaction, organic materials, Peierls critical temperature, power factor, renormalized phonon spectrum, Seebeck coefficient, thermal conductivity.*

Rezumat. În această lucrare, prezentăm o analiză a cristalelor organice cvasi-unidimensionale de TTT₂I₃ (tetratiotetracene-iodid) din perspectiva proprietăților lor termoelectrice. A fost utilizat un model fizic avansat. Hamiltonianul principal al modelului include contribuțiile electronice și fononice, interacțiunile electron - fonon și un termen asociat împrăștierii pe impurități. Pentru a descrie transportul de sarcină între lanțurile moleculare, modelul a fost extins la un cadru tridimensional. Au fost efectuate simulări numerice pentru a determina conductivitatea electrică, coeficientul Seebeck, conductivitatea termică, factorul de putere termoelectric și parametrul termoelectric de calitate în funcție de concentrația purtătorilor de sarcină, temperatură și conținutul de impurități. De asemenea, a fost analizată tranziția structurală de tip Peierls în lanțurile TTT, ceea ce a permis determinarea temperaturii critice a tranziției. Dispersia fononilor renormați a fost examinată în aproximația fazelor aleatorii pentru diferite temperaturi.

Cuvinte cheie: concentrația purtătorilor de sarcină, interacțiune electron-fonon, materiale organice, temperatura critică Peierls, factor de putere, spectrul renormalizat al fononilor, coeficient Seebeck, conductivitatea termică.

1. Introduction

A significant portion of global energy is currently produced using fossil-fuel heat engines, whose limited efficiency results in substantial waste heat. Developing cost-effective methods to convert part of this unused thermal energy into electricity has therefore become an important technological objective. Thermoelectric generators represent a promising solution, as they enable direct heat-to-electricity conversion and can also operate as solid-state refrigeration devices without mechanical components.

The dimensionless thermoelectric figure of merit, $ZT = \sigma S^2 T k^{-1}$, is the primary parameter determining a material's suitability for thermoelectric energy conversion. Here, σ is the electrical conductivity, S the Seebeck coefficient, $k = k^e + k^L$ the total thermal conductivity including electronic (k^e) and lattice (k^L) contributions, and T the operating temperature. Achieving high ZT values is essential for efficient energy conversion, yet these parameters are interdependent: increasing σ can reduce S and increase k^e , making the simultaneous optimization of all factors challenging. In recent decades, significant progress has been made, particularly in low-dimensional inorganic structures. High ZT values, such as ~ 2.4 has been measured [1] at room temperature in p -type $\text{Bi}_2\text{Te}_3/\text{Sb}_2\text{Te}_3$ superlattices. $ZT \sim 3$ has been reported in PbTeSe quantum dot superlattices [2], and even $ZT \sim 3.5$ [3,4] have been reported. However, these materials typically involve complex and costly fabrication processes, and commercially available Bi_2Te_3 -based thermoelectrics currently reach $ZT \sim 1$ around 500 K. Attaining $ZT > 3$ would make solid-state thermoelectric devices economically competitive with conventional energy conversion technologies, but large-scale commercialization remains limited. Despite these challenges, miniaturized thermoelectric modules are already mass-produced for practical applications, including temperature stabilization in laser diodes [5], climate-controlled automotive seats [6], portable coolers [7], and space technologies. Recent strategies for scaling up production suggest combining high-energy ball milling with aerosol jet printing to produce devices with high electrical conductivity and low thermal conductivity, resulting in a maximized ZT . This approach highlights the ongoing potential of thermoelectric materials for energy harvesting and refrigeration applications [8-10]. A detailed review of thermoelectric materials and their applications was presented in [11].

Organic compounds have recently gained attention due to their cost-effectiveness, abundance, environmental friendliness, and unique properties. Examples include poly(3,4-ethylenedioxythiophene) (PEDOT) doped with poly(styrenesulphonate) (PSS), where thin films of p -type have registered a ZT value of 0.42 at room temperature [12]. Even higher ZT values, reaching around 1, have been reported in films of PP-PEDOT/Tos [13]. Such results underline the opportunities offered by organic materials for next-generation thermoelectric devices.

High ZT values have been predicted in certain quasi-one-dimensional (Q1D) organic crystals, such as TTT_2I_3 [14] and $\text{TTT}(\text{TCNQ})_2$ [15]. These materials consist of linear molecular chains or stacks within a three-dimensional lattice, with interactions along the chains dominating over interchain interactions. Many organic compounds, including ion-radical salts like TTF-TCNQ, crystalline polymers, and specific charge transfer complexes, exhibit this

quasi-one-dimensional structure, which often leads to unique properties. Previous predictions of high ZT were based on simplified one-dimensional models. In existing Q1D crystals of tetrathiotetracene-iodide, TTT₂I₃, grown from solution [16] with measured electrical conductivity $\sigma_{xx} = 1.8 \cdot 10^5 \Omega^{-1}m^{-1}$, Seebeck coefficient $S_{xx} = 39 \mu VK^{-1}$ and thermal conductivity $k_{xx} = 1.0 Wm^{-1}K^{-1}$ along the conductive chains only $ZT \cong 0.1$ was obtained at room temperature [17]. Such low value of ZT is explained by the fact that the crystals were not very pure and the parameters were not optimized. We have developed a more realistic three-dimensional model, enabling more accurate simulations of the thermoelectric behavior of selected Q1D organic crystals.

The objective of this paper is to present the results of recent investigations on quasi-one-dimensional (Q1D) organic crystals and their potential applications in thermoelectric systems. The study provides a comprehensive analysis, including modeling and evaluation of key properties such as electrical conductivity, Seebeck coefficient, thermal conductivity, thermoelectric power factor and the thermoelectric figure of merit. Additionally, the research examines the Peierls structural transition in the TTT molecular chains of TTT₂I₃, determining the critical transition temperature and analyzing the dispersion of renormalized phonons across different temperatures and dimensionless Fermi momentum (k_F) values.

2. Three-Dimensional Physical Model of the Crystal

The Q1D crystals of TTT₂I₃ are formed from segregate stacks or chains of TTT molecules and iodine. However, only the TTT chains are conductive, owing to the strong π -electron overlap along their stacks. Each pair of TTT molecules donates one electron to the iodine chain composed of I_3^- ions, which act as electron acceptors. The electrons on the I_3^- ions are strongly localized and therefore do not contribute to charge transport. As a result, the carriers are holes. The electrical conductivity along the TTT chains is nearly three orders of magnitude higher than in the transverse directions. In earlier studies, this pronounced anisotropy justified the use of a simplified 1D model [18,19], where the crystal was regarded as consisting of independent one-dimensional chains arranged in a 3D crystal framework. In reality, the 1D conductive chains are not completely independent, as weak interchain interactions are present. It is evident that this interchain interaction affects the validity of the 1D approximation - most notably in very pure crystals, where it restricts the mobility of charge carriers. Therefore, it is crucial to determine how interchain interactions influence the thermoelectric properties of real crystals and, in this context, to establish the conditions under which the simpler 1D model remains applicable.

In this Section we will present the physical model of the TTT₂I₃ crystal that was described in more detail in [20]. The Hamiltonian of the 3D crystal model was formulated using the tight-binding approach and the nearest-neighbor approximation. It can be expressed as follows:

$$H = \sum_k \varepsilon(k) a_k^\dagger a_k + \sum_q \hbar \omega_q b_q^\dagger b_q + \sum_{k,q} A(k,q) a_k^\dagger a_{k-q} (b_q + b_{-q}^\dagger). \quad (1)$$

The first term in Eq. (1), $\sum_k \varepsilon(k) a_k^\dagger a_k$, represents the energy operator of free electrons in the periodic field of the lattice. The second term, $\sum_q \hbar \omega_q b_q^\dagger b_q$, corresponds to the energy

operator of longitudinal acoustic phonons. The third term, $\sum_{\mathbf{k}, \mathbf{q}} A(\mathbf{k}, \mathbf{q}) a_{\mathbf{k}}^+ a_{\mathbf{k}-\mathbf{q}} (b_{\mathbf{q}} + b_{-\mathbf{q}}^+)$ describes the two most significant electron-phonon interactions. Here, $\varepsilon(\mathbf{k})$ represents the carrier energy, where $a_{\mathbf{k}}^+, a_{\mathbf{k}}$ are the electron creation and annihilation operators associated with the 3D quasi-wave vector $\mathbf{k}$ (k_x, k_y, k_z). Analogously, $b_{\mathbf{q}}^+, b_{\mathbf{q}}$ denote the acoustic phonon creation and annihilation operators for a 3D wave vector $\mathbf{q}$ and frequency $\omega_{\mathbf{q}}$. The interaction matrix element is given by $A(\mathbf{k}, \mathbf{q})$. The electron energy $\varepsilon(\mathbf{k})$, measured relative to the top of the band, takes the following form:

$$\varepsilon(\mathbf{k}) = -2w_1(1 - \cos k_x b) - 2w_2(1 - \cos k_y a) - 2w_3(1 - \cos k_z c), \quad (2)$$

where: w_1 , w_2 and w_3 are the transfer energies of a hole along the chain (x direction) and perpendicular to it (y and z directions), respectively.

It is established that the Peierls transition happens at low temperatures. Consequently, the interaction of electrons with optical phonons can be ignored, and the acoustic phonon spectrum of a simple one-dimensional chain can be represented by [21]:

$$\omega_{\mathbf{q}}^2 = \omega_1^2 \sin^2(q_x b / 2) + \omega_2^2 \sin^2(q_y a / 2) + \omega_3^2 \sin^2(q_z c / 2). \quad (3)$$

In Eq. (3) the limit frequencies in the x , y , and z directions are denoted by ω_1 , ω_2 and ω_3 , respectively. In this model, two hole – phonon interactions are considered. The first has coupling constants proportional to the derivatives w_1' , w_2' and w_3' of w_1 , w_2 , and w_3 with respect to intermolecular distances, while the second depends on the molecule's average polarizability α_0 . The latter is especially important for crystals of large molecules, such as TTT, since α_0 scales approximately with molecular volume.

The square module of matrix element $A(\mathbf{k}, \mathbf{q})$ from Eq. (1) has the following form:

$$\begin{aligned} |A(\mathbf{k}, \mathbf{q})|^2 = & 2\hbar w_1'^2 / (NM \omega_{\mathbf{q}}) \left\{ [\sin(k_x b) - \sin(k_x - q_x, b) - \gamma_1 \sin(q_x b)]^2 + \right. \\ & + d_1^2 [\sin(k_y a) - \sin(k_y - q_y, a) - \gamma_2 \sin(q_y a)]^2 + \\ & \left. + d_2^2 [\sin(k_z c) - \sin(k_z - q_z, c) - \gamma_3 \sin(q_z c)]^2 \right\}. \end{aligned} \quad (4)$$

In Eq. (4), M denotes the mass of a TCNQ molecule, and N represents the number of TCNQ molecules in the basic region of the crystal; $d_1 = w_2 / w_1 = w_2' / w_1'$; $d_2 = w_3 / w_1 = w_3' / w_1'$; parameters γ_1 , γ_2 and γ_3 correspond to the ratio of the polaron - type interaction amplitude to the deformation potential in the x , y , and z directions, respectively.

$$\gamma_1 = 2e^2 \alpha_0 / b^5 w_1'; \quad \gamma_2 = 2e^2 \alpha_0 / a^5 w_2'; \quad \gamma_3 = 2e^2 \alpha_0 / c^5 w_3'. \quad (5)$$

The analysis indicates that the Hamiltonian from Eq. (1) cannot account for the sharp decrease in electrical conductivity observed at temperatures below $T_{max} = 35 \text{ K}$, even when varying crystal parameters and considering only the first interaction mechanism. It is therefore necessary to include the dynamic interaction of carriers with defects. The static interaction contributes to the renormalization of the hole spectrum. In TTT₂I₃ crystals, defects

arise due to the different thermal expansion coefficients of the TTT and iodine chains. The Hamiltonian describing this interaction is expressed as follows:

$$H_{def} = \sum_{k,q} \sum_{n=1}^{N_d} B(q_x) \exp(-iq_x x_n) a_k^\dagger a_{k-q} (b_q + b_q^-). \quad (6)$$

In Eq. (6), the variable x_n indexes the defects, which are assumed to be linear along the x - direction of the TTT chains and randomly distributed. $B(q_x)$ represents the matrix element describing the interaction of a hole with a defect, and is given by the following expression:

$$B(q_x) = \sqrt{\hbar / (2NM\omega_q)} \cdot I(q_x), \quad (7)$$

where: $I(q_x)$ is the Fourier transformation of the derivative of the carrier–defect interaction energy with respect to the intermolecular distance.

$$I(q_x) = D(\sin(bq_x))^2. \quad (8)$$

In this context, D characterizes the strength of the hole–defect coupling, equivalent to w_1' in Eq. (5), and the Peierls transition is strongly influenced by its value.

The renormalized phonon spectrum $\Omega(q)$ is obtained from the pole of the Green function, which is defined by the transcendent dispersion equation.

$$\Omega(q) = \omega_q [1 - \bar{\Pi}(q, \Omega)]^{1/2}, \quad (9)$$

where the principal value of the dimensionless polarization operator is given by

$$\text{Re } \bar{\Pi}(q, \Omega) = -\frac{4}{\hbar\omega_q} \sum_k \frac{[|A(\mathbf{k}, -\mathbf{q})|^2 + |B(\mathbf{q}_x)|^2](n_k - n_{k+\mathbf{q}})}{\varepsilon(\mathbf{k}) - \varepsilon(\mathbf{k} + \mathbf{q}) + \hbar\Omega}. \quad (10)$$

n_k represents the Fermi distribution function. We note, that Eq. (9) is solvable exclusively by numerical methods.

Transport properties. Consider the case in which a weak electric field and a small temperature gradient are imposed along the conductive chains. At room temperature, the phonon energy and the transversal kinetic energy of the holes can be neglected in the scattering processes, as both are much smaller than the kinetic energy associated with motion along the chains. Under these conditions, the linearized kinetic equation can be solved analytically, allowing the electrical conductivity σ_{xx} , the Seebeck coefficient S_{xx} , the electronic thermal conductivity k_{xx}^e and the thermoelectric figure of merit $(ZT)_{xx}$ to be expressed in terms of the transport integrals R_n as follows:

$$\sigma_{xx} = \sigma_0 R_0, \quad S_{xx} = (k_0 / e)(2w_1 / k_0 T) R_1 / R_0, \quad (11)$$

$$k_{xx}^e = [4w_1^2 \sigma_0 / (e^2 T)] (R_2 - R_1^2 / R_0), \quad (ZT)_{xx} = \sigma_{xx} S_{xx}^2 T / (k_{xx}^L + k_{xx}^e), \quad (12)$$

where

$$\sigma_0 = (2e^2 M v_{s1}^2 w_1^3 r) / (\pi^2 \hbar abc (k_0 T)^2 w_1'^2). \quad (13)$$

Here, $r = 4$ denotes the number of molecular chains present in the transverse cross-section of the unit cell. The term k_{xx}^L represents the lattice thermal conductivity, v_{s1} is the sound velocity along the chains, and R_n are the transport integrals.

$$R_n = \int_0^2 d\varepsilon \int_0^\pi d\eta \int_0^\pi d\zeta \varepsilon (2 - \varepsilon) n_{\varepsilon, \eta, \zeta} (1 - n_{\varepsilon, \eta, \zeta}) \times \frac{[\varepsilon + d_1(1 - \cos \eta) + d_2(1 - \cos \zeta) - (1 + d_1 + d_2)\varepsilon_F]^n}{\gamma_1^2(\varepsilon - \varepsilon_0)^2 + D_0 + \{d_1^2(1 + \gamma_2^2 + 2\sin^2 \eta - 2\gamma_2 \cos \eta) + d_2^2(1 + \gamma_3^2 + 2\sin^2 \zeta - 2\gamma_3 \cos \zeta)\} / (8\varepsilon(2 - \varepsilon))}. \quad (14)$$

For comparison with the 1D model, the following dimensionless variables were introduced:

$\varepsilon = 1 - \cos(bk_x)$, $\eta = k_y a$, and $\zeta = k_z c$. In this representation, $n_{\varepsilon, \eta, \zeta}$ denotes the Fermi distribution function expressed in terms of the new variables. The dimensionless resonance energy appearing in the relaxation time is given by $\varepsilon_0 = (\gamma_1 - \gamma) / \gamma_1$ and is measured in units of $2w_1$. The parameters $d_1 = w_2 / w_1 = w_2' / w_1'$; $d_2 = w_3 / w_1 = w_3' / w_1'$; characterize the ratios of the transfer integrals. The 1D Fermi energy is defined as $\varepsilon_F = E_F / (2w_1)$, while the corresponding 3D Fermi energy becomes $2w_1(1 + d_1 + d_2)\varepsilon_F$. The parameter D_0 quantifies the scattering of holes by impurities.

$$D_0 = n_{im}^{3D} I^2 V_0^2 \frac{M v_s^2}{4b^3 a c w_1'^2 k_0 T}, \quad (15)$$

where: n_{im}^{3D} denotes the impurity density, I represents the amplitude of the impurity potential, and V_0 is the spatial region over which this potential acts.

To determine the parameters d_1 and d_2 , the electrical conductivity in the transverse directions, σ_{yy} and σ_{zz} , was calculated. In these directions, the overlap of hole wave functions is minimal, making it convenient to express the system Hamiltonian in the representation of localized states on the TTT molecules. Consequently, for motion along the y and z axes, the dominant term in the Hamiltonian is the hole - phonon interaction, while the term describing hole motion within the periodic lattice potential is treated as a small perturbation. A canonical transformation is applied to the Hamiltonian, allowing the main contribution of the hole - phonon interaction to be accounted for already at the zero - order approximation. This procedure also results in a substantial narrowing of the original conduction band along the conductive chains. As a result, transport in the transverse directions occurs via a hopping mechanism, with the charge carriers effectively behaving as small polarons.

The expressions for σ_{yy} and σ_{zz} were computed numerically. By comparing these results with the experimental data, $\sigma_{yy} \approx \sigma_{zz} = 3.3 \Omega^{-1} \text{cm}^{-1}$, it can be inferred that $w_2 = w_3 = 0.015w_1$. The similarity of these values reflects the close correspondence between the lattice constants a and c along the y and z directions, respectively.

3. Results and Discussions

Computer simulations were carried out using the following set of parameters [17]: $M = 6.5 \times 10^5 m_e$ (where m_e is the free-electron mass), $w_1 = 0.16 \text{ eV}$, $w_1' = 0.26 \text{ eV} \cdot \text{\AA}^{-1}$, $a = 18.35 \text{ \AA}$, $b = 4.96 \text{ \AA}$, $c = 18.46 \text{ \AA}$. The sound velocity at low temperatures along the chains (i.e., in the b direction) is $v_{s1} = 1.5 \times 10^5 \text{ cm} \cdot \text{s}^{-1}$. The parameter d is set to 0.015, while $\gamma_1 = 1.7$ and γ_2 is obtained from the relation $\gamma_2 = \gamma_1 b^5 / a^5 d$. For the transversal sound velocity in the a direction, the value $v_{s2} = 1.35 \times 10^5 \text{ cm} \cdot \text{s}^{-1}$ was used. The dimensionless parameters for describing the impurity scattering were considered as follows: $D_0 = 0.001$ for static impurities and $D_1 = 0.2$ for lattice defects, thermally activated.

First of all, the computer calculations were performed for different values of free parameters in order to adjust the physical model by comparison with experimentally reported data (Figures 1, 2). In Figure 1 the experimental data from [16] were considered for TTT₂I₃ crystals. The measured electrical conductivity along TTT chains at room temperature is $1.8 \cdot 10^5 \Omega^{-1} \text{ m}^{-1}$. It can be observed that for temperatures, lower than a threshold, the computer simulations are diverging from experiment. This can be explained by the reshape of crystalline lattice with temperature decrease, a smooth metal-dielectric transition takes place for temperatures below 100 K. The three lines represent calculated values for electrical conductivity for three values of dimensionless Fermi energy $\varepsilon_F = 0.27, 0.3, 0.33$. For stoichiometric crystals $\varepsilon_F = 0.37$. It is observed that the theory describes well the temperature dependence of electrical conductivity in the range from 180 K to 400 K. It has to be mentioned that electrical conductivity in such crystals is provided by holes, due to the presence of iodine atoms. With the increase of temperature, the iodine evaporates and the physical model is not more applicable.

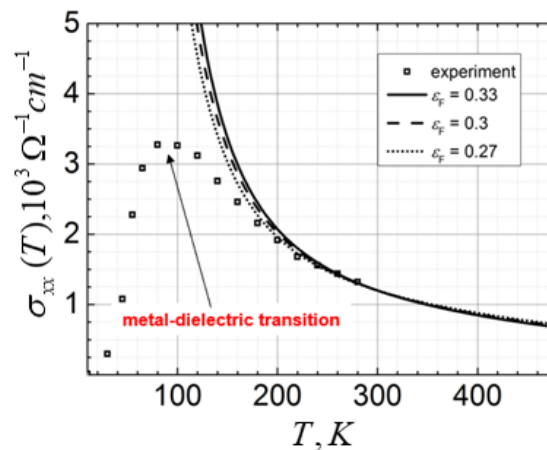


Figure 1. Electrical conductivity as a function of temperature T : rhombs – experiment [16]; lines – numerical results.

In Figure 2 the results for thermopower S_{xx} are presented as a function of temperature for different levels of dimensionless Fermi energy. It is observed that in this case the fluctuations of the dielectric phase practically do not affect the behavior of S_{xx} for a wide interval of temperatures. For crystals with near-to stoichiometric concentration of conducting holes (continuous line), the numerical results are fitting the experimentally reported ones. With the decrease of ε_F one can obtain higher values for thermopower, especially for near-to room temperatures, a perspective results for thermoelectric applications.

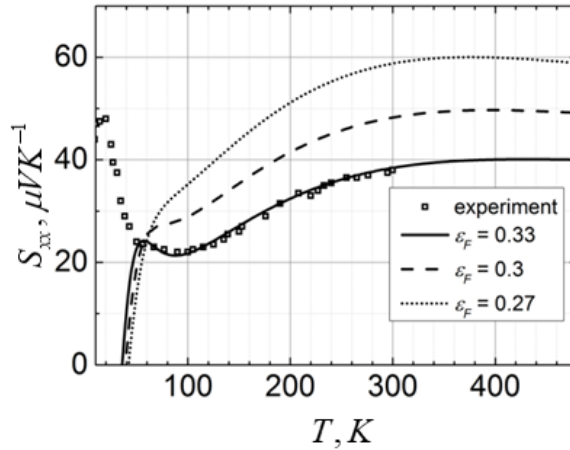


Figure 2. Thermopower as a function of temperature T : rhombs – experiment [16]; lines – numerical results.

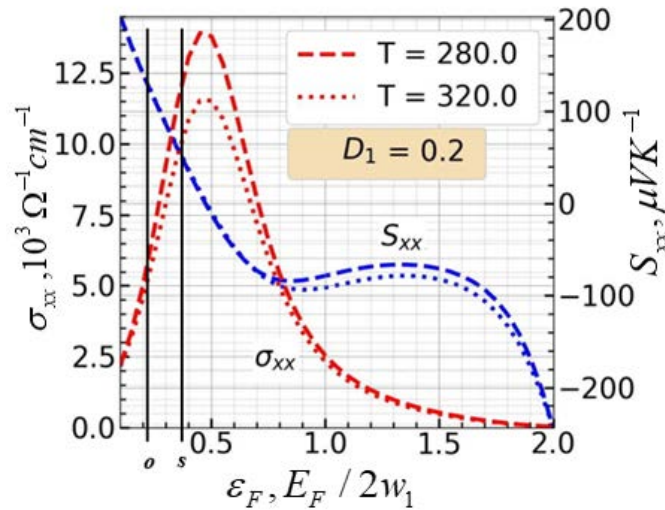


Figure 3. Electrical conductivity and Seebeck coefficient as a function of dimensionless Fermi energy. D_1 – scattering on lattice defects.

Considering that crystals are organic and for temperatures higher than 350–400 K the iodine evaporates, we calculated in detail the electrical conductivity and the thermopower for two values of T : 280 K and 320 K for a wide range of dimensionless Fermi energy (Figure 3). It is observed that electrical conductivity is more sensitive to temperature changes, mainly at $\varepsilon_F = 0.5$ (red line), while the thermopower is less sensitive. The two vertical lines, signed with o and s mean the **stoichiometric** and optimized values of dimensionless Fermi energy.

From Figure 3 one can deduce that for stoichiometric crystals with $\varepsilon_F = 0.37$, $\sigma_{xx} = 10 \cdot 10^3 \Omega^{-1} \text{cm}^{-1}$ and $S_{xx} = 60 \mu\text{VK}^{-1}$. When the concentration of conducting holes is diminished, the dimensionless Fermi energy decreases and for optimized crystals one can obtain high values of S_{xx} : $\varepsilon_F = 0.22$, $\sigma_{xx} = 5 \cdot 10^3 \Omega^{-1} \text{cm}^{-1}$ and $S_{xx} = 120 \mu\text{VK}^{-1}$. This can be explained in such manner: the less holes, the lower electrical conductivity but each hole carries more energy within the same temperature gradient.

In Figure 4 the electrical conductivity and electronic thermal conductivity are plotted as a function of dimensionless Fermi energy for the same temperature values.

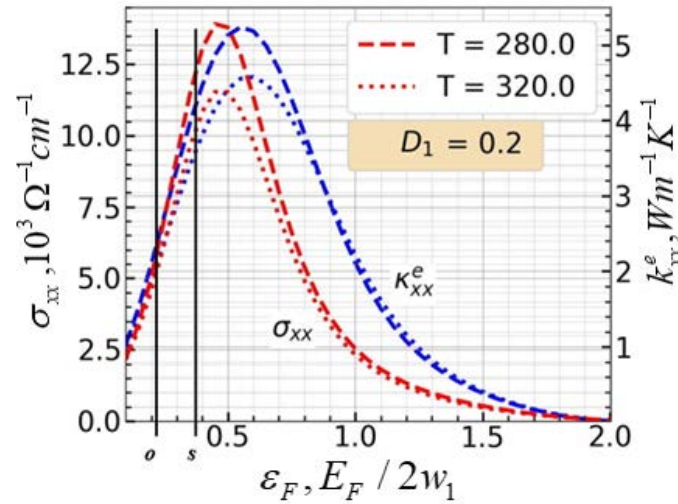


Figure 4. Electrical conductivity vs electronic thermal conductivity as a function of dimensionless Fermi energy. D_1 – scattering on lattice defects.

It can be observed that the manipulations with carrier concentration results in the creation of more lattice concentration. Also, with the increase of the temperature, the electrical conductivity and electronic thermal conductivity decreases. For *stoichiometric* values of dimensionless Fermi energy the following values were calculated: $\sigma_{xx} = 12 \cdot 10^3 - 10 \cdot 10^3 \Omega^{-1} \text{cm}^{-1}$, $k_{xx}^e = 3.8 - 4 \text{ Wm}^{-1} \text{K}^{-1}$ for $T = 280 \text{ K}$ and $T = 320 \text{ K}$ respectively. For *optimized* crystals: $\sigma_{xx} = 5 \cdot 10^3 \Omega^{-1} \text{cm}^{-1}$, $k_{xx}^e = 2.2 \text{ Wm}^{-1} \text{K}^{-1}$ and practically do not depend on T . The displacement of the maximums of σ_{xx} and k_{xx}^e indicate on the violation of the Wiedemann-Franz law in these crystals.

The obtained results are in accordance with the experimentally reported data, which indicates on the reliability of the physical model to perform numerical calculations and some predictions for a wider range of temperatures and carrier concentrations. It was shown that for such organic crystals, discussed above, a metallic-like electrical conductivity can be obtained with the tuning of charge carrier concentration. Also, due to the violation of the Wiedemann-Franz law, the electronic thermal conductivity increases slowly than the electrical conductivity when manipulating with carrier concentration. This phenomenon is very prospective for future thermoelectric applications. High values of thermopower, up to $120 \mu\text{VK}^{-1}$, combined with low electronic thermal conductivity, $2.2 \text{ Wm}^{-1} \text{K}^{-1}$, is related to high thermoelectric figure-of merit that can be obtained in these crystals by evaporating iodine and lowering the Fermi level.

In what follows, we examine the characteristics of the Peierls transition in quasi-one-dimensional (Q1D) organic crystals of TTT₂I₃ [22]. The Peierls transition has been extensively investigated in the literature [23, 24]. In previous studies, its behavior has been explored in TTF–TCNQ crystals [25] as well as in TTT₂I₃ organic compounds [20, 22]. The objective of this work is to provide a comprehensive theoretical framework for the Peierls transition in TTT₂I₃ crystals, employing a three-dimensional physical model that simultaneously considers the two most relevant electron–phonon interaction mechanisms previously mention.

Figures 5 and 6 present the dependence of the renormalized phonon frequencies, $\Omega(q_x)$, on the wave-vector component q_x , for various temperatures, q_y and q_z values, and different Fermi quasi-momentum (k_F). Here, q_x , q_y and q_z denote the projections of the phonon quasi-wave vector along the x , y , and z directions, respectively.

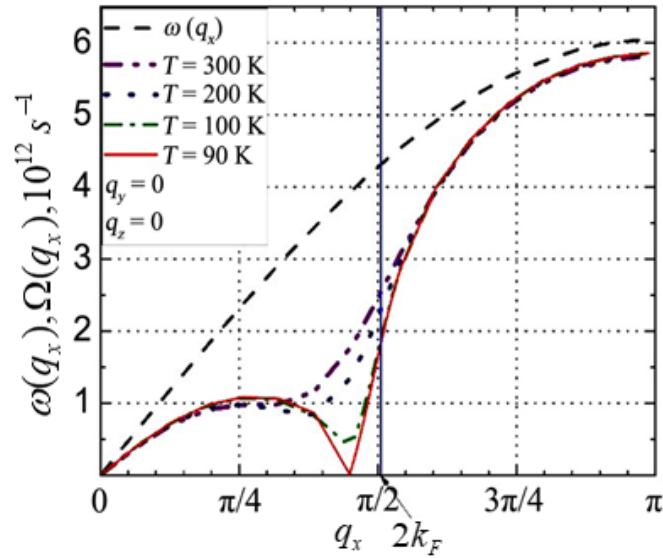


Figure 5. The renormalized phonon spectrum $\Omega(q_x)$ is plotted for several temperatures using $\gamma_1 = 1.7$, where the dashed curve represents the free-phonon spectrum [22]. The specific parameters used for this plot are $k_F = 0.502 \cdot \pi / 2$, $D = 1.057 \text{ eV} \cdot \text{\AA}^{-1}$.

The initial (unrenormalized) phonon frequency is represented as $\omega(q_x)$, while (k_F) denotes the dimensionless Fermi quasi-momentum, determined by the carrier concentration. It is evident from all figures that $\Omega(q_x)$ exhibits lower values than $\omega(q_x)$ in the absence of electron-phonon coupling. This reduction reflects the softening of the lattice elastic constants due to electron-phonon interactions. Additionally, decreasing the temperature T leads to a noticeable change in the shape of the curves. A minimum develops in the $\Omega(q_x)$ dependence, becoming increasingly pronounced at lower temperatures. The expected behavior was that $\Omega(q_x)$ would vanish at $q_x = 2k_F$ at a certain critical temperature, indicating the occurrence of the Peierls structural transition.

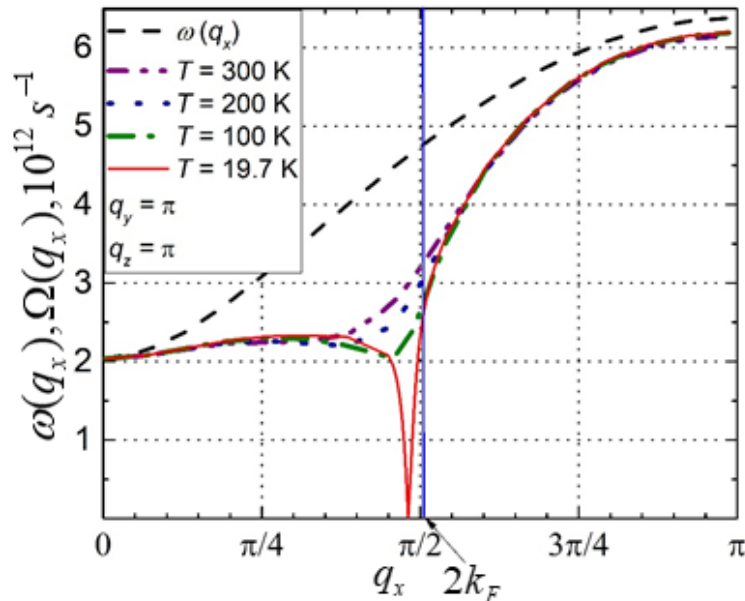


Figure 6. The renormalized phonon spectrum $\Omega(q_x)$ is plotted for several temperatures using $\gamma_1 = 1.7$, where the dashed curve represents the free-phonon spectrum [22]. The specific parameters used for this plot are $k_F = 0.502 \cdot \pi / 2$, $D = 1.055 \text{ eV} \cdot \text{\AA}^{-1}$.

However, our calculations show that the renormalized phonon frequency reaches zero at a value $q_x \neq 2k_F$. This discrepancy arises from the deviation of k_F from $\pi/4$. Figure 5 represents the case where $q_y = 0$ and $q_z = 0$, with the dimensionless Fermi momentum $k_F = 0.502 \cdot \pi / 2$. The parameter D is set to $1.057 \text{ eV} \cdot \text{\AA}^{-1}$, and the interaction between TTT chains is neglected. In this scenario, the Peierls transition begins at $T = 90 \text{ K}$, at which point the electrical conductivity reaches its maximum. As the temperature decreases, the electrical conductivity gradually diminishes. This transition temperature value is very close to that presented in the experimental curve in [16].

Figure 6 corresponds to the case where the interaction between TTT chains is included ($q_y = \pi, q_z = \pi$), with $D = 1.055 \text{ eV} \cdot \text{\AA}^{-1}$ and $k_F = 0.502 \cdot \pi / 2$. As shown in the figure, the transition is completed at $T = 19.7 \text{ K}$. It is evident from Figure 1 that the electrical conductivity drops sharply and approaches zero at approximately $T \sim 20 \text{ K}$. Furthermore, it is observed that the parameter D decreases, indicating a weaker interaction between holes and defects in this case. Also, it was observed that the interchain interaction, reduces the transition temperature. The interactions between holes and phonons, as well as those with structural defects, lead to a reduction in $\Omega(q_x)$ and a decrease in the sound velocity over a wide temperature range.

4. Conclusions

A more complete physical model for nanostructured organic crystals of tetratitetracene-iodide was developed and applied for numerical calculations of electrical conductivity, electronic thermal conductivity and thermopower (Seebeck coefficient). The calculations were performed for a range of temperatures from 20 K to 400 K , revealing a strange behavior near $T = 90 \text{ K}$, explained by the metal-dielectric transition of the Peierls type. For temperatures near to 300 K , the physical model is completely applicable for calculating the thermoelectric properties of the crystal. It was demonstrated by numerical calculations that when manipulating with charge carrier concentration, which leads to the variation of the Fermi level, the thermopower can be increased significantly, with a slow diminution in electrical conductivity. Moreover, the thermal conductivity carried by electrons decreases also, which indicates that in such materials a high value of thermoelectric figure-of-merit can be obtained.

Acknowledgments: This work was funded by Ministry of Education and Research from the Republic of Moldova (institutional subprogram #02.04.02) "Development of technologies and investigation of the properties of layered semiconductor compounds, hybrid nanostructures and laser sources."

Conflicts of Interest: The authors declare no conflict of interest.

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Citation: Andronic, S.; Sanduleac, I. TTT₂I₃ organic crystals – a promising platform for low-dimensional thermoelectric devices. *Journal of Engineering Science*. 2025, XXXII (4), pp. 7-18. [https://doi.org/10.52326/jes.utm.2025.32\(4\).01](https://doi.org/10.52326/jes.utm.2025.32(4).01).

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Submission of manuscripts:

jes@meridian.utm.md

[https://doi.org/10.52326/jes.utm.2025.32\(4\).02](https://doi.org/10.52326/jes.utm.2025.32(4).02)
UDC 621.791



PRACTICAL ASPECTS OF PART RECONDITIONING BY WELDING

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Received: 11. 21. 2025

Accepted: 12. 27. 2025

Abstract. The restoration of machine functionality through the reconditioning of worn parts is an effective method of reducing repair costs and time, eliminating the need to purchase replacement parts and, in some cases, extending the service life of components. The process includes stages such as cleaning, defect identification, determination of material properties, and the selection of suitable reconditioning methods and materials. Due to its versatility and low cost, welding is used in more than 80% of metal reconditioning cases. The article presents the stages, procedures, and technological parameters of part reconditioning by welding, the criteria for selecting the appropriate processes, and the analysis of possible defects, while also providing recommendations for their prevention.

Keywords: *reconditioning, welding, surfacing, defects, thermal cycle, technological parameters.*

Rezumat. Restabilirea funcționalității mașinilor prin recondiționarea pieselor uzate este o metodă eficientă de reducere a costurilor și duratei de reparație, eliminând necesitatea procurării pieselor de schimb și, în unele cazuri, prelungind durata de funcționare a componentelor. Procesul include etape precum curățarea, identificarea defectelor, determinarea proprietăților materialului, alegerea metodei și a materialelor de recondiționare. Datorită versatilității și costurilor reduse, sudarea este utilizată în peste 80% din cazurile de recondiționare metalică. Articolul prezintă etapele, procedurile și parametrii tehnologici ai recondiționării pieselor prin sudare, criteriile de alegere a procedeelor și analiza defectelor posibile, oferind totodată recomandări pentru prevenirea acestora. Articolul dat tratează detaliat etapele și procedurile recondiționării pieselor prin sudare, regulile alegerii metodelor de sudare, defectele posibile și căile de prevenire a lor.

Cuvinte cheie: *recondiționare, sudare, încărcare, defecte, ciclul termic, parametri tehnologici.*

1. Introduction

The reconditioning of worn industrial parts is an increasingly important solution for extending the service life of equipment and reducing maintenance costs. Statistics show that approximately 85% of machine failures originate from wear of contact surfaces rather than structural damage [1, 2], which highlights the practical relevance of reconditioning technologies.

International experience (Bosch, DelcoRemi, Cardone Industries, TRW) demonstrates that reconditioned parts represent up to 40% of all replacement components in highly industrialized economies [3]. From an economic perspective, reconditioning requires only 10–25% of the cost of a new part and reduces execution time by approximately 45% [4], also decreasing material consumption by nearly 80%. Environmentally, the process significantly reduces metal waste and CO₂ emissions [5]. Despite its advantages, welding-based reconditioning presents challenges related to thermal impact, residual stresses, geometric distortion, and the occurrence of defects such as cracks, porosity, or slag inclusions. These issues mainly arise from incorrect parameter selection, improper surface preparation, or inadequate post-weld treatment.

The aim of this article is to analyze the stages, technological parameters, and procedures used in welding-based part reconditioning, emphasizing the criteria for selecting appropriate processes and the typical defects that may occur. To achieve this aim, the following objectives were established:

1. To review the stages of part reconditioning by welding and describe the preparation and execution steps.
2. To present the main welding processes applicable to reconditioning and the technological parameters influencing the quality of the deposited layer.
3. To analyze typical defects occurring during reconditioning and identify their causes.
4. To evaluate the influence of thermal cycles and welding parameters on structural integrity and durability.

2. Materials and Methods

2.1. General Stages of Reconditioning

The reconditioning process consists of several well-defined stages [6–9]:

1. Cleaning and inspection– removal of impurities and detection of cracks, voids, or inclusions.
2. Surface preparation– mechanical processing (e.g., chamfering, milling), protection of adjacent areas (Figures 1-3).

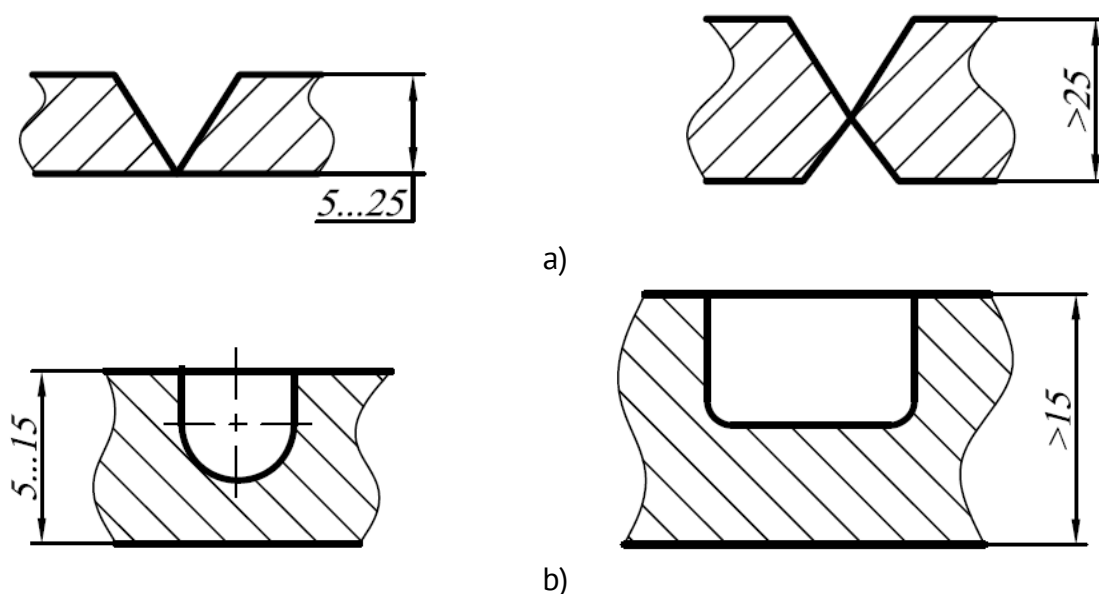


Figure 1. Preparation of weld joints for crack removal:

a) for removing single penetrated cracks; b) for removing multiple embedded cracks.

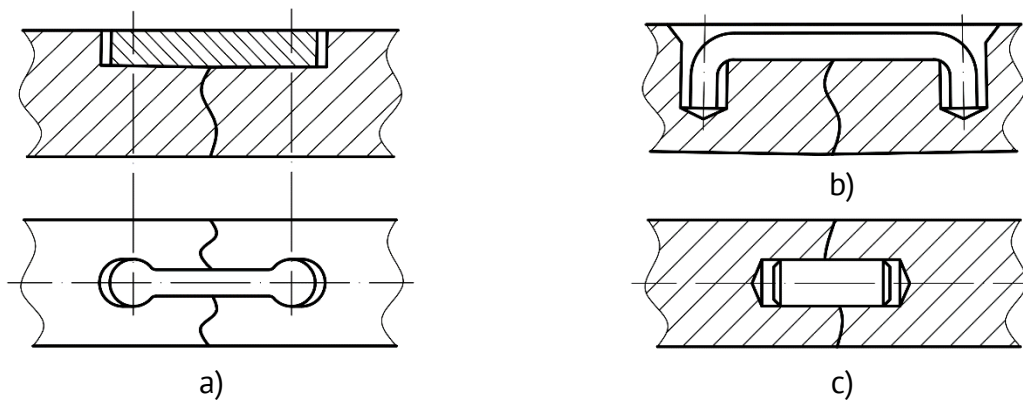


Figure 2. Introduction of additional elements: a) plates; b) clamps; c) pins.

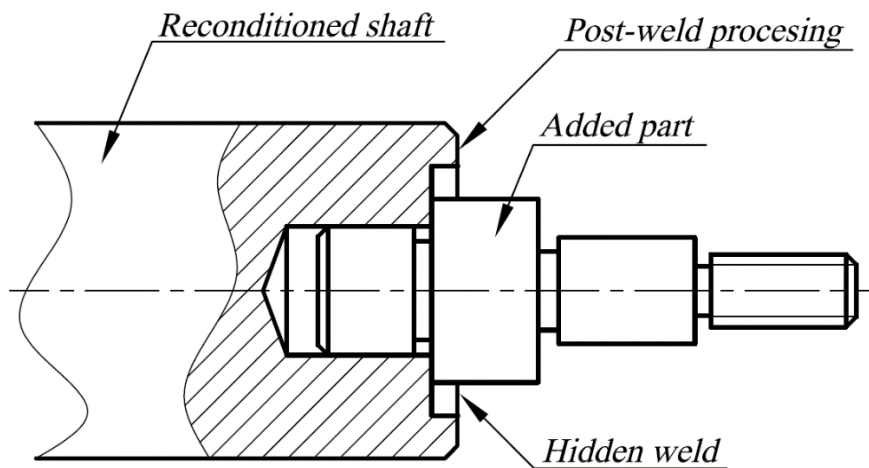


Figure 3. Shaft reconditioning by replacing a broken section.

3. Selection of welding method and filler metal – based on material type and defect characteristics.
4. Welding/Surfacing– gradual deposition of thin layers to minimize internal stresses [10], (Figures 4 and 5).
5. Post-welding treatments– thermal processing to reduce stresses and mechanical machining to restore geometry [11].
6. Final inspection– non-destructive testing of deposited layers.

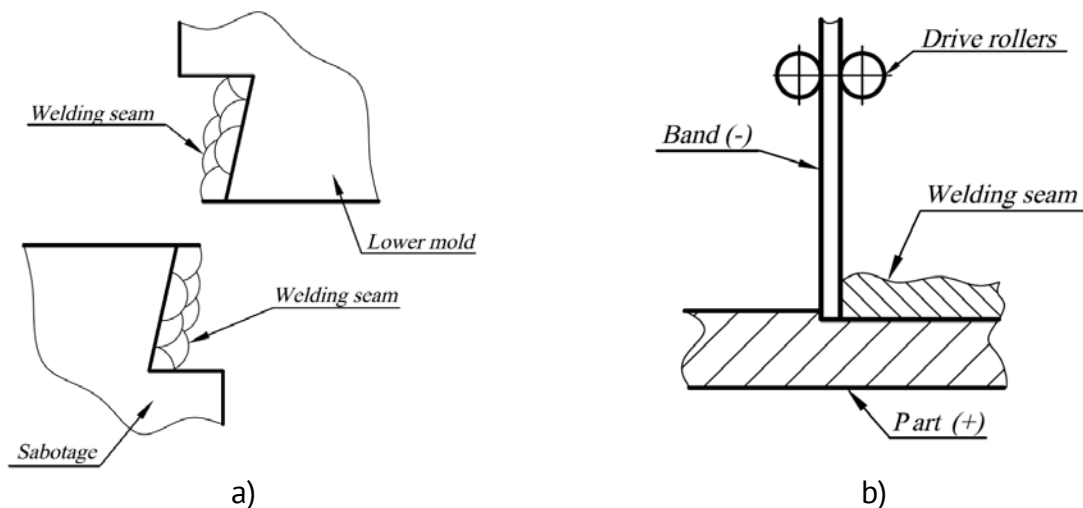


Figure 4. Surface reconditioning by overlaying: a) with wire; b) with strip.

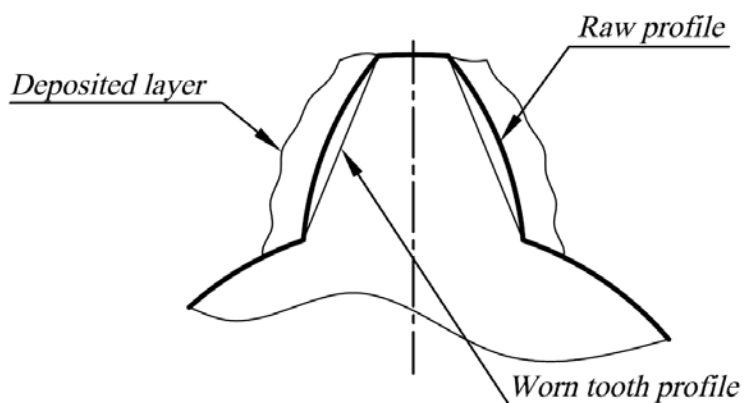


Figure 5. Gear tooth surface reconditioning by mechanical processing.

2.2. Classification of Parts and Functional Requirements

Based on operating conditions, reconditioned parts fall into several categories [12–14]:

- Metal-to-metal friction parts (shafts, gears): require hardness similar to base metal [15,16].
- Abrasive-load parts: require chromium-rich filler materials.
- High-temperature components (dies, rolling mill rolls): require heat-resistant alloys.
- Sliding bearings: copper or cast-iron based filler materials.
- Cutting tools: high-alloy steels.

2.3. Welding Methods Used in Reconditioning

The choice of welding process depends on geometry, thickness, and required mechanical properties [17–21], (Table 1).

Table 1

Welding Methods in Reconditioning	
Method	Typical use
Shielded metal arc welding (SMAW)	Simple, economic; thickness > 50 mm
Submerged arc welding (SAW)	Cylindrical parts $D > 200$ mm
Gas metal arc welding (GMAW)/CO ₂	Thin parts (1–4 mm), high productivity
Tungsten inert gas (TIG)	High-quality surfacing, fine structure

2.4. Calculation Formulas and Technological Parameters

When the electric arc is initiated, a molten metal pool forms, and as the electrode moves, it leaves behind a weld bead characterized by its length (L), width (B), height (h), and penetration depth (H) (Figure 6) [17,18].

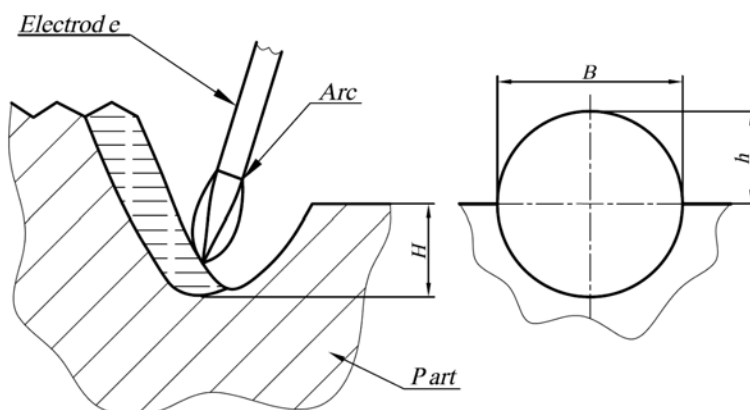


Figure 6. General view of the weld pool.

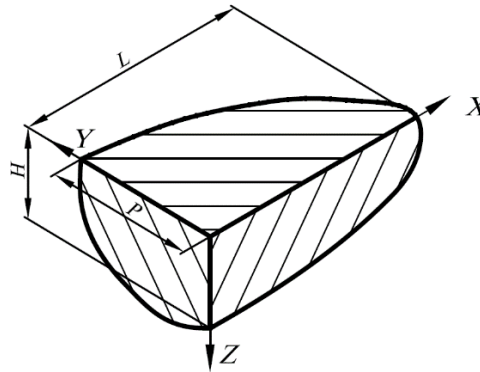


Figure 7. Shape of the weld pool.

For engineering calculations, it is sufficient to approximate the weld pool volume as an ellipsoid with dimensions L (length), P (width), and H (depth), aligned with a coordinate system X, Y, Z (Figure 7).

According to the simplified calculation, the volume of the weld pool is determined using the following relationship:

$$V = L \times P \times H; \quad (1)$$

The length of the weld pool can be determined using the following relationship:

$$L = K \frac{(I \times U)^2}{V_s \times G^2} \quad (2)$$

The depth (which is approximately equal to the height H):

$$H = K \frac{I \times U}{V_s \times G}, \quad (3)$$

where: K is the welding parameters coefficient, taking into account the type of current, polarity, and electrode (wire) diameter; I and U are the welding current intensity and voltage; V_s is the welding speed; and G is the thickness of the welded material.

Depending on the welding voltage, and welding wire diameter 1,2 mm, the welding current value can be selected from the nomogram shown in Figure 8. The current is adjusted more precisely after the test passes are made.

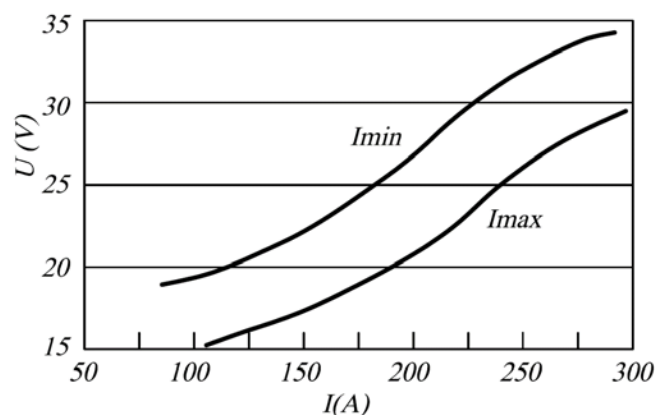


Figure 8. Welding current nomogram as a function of voltage for a wire with a diameter of 1.2 mm.

The mass of molten metal per unit of time effectively represents the welding productivity. Increasing the welding current and the wire feed speed leads to an increase in this productivity (Figure 9).

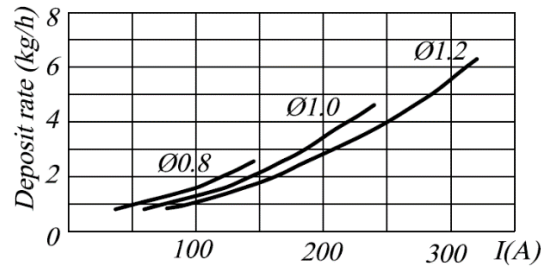


Figure 9. Deposition rate as a function of welding current and wire diameter.

2.5. Thermal Analysis of the Welding Process

As already mentioned, the thermal influence on the parts can lead to changes in the properties of the base material. Therefore, it is important to know the heating speed and temperature of the reconditioned layer of the part, as well as the cooling time until the initial temperature is reached.

The thermal cycle during welding can be characterized by the graph shown below (Figure 10).

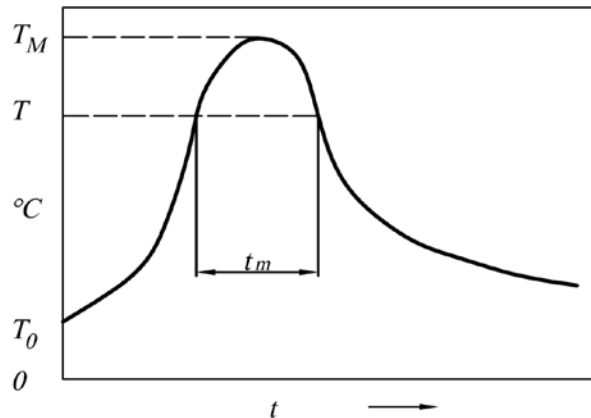


Figure 10. Thermal cycle during welding. T_M is the maximum temperature, T is the holding temperature, T_0 is the initial temperature (500-600 °C), and t_m is the time for holding the initial temperature.

In the case of electric welding, the cooling rate is determined using the following relation:

$$V_R = \lambda \times (T - T_0 \times V_S) / q, \quad (4)$$

where: q is the effective power of the welding source, obtained by subtracting the arc power q_a and the energy lost through conduction and convection q_p :

$$q = q_a - q_p \quad (5)$$

The analytical determination of energy losses is quite difficult, so for practical calculations, a reduction of 8...15% is assumed:

$$q = (0.85 \dots 0.92) \times \frac{U \times I}{60 \times V_S} \quad (6)$$

The time for holding the initial temperature:

$$t_m = \frac{2K \times q}{V_S \times \lambda \times (T_M - T)}, \quad (7)$$

where: λ is the coefficient that takes into account the volume of the material in the part.

If the holding time is exceeded, welding parameters are modified to avoid potential defects. Defects in the form of slag inclusions can be caused by factors such as excessive humidity, contaminants in the welding area, or incorrect arc length.

3. Results and Discussions

3.1 Influence of Welding Parameters on Bead Geometry

Using the formulas presented in Section 2.4, the influence of current I , voltage U and travel speed V_s on bead geometry was evaluated.

The calculated examples show that:

Increasing welding current from 120A to 160A increases the bead depth H by approximately 18–25%, which confirms the direct proportionality described by formula (3).

A 20% increase in travel speed reduces bead width and penetration by 12–15%, increasing the risk of lack of fusion.

For thin parts (1–4 mm), the recommended parameter interval (Figure 8) corresponds to stable values of L and H , reducing overheating and distortion.

These correlations support the practical rule that stable and moderate heat input ensures uniform bead formation and fewer defects.

3.2 Thermal Cycle Evaluation and Structural Effects

The thermal cycle computed using relations (5) – (7) confirms that:

The cooling rate V_R increases significantly when welding speed is raised.

For example, doubling travel speed increases V_R by about 70%, which may cause excessive hardness in alloy steels.

The effective heat input q varies between 0.42 and 0.55 kJ/mm for the analyzed values of U , I and V_s .

Values above this interval correlate with:

- coarsened microstructure,
- increased deformation,
- formation of tension zones promoting cracking.

Holding time t_m in the critical temperature range (600–800 °C) decreases with higher thermal conductivity λ and higher welding speeds.

These results support the practical necessity of controlling interphases temperature, preheating and bead sequencing for ensuring structural stability.

3.3 Comparative Performance of Welding Methods

Analysis of the main processes (SMAW, SAW, GMAW/CO₂, TIG) using the data in Section 2.3 shows (Table 2):

Table 2

Comparative Performance of Welding Methods					
Process	Advantages		Limitations		
SMAW	Flexibility, low equipment cost.		Higher slag probability.	slag	inclusion
SAW	Ideal for cylindrical parts; deep penetration.		Higher slag probability.	slag	inclusion

Continuation Table 2

GMAW/CO ₂	Highest productivity; uniform bead; low porosity.	Sensitive to rust/impurities.
TIG	Excellent surface finish; minimal dilution.	Low deposition rate.

Note: SMAW - shielded metal arc welding; SAW - submerged arc welding; GMAW - gas metal arc welding; TIG - tungsten inert gas.

Experimental and industrial data [7, 10, 23, 24] indicate that GMAW and TIG produce reconditioning layers with fewer discontinuities and up to 30% lower residual stresses compared to SMAW.

3.4 Analysis of Defects and their Causes

Correlating industrial observations with parameter calculations, the following trends were identified:

Cracks appear at high cooling rates (low q , high V_s) and insufficient preheating.

Porosity is primarily caused by:

- contamination (oil, rust),
- improper shielding gas conditions for GMAW/TIG.

Slag inclusions occur mainly in SMAW and SAW when bead overlaps are insufficient.

Geometric distortion correlates directly with excessive heat input and irregular bead sequencing.

A properly optimized process—correct I , U , V_s , adequate preheating and post-weld treatment—reduces defect occurrence by up to 40–60%, as indicated by literature and industrial reports.

3.5 Environmental and Economic Considerations

The results confirm that:

Reconditioning reduces CO₂ emissions by over 60% compared to producing new parts.

Material consumption is reduced by approximately 75–80%, especially for large shafts, housings and gears.

For typical industrial components, reconditioning costs represent only 10–25% of the cost of a new part.

Thus, the analyzed technological parameters directly affect economic efficiency, because lower defect rates imply fewer reworks and less material waste.

4. Conclusions

1. The analytical correlations obtained from bead geometry and thermal cycle formulas confirm the decisive role of current, voltage and travel speed in determining penetration depth, bead width and cooling rate. Proper parameter selection reduces defect probability and improves structural integrity.

2. Thermal cycle evaluation demonstrates that excessive heat input leads to microstructural degradation and geometric distortion, while optimal heat input ensures dimensional stability and uniform hardness in the deposited layer.

3. Comparative process analysis shows that GMAW and TIG offer the best quality for reconditioning, producing smooth layers, minimal porosity and lower residual stresses, while SMAW and SAW remain suitable for thick or large cylindrical parts.

4. The main defects—cracks, slag inclusions, porosity and local deformations—are strongly correlated with improper parameter selection, insufficient surface preparation and inadequate preheating. Optimizing these factors reduces defect occurrence by up to 60%.

5. Environmental and economic evaluation confirms the high efficiency of welding-based reconditioning, with material savings of 75–80%, CO₂ reduction above 60%, and total costs representing only 10–25% of manufacturing new parts.

Conflicts of interest: The author declares no conflict of interest.

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Citation: Botez, A. Practical aspects of part reconditioning by welding. *Journal of Engineering Science.* 2025, XXXII (4), pp. 19-28. [https://doi.org/10.52326/jes.utm.2025.32\(4\).02](https://doi.org/10.52326/jes.utm.2025.32(4).02) .

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Submission of manuscripts:

jes@meridian.utm.md

[https://doi.org/10.52326/jes.utm.2025.32\(4\).03](https://doi.org/10.52326/jes.utm.2025.32(4).03)
UDC 681.586:61:004.738.5



SENSING LAYER TECHNOLOGIES AND NETWORKING ON THE INTERNET OF MEDICAL THINGS

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Received: 12. 04. 2025

Accepted: 12. 27. 2025

Abstract. Internet of Medical Things (IoMT) apply engineering principles and design to medicine and biology to improve healthcare outcomes. This interdisciplinary field bridges engineering and medicine by integrating the innovative and analytical strengths of engineering with medical and biological expertise, resulting in more effective approaches to diagnosing, monitoring, and treating patients. This article focuses on exploring the key components and strategies of the sensing layer in the IoMT, including medical devices, smart sensors, biomedical sensors, wireless technologies, communication protocols, and the associated challenges of reliability and security. Several critical factors must be taken into account when designing IoMT sensing layer networks, such as body movement, temperature variation, energy efficiency, transmission range and heterogeneous environments.

Keywords: *Internet of Medical Things, smart sensors, biosensors, sensor layer protocols, wireless sensor body network.*

Rezumat. Internetul Lucrurilor Medicale (ILM) aplică principii ingineresti de proiectare în medicină și biologie pentru a îmbunătăți rezultatele asistenței medicale. Acest domeniu interdisciplinar face legătura între inginerie și medicină prin integrarea punctelor forte inovatoare și analitice ale ingineriei cu expertiza medicală și biologică, rezultând abordări mai eficiente pentru diagnosticarea, monitorizarea și tratarea pacienților. Acest articol se concentrează pe explorarea componentelor și strategiilor cheie ale nivelului de detectare în ILM, inclusiv dispozitive medicale, senzori inteligenți, senzori biomedicali, tehnologii wireless, protocoale de comunicație și provocările asociate legate de fiabilitate și securitate. La proiectarea stratului de percepție al ILM trebuie luați în considerare mai mulți factori critici, cum ar fi mișcarea corpului, variația temperaturii, eficiența energetică, distanța de transmisie și mediile eterogene.

Cuvinte cheie: *Internetul Lucrurilor Medicale, senzori inteligenți, biosenzori, protocoale ale stratului de percepție, rețea corporală de senzori fără de fir*

1. Introduction

Biomedical systems apply engineering concepts and design principles to the fields of medicine and biology to improve healthcare outcomes. This discipline seeks to bridge the gap between engineering and medicine by merging the innovative and analytical capabilities of engineering with expertise in medical and biological sciences, resulting in more advanced techniques for diagnosis, monitoring, and treatment. These systems involve a wide range of applications, including medical imaging, prosthetics, implantable devices, and healthcare information systems. Additionally, the integration of biomedical systems with artificial intelligence represents a growing and dynamic area of research.

The advancement of biomedical systems typically requires a multidisciplinary collaboration, drawing on knowledge from medicine, engineering, and computer science. Progress in this field holds the promise of enhancing patient care and transforming the delivery of healthcare services.

One of the most effective and beneficial methods to put biomedical systems into practice is by using established and widely recognized approaches such as Wireless Sensor Networks. These networks gain additional significance when we take into account their connection with the Internet of Things (IoT) and their application in the healthcare domain [1]. This combination allows for greater connectivity and data exchange between medical devices and applications, leading to improved health monitoring and patient care. By harnessing these technologies, healthcare providers can collect and analyze patient data more efficiently, enabling timely interventions and better management of health conditions. Overall, making use of along with the IoT [2] represents a significant advancement in the implementation of biomedical systems, paving the way for innovative solutions in health care.

The Internet of Medical Things (IoMT) [3], a specific application of IoT in biomedicine, plays an important role in the advancement of biomedical systems and presents various advantages for the healthcare sector. By integrating the core technologies and functionalities of the IoT into healthcare, IoMT creates considerable opportunities to enhance patient care, streamline clinical processes, and support healthcare providers in making informed decisions based on data.

The IoMT defines a network of interconnected medical sensors, devices, applications, and services that utilize internet-enabled technologies to facilitate real-time health monitoring and management.

2. Sensing Layer as Element of IoMT Architecture

When creating solutions for the IoMT there are several critical elements that must be thoughtfully addressed to achieve a successful outcome. A primary consideration is the security of both the devices and the data they process since these products frequently gather sensitive information from patients. Another vital element is interoperability which pertains to the ability of various devices to communicate and collaborate effectively within a healthcare framework. Additionally, usability plays a key role because it is important for devices to be accessible for both medical practitioners and patients ensuring they can be used easily and efficiently.

In addition, it is crucial to assess the reliability and sturdiness of the devices as they must operate correctly in diverse healthcare settings and scenarios. This means ensuring they can endure regular use and any potential physical challenges they might encounter. Finally

adhering to regulatory standards is another important consideration since all medical devices are required to follow specific regulations and guidelines established by health organizations to guarantee safety and efficacy. In summary these elements are essential for the effective design and implementation of IoMT solutions within the healthcare sector.

Aspects to consider in the design of IoMT:

- *User mobility.* One challenge lies in maintaining communication strength when the network topology changes due to a user's movement, as sensor devices and medical equipment may move with the user. To tackle this unpredictable issue, the IoMT routing protocol should be adaptable, ensuring robust communication while not compromising quality.
- *Energy consumption optimization.* Energy efficiency plays a vital role in influencing the size, operational lifespan, and overall usability of a device. The used by IoMT routing protocol should, thus, focus on optimizing energy consumption to ensure adequate device performance. For implantable devices, a battery life of at least 10 to 15 years is necessary to avoid frequent surgeries and their associated costs. Wearable devices depend on frequent battery replacements to maintain usability.
- *Connectivity and transmission range.* Shorter transmission ranges may lead to disconnections and reorganization among sensor nodes in an IoMT system due to body movements.
- *Heterogeneous environment.* The IoMT routing protocol for Wireless Body Sensor Networks (WBSNs) should be capable of handling heterogeneous environments common in WBSN applications.
- *Quality of Service (QoS).* In real-time applications, like ECG, data loss and delays can be detrimental. Consequently, QoS requirements should be addressed to ensure efficient handling of such scenarios, especially for implanted smart sensors with fixed memory and computational capabilities.
- *Interoperability and compatibility* are essential within the IoMT framework to enable seamless communication and data sharing among different healthcare devices and systems. The use of standardized communication protocols and health data exchange formats is key to achieving effective interaction between diverse IoMT components.
- *User interfaces and applications* serve as access points for healthcare professionals, caregivers, and patients to interact with the IoMT system. Tools like mobile applications, web platforms, and specialized medical software support real-time health monitoring, data visualization, and remote healthcare services.
- *Security and privacy* are ensured through robust measures including encryption, authentication, and access control, which protect sensitive patient information from unauthorized access. While cloud storage enables quick and accurate responses from IoMT devices, it also introduces potential risks such as data breaches or misuse.

The structure of the Internet of Medical Things consists of a complex and detailed architecture. This system is essential for connecting an array of medical devices, specific software solutions, and a variety of healthcare platforms. The main objective of this integration is to greatly enhance patient care while optimizing different healthcare processes. Through this technological and healthcare fusion, IoMT strives to facilitate smoother operations that are advantageous for both patients and healthcare professionals. Ultimately, this integrated network is designed to raise the quality of medical care and optimize the efficiency and effectiveness of healthcare delivery. In the simplest case there are four primary

elements in IoMT: medical devices and sensors, connectivity, data management and application, respectively four layers in the IoMT architecture: sensing, networking, cloud infrastructure and application and decision-making layers as shown in Figure 1.

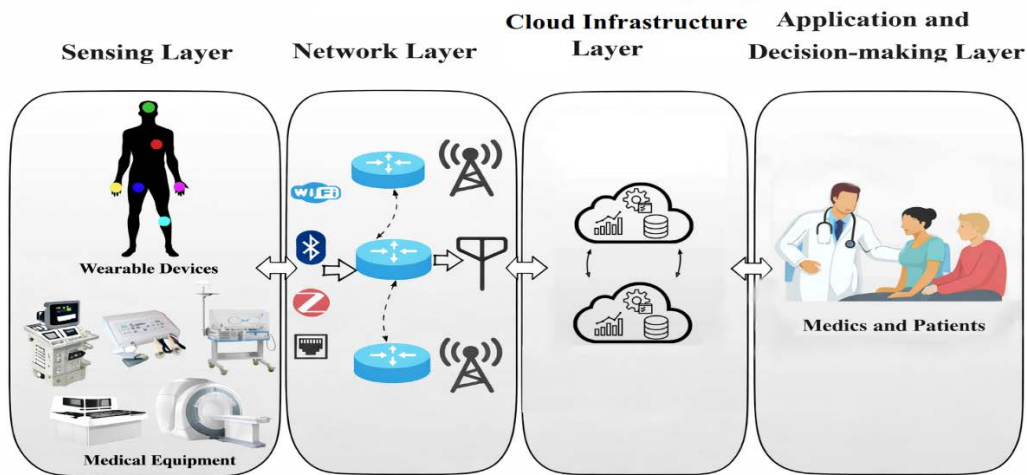


Figure 1. Four layers IoMT architecture.

The sensing layer, also referred to as the perception layer, forms the foundation of the IoMT architecture and plays a crucial role in collecting and organizing data from various physical devices. Its importance is particularly evident in IoMT systems, as it directly interacts with medical sensors and equipment. In this context, the sensing layer captures information from devices like heart rate monitors, glucose meters, and various wearable or implantable technologies. It acts as a critical bridge between the physical medical environment and the digital domain where data is processed and analyzed.

Sensors are instruments that detect physical, chemical, or biological signals and enable the measurement and recording of those signals. For instance, tactile sensors, often made from piezoelectric materials, produce voltage in response to touch, pressure, bending or temperature changes.

Actuators, on the other hand, perform specific actions based on data received from sensors or user interaction. An example is an actuator with an integrated reservoir and pump that delivers the appropriate dose of insulin to a diabetic patient according to glucose level readings.

3. Medical Sensors and Devices

The IoMT sensing layer operates with various devices and different kinds of sensors, which can be classified as wearable devices and medical equipment

Wearable devices are made to be worn or attached to the body to track health and provide important medical data. Depending on their use, these devices can be divided into consumer wearables, clinical wearables, implantable devices, and ambient devices.

Consumer wearables are mainly used for tracking body signals in real-time, like blood pressure and heart activity, as well as behaviors such as movement and speed.

An implantable device is a medical instrument inserted into a patient's body through surgery. Notable examples of advanced implantable technology include pacemakers and digital pills, also referred to as smart pills. Modern ingestible devices, such as capsule

endoscopes, are capable of gathering gastrointestinal data over extended periods outside clinical settings, with the potential for additional sensors to enhance data collection.

Ambient devices include a range of environmental sensors such as motion detectors, contact switches, beam-break sensors, and pressure mats used to monitor environmental conditions or detect changes in locations like homes and workplaces.

Medical equipment refers to tools for medical imaging, biochemistry analysis, and bedside monitoring used to detect diseases in hospitals.

Medical imaging equipment consists of diagnostic tools that provide information about the body's structure using techniques such as MRI, CT scans, and ultrasounds. The images produced are usually large and don't need to be processed in real-time.

The biochemistry analyzer is used to identify different biochemical markers in the body, and it produces smaller amounts of data with less need for immediate processing.

The bedside monitor is used to continuously watch a patient's body readings in hospital rooms, relying on real-time data to quickly alert staff to any urgent situations.

One of the most important groups of sensors that we can find in various applications is known as biosensors. These devices are specially designed to detect and measure biological changes or substances in a given environment (see the Figure 2). A biosensor is a device designed to detect biological or chemical reactions by producing signals that correspond to the concentration of a specific analyte involved in the reaction [5]. These sensors are used in various applications, including disease tracking, pharmaceutical research, and the identification of environmental pollutants, harmful microorganisms, or disease-related markers present in bodily fluids such as blood, urine, saliva and sweat.

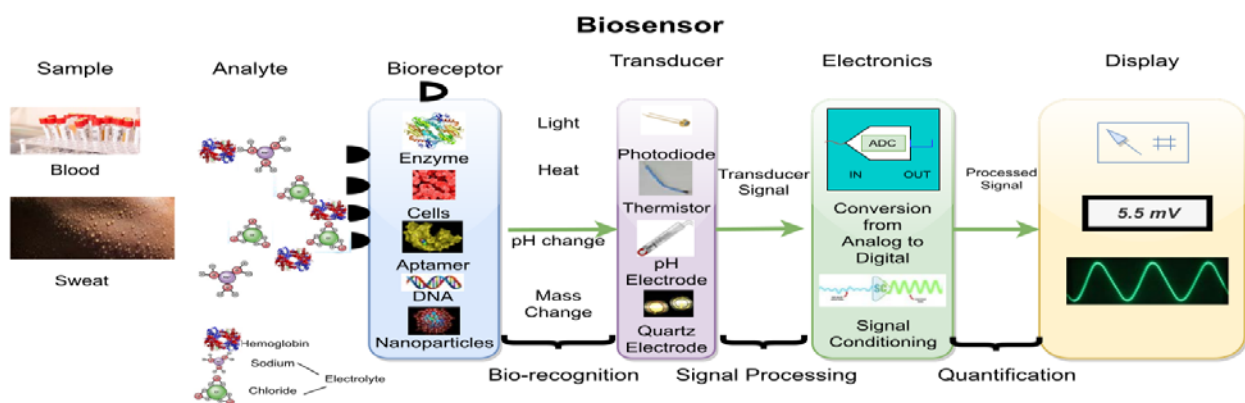


Figure 2. Schematic representation of a biosensor [6].

All body sensors [7,8] are integrated into a wireless body sensor network, which can be defined as a specialized form of mobile personal area network. This network allows multiple sensors, strategically embedded or worn on the body, to communicate without the need for wires or cables. The wireless aspect of this network enables the sensors to transmit vital health data in real-time, facilitating continuous monitoring of physiological parameters. By creating this interconnected system, it becomes possible for the sensors to work collaboratively, providing comprehensive insights into an individual's health status while also enhancing the overall effectiveness of medical interventions.

Each body sensor needs to connect wirelessly to a specific device that is referred as mobile multiprotocol Personal Access Point (PAP) as shown in Figure 3. This important device is responsible for collecting all the information from these sensors. Once it gathers the data,

PAP transmits this information to the next level of the IoMT system, ensuring that the collected data are effectively shared and utilized in a connected healthcare network.

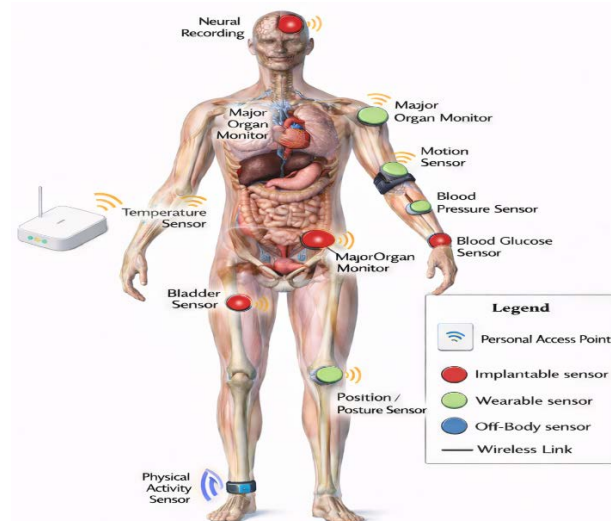


Figure 3. Wearable wireless body sensor network.

4. IoMT Sensing Layer Networking and Communications

The sensor layer is the essential component of the IoMT system, tasked with collecting data from patients using various sensors and transmitting this information to the network layer for further transmission and processing. It includes hardware components like sensors, controllers, and actuators, which allow for accurate detection of health-related parameters.

The sensor layer consists of two main sublayers: data-processing and data-entry. The primary role of the data-processing sublayer is to analyze the incoming data by employing various signal acquisition and medical perception technologies. The collected data are then sent to the next stage through the data-entry sublayer, which uses short-range and lower power transmission methods. The main technologies and protocols used on this layer are described below.

Infrared Data Association (IrDA). Infrared technology developed by the Infrared Data Association (IrDA) [10] enables short-range communication between devices using infrared light. IrDA establishes standards for wireless infrared communication that operates over a few meters through focused infrared beams. The IrDA protocol consists of three key layers: the physical layer, the link access protocol (IrLAP), and the link management protocol (IrLMP). IrLAP is responsible for establishing a basic connection between two devices, using the High-level Data Link Control standard to manage connections and data transmission. IrLMP allows different components within IrDA devices to communicate simultaneously through a single IrLAP connection and supports service discovery between devices. The device discovery process identifies the address of nearby devices, their IrLAP version, and additional discovery data defined by the IrLMP layer. In the healthcare field, IrDA technology is applied in devices such as thermometers and infrared cameras, particularly in temperature sensors. Additionally, infrared thermal imaging is used to capture body images and measure surface temperature.

Radio Frequency Identification (RFID) [11] is a wireless technology that helps identify things using radio signals for short-distance communication. The two main parts of RFID are the RFID reader and the RF tag, which is the device that responds to the signals. The RFID reader can store data and read information from a distance. When the RF tag gets a signal from the reader, it replies with identification details and other information. This identification

usually includes a unique serial number, and the extra information might include product details like stock numbers and production dates. There are two main types of RFID tags: active tags and passive tags. Active tags have their own power source and work on higher frequency bands, making them more expensive. Passive tags, on the other hand, operate at lower frequencies and do not have a power supply inside. The common frequency ranges for RF communications are: (a) Low Frequency (LF) at 125 kHz, (b) High Frequency (HF) at 13.56 MHz, (c) Ultra High Frequency (UHF) from 433 MHz to 860–960 MHz, and (d) Microwave frequencies at 2.45 GHz and 5.8 GHz. RFID tags that are placed inside or near a patient's body are important for creating healthcare systems that do not bother the patient. Passive RFID tags can also be used to monitor the patient's surroundings, helping to spot changes in physical or chemical conditions. RFID technology is also used for controlling access; for example, hospitals might use RFID cards to secure entry. Another use of RFID involves tags and sensors that monitor temperature closely for storing medications, ensuring the right temperature is maintained for different drugs.

Near Field Communication (NFC) [12] is a way for devices to communicate over very short distances, usually just a few centimeters. NFC is set by standards called ECMA-340 and ISO/IEC 18092. It works by using magnetic fields to connect the sending and receiving devices and operates at a frequency of 13.56 MHz, allowing data to transfer at speeds up to 424 Kbps. Like RFID, NFC can work in both active and passive ways. In passive mode, one device creates a radio field, which powers the other device using the energy from the first device. NFC makes it easy and cheap to connect Internet of Things (IoT) devices.

Bluetooth/BLE is a popular wireless technology that follows the IEEE 802.15.1 standard. It is made for devices that use little power and are affordable, working in the 2.4 GHz frequency range. Bluetooth allows for Personal Area Networks (PAN) in a star layout, which means it uses less power, sets up quickly, and can connect many devices easily. It is great for short-distance data transfer between mobile devices. The range can vary by version; for example, version 2.1 can go up to 100 meters indoors, while version 5 (BLE) can reach up to 400 meters. Different Bluetooth versions can send data at speeds of up to 2 Mbps. The low-power version is called Bluetooth Low Energy (BLE) [13] or Bluetooth Smart. The latest version, BLE 5.0, also has a data speed of 2 Mbps and uses less power because it transmits data faster. This shorter transmission time helps devices work better together. When compared to other low-power wireless technologies like ZigBee or Z-Wave, BLE has the highest data speeds, even at its original rate of 1 Mbps. Bluetooth does need devices to pair, but it uses more power than NFC.

Bluetooth Low Energy (BLE) is commonly used in IoT networks involving battery-operated, energy-efficient, and cost-effective devices. It enables fast connectivity and supports straightforward device-to-device communication or star network configurations. BLE is particularly well-suited for IoMT applications that require short-range communication, rapid response times, and minimal data transmission. This makes it ideal for Human Interface Devices (HIDs), fitness trackers and portable medical equipment.

Z-Wave [14] is a smart home communication protocol that uses various techniques to keep your information safe and secure. It incorporates encryption, which is a method of scrambling data to protect it from unauthorized access. In addition to encryption, Z-Wave also employs behavior detection, which helps to identify and respond to unusual activities that might indicate a security threat. Another important feature is proximity security, which

adds an additional layer of protection by ensuring that devices communicate only when they are physically close to each other.

The protocol has a specific mechanism called the "Security" command class, which plays a crucial role in safeguarding the data. This mechanism is designed to maintain the confidentiality of information, which means that only authorized users can access the data. It also ensures source integrity, meaning that the information comes from a legitimate source, and data integrity, which guarantees that the information has not been altered or compromised in any way.

To achieve these security goals, Z-Wave uses special techniques for securing both the payloads, which are the actual data being sent, and the frames, which are the structures that carry the data. The data is protected through a powerful method called AES encryption, which stands for Advanced Encryption Standard. This encryption method is widely recognized for its effectiveness in keeping information secure. Z-Wave utilizes three shared keys to help manage encryption and decryption processes, ensuring a robust defense against unauthorized access and maintaining a high level of security for all communications within its network.

The Ultra-Wideband (UWB) protocol [15], based on the IEEE 802.15.3 standard, is specifically designed to support high-speed, short-range indoor wireless communication. Capable of delivering data rates between 110 and 480 Mbps, UWB is ideal for bandwidth-intensive applications such as audio and video streaming in home networks. Due to its wide bandwidth, UWB can act as a wireless alternative to high-speed cable connections like USB 2.0 and IEEE 1394. Regulatory frameworks for UWB vary worldwide. In the U.S., the Federal Communications Commission allocated spectrum for UWB in February 2002, while the European Commission established regulatory guidelines in February 2007 for all EU member states. UWB operates differently from conventional wireless technologies. Instead of modifying the power, frequency, or phase of a continuous wave, it transmits information by emitting radio pulses at specific time intervals across a broad frequency range, using techniques such as pulse-position modulation. Its low power consumption and precise signal timing make UWB well-suited for real-time applications in environments sensitive to radio frequency interference, such as hospitals

ZigBee [16] is a wireless communication protocol that adheres to the IEEE 802.15.4 standard. It is known for being low-cost, low-power, and low-speed, with a communication range of up to 100 meters and data rates ranging from 40 to 250 Kbps. Operating at 915 MHz and 2.4 GHz, ZigBee is intended for Personal Area Networks (PANs) and supports multiple network topologies, including star, tree, and mesh configurations, accommodating up to 65,000 nodes per network.

Widely used in IoT applications, ZigBee is particularly valuable in healthcare for connecting sensors to central coordinators and facilitating communication between multiple coordinators. In 2009, the ZigBee Alliance introduced the ZigBee Health Care public application profile, which was developed for assistive, non-invasive healthcare devices. Based on ZigBee Pro, this profile offers a standardized application layer protocol tailored for healthcare settings, ensuring interoperability among various medical and non-medical devices.

A summary of the current communication protocols and technologies used in the IoMT sensing layer is presented in Table 1.

Table 1

IoMT Sensing Layer Communication Protocols and Technologies [10-17]

Protocol/Technology	Frequency	Data Rate	Range	Power consumption
IrDA	850–900 nm	14.4 Kbps	1 m	Not available
RFID	13.56 MHz	106 – 424 Kbps	20 cm	Very low
NFC	13.56 MHz	106 – 424 Kbps	20 cm	Very low
Bluetooth/BLE	2.4 GHz	1, 2, 3 Mbps	80 – 90 m	Low
ZWave	800–900 MHz	40 Kbps	30 m	Low
UWB	3.1–10.6 GHz	53 – 480 Mbps	10 m	Very low
ZigBee	2.4 GHz	250 Kbps	100 m	Low

5. Recent Related Work on Technologies and Networking in the IoMT Sensing Layer

Recent research on the Internet of Medical Things (IoMT) has increasingly focused on the *networking aspects of the sensing (perception) layer*, which serves as the primary interface between physiological data acquisition and higher-level processing layers. This layer is composed of wearable, implantable, and ambient sensors that collect biomedical signals and transmit them to local gateways or coordinators under strict constraints related to energy consumption, latency, reliability, and security.

A predominant architectural model discussed in recent literature (2023–present) is the WBAN, which enables short-range communication among heterogeneous medical sensors deployed on or inside the human body. WBAN-based architectures are widely regarded as the most suitable networking paradigm for the IoMT sensing layer due to their support for low-power operation and patient mobility. Zhou *et al.* [18] provide a comprehensive survey of wireless communication solutions for IoMT, identifying WBANs as the cornerstone of sensing-layer connectivity in modern medical monitoring systems.

With respect to communication technologies, recent studies highlight the dominance of *short-range wireless protocols* at the sensing layer. *Bluetooth Low Energy* is consistently reported as the most prevalent technology, owing to its low energy consumption, adequate throughput for physiological data, and native support in consumer devices such as smartphones and smartwatches. BLE is particularly suitable for continuous health monitoring applications requiring long device lifetimes. In parallel, *ZigBee and IEEE 802.15.4-based protocols* remain relevant in multi-sensor scenarios, where mesh networking and scalability are required [19]. Additionally, *Ultra-Wideband* has gained attention for its high localization accuracy and robustness against interference, although its practical deployment in IoMT sensing systems remains limited due to higher complexity and power requirements.

Recent works also emphasize the increasing use of *hybrid and multi-tier communication architectures*. In these architectures, short-range wireless technologies are employed for intra-WBAN communication, while data forwarding to remote servers is handled by Wi-Fi or cellular-enabled gateways. He notes that such hybrid approaches improve scalability and quality of service while preserving energy efficiency at the sensor level [19]. This layered communication strategy reflects the growing heterogeneity of IoMT systems and the need for flexible networking solutions.

A notable trend in post-2023 literature is the integration of *local data pre-processing and edge intelligence* within sensing devices. Instead of transmitting raw biomedical signals, sensors increasingly perform signal conditioning, feature extraction, or event detection prior to communication. This approach significantly reduces network traffic and latency while extending sensor battery life, which is critical for long-term and dense sensing deployments. Zhou *et al.* [18] identify communication-aware sensing and edge-assisted data reduction as key enablers for scalable IoMT networks.

Security and interoperability remain critical challenges at the sensing layer. Recent studies indicate that conventional IoT security mechanisms are often inadequate for medical applications due to the sensitivity of health data and the life-critical nature of IoMT services. Consequently, current research focuses on lightweight encryption, authentication, and key management schemes tailored to resource-constrained sensing devices. Jayousi [20] further emphasizes that the lack of standardization across communication protocols hinders interoperability and large-scale deployment of IoMT systems, motivating ongoing efforts toward unified communication frameworks.

Emerging research directions also consider the indirect impact of *5G-enabled gateways and future 6G communication paradigms* on sensing-layer networking. While cellular technologies are not directly employed by sensing nodes, their support for ultra-reliable low-latency communication influences system-level design requirements and gateway-sensor interaction models, particularly for real-time and remote healthcare applications.

In summary, recent related work characterizes the IoMT sensing layer as a resource-constrained yet highly dynamic networking environment. Current trends emphasize WBAN-centric architectures, energy-efficient short-range wireless technologies, intelligent data reduction at the source, and enhanced security and interoperability mechanisms. These developments aim to support reliable, scalable, and patient-centric IoMT systems capable of meeting the stringent demands of next-generation digital healthcare.

6. Conclusions

The IoMT sensing layer allows healthcare professionals to make informed decisions using real-time data from physical devices, which is crucial for better patient care and health monitoring. It serves as a vital connection between the physical world of medical devices and sensors and the digital realm where data is processed and analyzed. By enabling continuous and non-invasive monitoring, the sensing layer contributes to early diagnosis, timely intervention, and personalized treatment plans.

The IoMT sensor layer's emerging communication technologies must utilize short-range and low-power approaches to minimize inter-body interference and reduce battery consumption. Protocols such as BLE, Zigbee, and NFC are being increasingly adopted due to their efficiency in maintaining reliable connectivity while preserving energy. These technologies ensure the seamless transmission of patient data from wearable or implantable devices to local hubs or gateways for further analysis.

As the sensor layer continues to evolve, emphasis must also be placed on ensuring data security and patient privacy. Implementing robust encryption protocols and authentication mechanisms is essential to prevent unauthorized access to sensitive health information. Together, these technological and structural enhancements reinforce the IoMT sensing layer as a foundational component of modern, intelligent healthcare ecosystems.

This research was conducted within the LifeTech project at the Technical University of Moldova, the results were presented in the Biomedical Section of the 13th International Conference on Electronics, Communications, and Computing (IC ECCO-2024), organized by the Technical University of Moldova, held in Chişinău, Moldova, on October 17–18, 2024.

Conflicts of Interest: The authors declare no conflict of interest.

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Citation: Moraru, V.; Gribincea, D.; Guțuleac, E. Sensing layer technologies and networking on the internet of medical things. *Journal of Engineering Science*. 2025, XXXII (4), pp. 29-40. [https://doi.org/10.52326/jes.utm.2025.32\(4\).03](https://doi.org/10.52326/jes.utm.2025.32(4).03).

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Submission of manuscripts:

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[https://doi.org/10.52326/jes.utm.2025.32\(4\).04](https://doi.org/10.52326/jes.utm.2025.32(4).04)
UDC 004.4:004.738.5:004.8



AUTOMATED WEB APPLICATION TESTING BASED ON ARTIFICIAL INTELLIGENCE

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Received: 11. 20. 2025

Accepted: 12. 18. 2025

Abstract. The evolution of web technologies and the increasing complexity of digital systems have transformed web application testing into an indispensable component of software quality assurance. Traditional automated testing frameworks – based on scripting and static data – remain effective but face scalability and adaptability challenges. This study hypothesizes that the integration of artificial intelligence (AI), particularly large language models (LLMs) and reinforcement learning, can significantly improve the efficiency and autonomy of testing processes. The paper aims to analyze comparatively traditional and AI-assisted methods for functional testing of web applications, using a synthesis of recent academic and industrial research. The analysis identifies the main advantages of AI-based testing, such as rapid test generation, extended coverage, and enhanced adaptability, while highlighting limitations related to transparency and integration within continuous integration and continuous delivery (CI/CD) environments. The findings contribute to a better understanding of intelligent automation in software testing and provide guidance for quality assurance (QA) professionals and researchers toward adopting sustainable AI-driven testing practices.

Keywords: *artificial intelligence, AI-assisted testing, functional and non-functional evaluation, machine learning, quality assurance, test automation architecture, web systems.*

Rezumat. Evoluția tehnologiilor web și complexitatea tot mai mare a sistemelor digitale au transformat testarea aplicațiilor web într-o componentă indispensabilă a asigurării calității software. Framework-urile tradiționale de testare automată, bazate pe scripturi și date statice, rămân eficiente, dar se confruntă cu limitări legate de scalabilitate și adaptabilitate. Studiul de față pornește de la ipoteza că integrarea inteligenței artificiale (IA), în special a modelelor lingvistice de mari dimensiuni (LLM) și a învățării prin întărire, poate îmbunătăți semnificativ eficiența și autonomia proceselor de testare. În lucrare s-au analizat comparativ metodele tradiționale și cele asistate de IA pentru testarea funcțională a aplicațiilor web, printr-o sinteză a cercetărilor academice și industriale recente. Au fost evidențiate principalele avantaje ale testării bazate pe IA, precum generarea rapidă de teste, extinderea

acoperirii funcționale și creșterea adaptabilității, subliniind totodată limitele legate de transparență și includerea în mediile de integrare și livrare continuă (CI/CD). Rezultatele contribuie la o mai bună înțelegere a automatizării inteligente în testarea software și oferă specialiștilor în asigurarea calității (QA) și cercetătorilor repere pentru adoptarea sustenabilă a practicilor de testare bazate pe IA.

Cuvinte-cheie: *inteligență artificială, testare asistată de inteligență artificială, evaluare funcțională și nefuncțională, învățare automată, asigurarea calității, arhitectura automatizării testării, sisteme web.*

1. Introduction

The automation of software testing has become a cornerstone of software quality assurance, particularly within Agile and Development Operations (DevOps) workflows. It involves the use of specialized tools and frameworks to automatically execute predefined test cases, assess outputs, and generate reports with minimal human intervention [1]. Such automation enables rapid feedback loops, improves scalability, and minimizes the risk of human error during repetitive or regression testing cycles.

Automated testing frameworks, such as Selenium, JUnit, and REST Assured, have proven essential for ensuring consistency and repeatability in large-scale software projects [2]. They are applicable across multiple domains – from web and mobile applications to embedded and distributed systems – providing advantages in speed, efficiency, and cost reduction. However, traditional automation remains limited by its reliance on static scripts and predefined data sets. Maintaining these scripts is often labor-intensive, and their rigidity makes them fragile in the face of continuous interface evolution or dynamic web content [3].

In recent years, the rise of artificial intelligence (AI) and machine learning (ML) has brought new perspectives to test automation. AI-driven tools promise to overcome traditional limitations by introducing adaptive and self-learning mechanisms capable of identifying patterns, generating intelligent test data, and predicting failures [4]. Techniques based on large language models (LLMs), reinforcement learning, and anomaly detection are now being explored to automate functional and non-functional testing tasks more intelligently [5,6].

Despite these promising developments, the integration of AI into testing pipelines introduces its own set of challenges, including data quality dependencies, lack of transparency in model behavior, and difficulty ensuring reproducibility in testing outcomes [7]. Furthermore, questions remain about the explainability of AI-driven decisions and their compatibility with established QA standards.

2. Materials and Methods

Research approach. This study was conducted as a documentary and comparative analysis between traditional and AI-assisted testing frameworks for web applications. The methodology was based on a systematic review of academic publications (2019–2025) indexed in Institute of Electrical and Electronics Engineers (IEEE) Xplore, Association of Computing Machinery (ACM) Digital Library, and Multidisciplinary Digital Publishing Institute (MDPI) databases, complemented by industrial reports and tool documentation. A total of 27 key sources were reviewed, out of which 9 core studies were selected for detailed synthesis based on relevance to functional and non-functional testing automation. The research focused on three main domains:

1. Functional testing – User interface (UI) and application programming interface (API) level test automation.
2. Non-functional testing – performance and load testing in continuous integration (CI) / continuous delivery (CD) contexts.
3. AI-based augmentation – adoption of LLMs, reinforcement learning, and anomaly detection for intelligent automation.

Software and Tools. All comparative analyses were performed using a set of open-source and research-grade frameworks widely recognized in the software testing community:

- Traditional tools: Selenium 4.20, REST Assured 5.4.0, JUnit 5.10, Apache JMeter 5.6.
- AI-assisted tools: FormAgent (LLM-based input classification) [5], QTypist (context-aware form completion) [5], and reinforcement-learning-based test agents [7].
- Data visualization and comparative tables were created using Python 3.11 and Pandas 2.2, while all code examples were implemented in Java 17 (Spring Boot environment).

The frameworks were selected to represent the current industry-standard stack and next-generation intelligent testing paradigms. Each tool was evaluated based on its ability to:

- Automate test generation and execution,
- Detect anomalies and adapt to changes,
- Integrate within DevOps pipelines.

Experimental workflow. The overall research workflow consisted of four key phases (Figure 1 and 2 in the paper):

1. Architecture Analysis – examination of the generic test automation architecture (gTAA) as a baseline model [2].
2. Data Collection – extraction of performance, coverage, and stability metrics from published benchmarks and documentation.
3. Comparative Evaluation – qualitative and quantitative comparison between traditional and AI-assisted frameworks (see Table 1).
4. Integration Modelling – conceptual mapping of AI mechanisms (self-healing, predictive analysis, reinforcement learning) into gTAA layers.

Where quantitative data were available, test execution duration, fault detection rate, and maintenance effort were normalized to comparable baselines. Descriptive statistics (mean, variance, relative improvement %) were computed to evaluate each approach's impact on efficiency and maintainability.

Data sources and reproducibility. The dataset for this review includes peer-reviewed articles and industrial case studies published between 2020 and 2025. Each paper was verified for credibility, citation count, and methodological clarity.

All tools used (Selenium, JMeter, REST Assured, FormAgent, etc.) are open-source or publicly available, allowing reproducibility of results. Configurations, test scripts, and framework setup instructions are provided in the supplementary material for replication.

Statistical processing and comparative metrics. Statistical comparison relied on secondary data extracted from referenced works [7,8]. Performance and coverage metrics were averaged across studies, and percentage improvements were calculated relative to traditional baselines. For instance, AI-based self-healing mechanisms demonstrated up to 70% reduction in maintenance time and 11.9% increase in statement coverage [5,6].

Reinforcement-learning-driven API testing showed a significant rise in fault detection efficiency over scripted methods.

The final comparative analysis integrated both quantitative (efficiency, coverage, stability) and qualitative (adaptability, usability, explainability) factors, enabling a comprehensive evaluation of AI-assisted testing frameworks in practical settings.

The present paper aims to explore the transition from traditional to AI-assisted test automation for web applications through a structured comparative analysis of academic and industrial sources. It hypothesizes that the integration of AI techniques – particularly LLM-based reasoning and reinforcement learning – can enhance scalability, resilience, and adaptability in automated testing while reducing maintenance overhead.

The study synthesizes the current state of research across UI, API, and performance testing domains, identifying key limitations in existing frameworks, and highlights opportunities for innovation. The main conclusions of the bibliographic review suggest that AI-assisted approaches offer significant advantages in coverage and efficiency but require further validation in real-world industrial environments. This research contributes to a better understanding of how intelligent automation can reshape software quality assurance practices in the context of CI/CD systems.

3. Results and Discussions

Test automation is a systematic engineering discipline that involves designing, developing, and maintaining testware and automation frameworks in a structured and measurable way [1]. Within this context, web application test automation plays a critical role in supporting both functional and non-functional testing efforts throughout the software lifecycle.

3.1. Traditional Web Application Testing

Traditional automation approaches follow a layered architecture and lifecycle that includes planning, tool selection, implementation, execution, and ongoing maintenance. These are often structured around modular frameworks such as data-driven, keyword-driven, or hybrid designs, using tools like Selenium for UI testing, REST Assured for API validation, and JUnit or TestNG for unit-level checks.

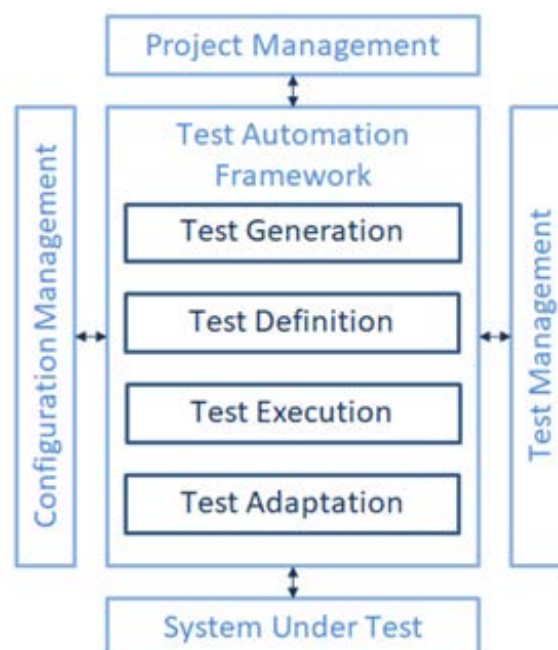


Figure 1. Generic Test Automation Architecture (gTAA) [16].

While traditional automation is mature and well-supported by industry tools and practices, its effectiveness is often challenged by the complexity and volatility of modern web applications. The rapid evolution of front-end frameworks, asynchronous client-server interactions, cross-browser variability, and continuous delivery pipelines expose limitations in test stability, scalability, and maintainability. The success of an automation solution depends not only on tool implementation, but also on alignment with test architecture, risk analysis, and return-on-investment (ROI) considerations [2].

In practice, the implementation of gTAA architectures diverges significantly depending on the testing scope. Functional and non-functional testing impose different constraints on automation strategies, tool selection, and maintenance practices—particularly in the context of web applications, where systems are dynamic, distributed, and constantly evolving.

Functional test automation. Functional testing aims to verify the behavior of the application against the defined requirements. It focuses on “what the application should do”, without analyzing the internal implementation. In the case of web applications, functional testing is mainly carried out at three levels: UI testing – tracks visible interactions: navigation, form filling, input validations, visual responses to actions; API testing – validates the behavior of exposed services (REST, SOAP), ensuring that responses are correct, structured, and compliant with API contracts; and database testing – involves checking CRUD (create, read, update, delete) operations, data consistency, and relationship integrity.

Traditional tools used include Selenium for UI, JUnit/TestNG for component testing, REST Assured, Postman and different IDE plugins for APIs, and direct connectivity via JDBC for database-level testing. Classic frameworks are based on principles such as data-driven, keyword-driven, or hybrid types. Despite their advantages, these methods require a large amount of effort to define, maintain, and update tests, especially in applications with frequently changing interfaces. Also, test scripts can become fragile in the face of minor changes, requiring constant maintenance effort.

Non-functional test automation. Performance testing is a type of non-functional testing that analyzes the application's ability to function under load. The goal is to determine how the system reacts to varying traffic volumes, under stress, or over extended periods of use. Common types of testing include Load testing – measures performance under normal or increased load; Stress testing – tests the limits of the application under extreme conditions. Spike testing – evaluates the application's reaction to sudden increases in traffic; Soak testing – analyzes the application's behavior over time, detecting memory leaks or degradations.

Traditional tools include Apache JMeter, LoadRunner, Gatling, and Blazemeter. They allow you to define complex scenarios and measure metrics such as response time, error rates, throughput, and resource utilization.

In the case of Java applications, system behavior is often affected by JIT compilation, which causes large variations in the first few minutes of execution. The traditional practice involves a fixed “warm-up” period before collecting performance data, but this method is inefficient and often inaccurate. Another major drawback of traditional methods is the lack of automatic anomaly detection, which forces QA teams to analyze graphs and logs to identify regressions. In addition, performance tests are difficult to integrate into DevOps pipelines due to their long execution time and high resource consumption, which limits their frequent use.

Automation workflow and CI/CD integration. Traditional test automation workflows follow a structured process that starts from formalized requirements and extends through scripting, execution, and continuous validation. Once test cases are designed, they are automated and integrated into CI/CD pipelines, where execution is triggered on code changes or at defined build stages. Test results are collected and evaluated using reporting tools, supporting decision-making throughout the development cycle.

These workflows, although well-established, require careful orchestration to maintain stability—particularly in web applications characterized by dynamic content, asynchronous interactions, and frequent updates. Full regression suites are often time-consuming and resource-intensive, leading teams to adopt selective test execution strategies such as nightly runs, smoke testing, or pipeline parallelization. Regardless of the test level UI, API, or performance—automation reliability heavily depends on the consistency of the system under test and the maintainability of the test artifacts.

Challenges and limitations of traditional automation framework (TAF). The generic test automation framework offers a robust architectural foundation for organizing and scaling automated testing activities across test levels and system layers. However, its implementation in modern development environments is increasingly constrained by system complexity, high change frequency, and the need for fast, reliable feedback. These challenges have stimulated the evolution of automation practices toward more intelligent and autonomous solutions. Rather than replacing the generic architecture, recent AI-driven approaches aim to augment and extend its components—introducing adaptability in test generation, self-healing in execution, and predictive capabilities in analysis. The next chapter explores how Artificial Intelligence is being integrated into the functional and non-functional testing lifecycle, and how its mechanisms can be embedded within or layered onto the gTAA structure to enhance automation resilience, efficiency, and scalability.

3.2. AI-assisted Web Application Testing

AI-based testing refers to the use of intelligent algorithms—such as machine learning, large language models, and reinforcement learning—to improve various stages of the testing process. Unlike traditional scripted testing, where testers explicitly define inputs and expected outputs, AI-based systems can generate, adapt, and validate test cases autonomously, based on data patterns, user behavior, or learned models [4].

Recent advances in natural language processing (NLP) and code understanding have enabled the development of tools that can generate test scripts directly from user story descriptions or acceptance criteria written in natural language. AI can also identify high-risk areas in code, suggest targeted test cases, and prioritize tests based on historical data about defects or code changes.

AI in user interface testing. User interface testing is one of the most challenging components of traditional automation, especially due to the visual and dynamic nature of front-end components. Frequent UI updates, layout changes, and asynchronous behaviors often cause traditional scripts, which rely on static locators and hard-coded values, to fail. These challenges make user interface testing an ideal candidate for AI optimization.

AI-assisted UI testing uses LLMs and computer vision techniques to simulate user interactions, generate test data, and validate expected behaviors in a robust and context-aware manner. Instead of relying solely on locators (e.g., XPath, CSS), intelligent tools can

interpret page layout, field labels, and button semantics to decide what actions to take – even when the UI structure changes.

One notable implementation of these capabilities is FormAgent [5], which leverages transformer-based LLMs to classify input fields and generate realistic values semantically. Combined with GPT-4o for post-submission validation, it improves both coverage and efficiency, achieving up to 11.9% higher statement coverage compared to prior tools. This exemplifies the practical value of integrating AI into UI test workflows and highlights the shift from rule-based to context-aware automation.

Tools like FormAgent and techniques like QTypist demonstrate how LLMs can automatically fill out web forms using meaningful and contextually relevant data. These systems do not insert random data, but analyze the purpose of each field (e.g., "First Name," "Email," "Address") and provide realistic and valid values. Furthermore, the success of a form submission is evaluated not only by changes in the DOM, but also by interpreting messages, UI states, or server responses – aspects that traditional tools cannot handle autonomously [6].

Another contribution of AI in UI testing is the implementation of self-healing mechanisms. When locators or UI elements become invalid due to interface changes, AI-powered frameworks can detect and map similar elements using historical patterns, metadata, and contextual analysis. This reduces the need for manual script updates and significantly improves test suite maintainability. A recent case study demonstrated this capability in practice, where a self-healing AI/ML framework was deployed on a dynamic e-commerce platform. The system used monitoring agents and machine learning models trained on historical test data to detect and autonomously repair broken tests. As a result, it achieved an 80% increase in test suite reliability and a 70% reduction in maintenance time, while also lowering costs and enhancing adaptability within CI/CD workflows [7].

In conclusion, AI improves UI testing across multiple dimensions: by generating semantically valid input data using LLMs, adapting to layout and structure changes, autonomously repairing broken selectors through self-healing techniques, and enhancing regression analysis through intelligent visual comparison. These advancements not only reduce the need for human intervention and ongoing maintenance effort but also increase the reliability and resilience of automated testing workflows in fast-changing web environments.

AI in web services testing. API testing is essential for validating modern web applications, as APIs are the building blocks of microservices, data exchange, and system integrations. Unlike UI testing, which interacts with visual elements, API testing focuses on request-response behavior, data integrity, and business logic – making it ideal for automation [8]. AI-assisted API testing brings a paradigm shift, enabling autonomous exploration, test case generation, and intelligent validation. Instead of being written manually, tests can be generated automatically by analyzing API documentation schemas (OpenAPI, Swagger), learning usage patterns, and generating hundreds or thousands of test cases with minimal involvement from testers.

A major contribution in AI-driven API testing is the use of intelligent agents capable of simulating realistic interactions with web services across a broad spectrum of input scenarios. These agents, typically based on reinforcement learning or anomaly detection models, generate test inputs using information such as parameter constraints, previously observed responses, known defect patterns, and relationships between endpoints. In experimental evaluations, such agents demonstrated the ability to autonomously generate

thousands of test cases and detect real faults in public APIs—without relying on manually defined oracles. This highlights their potential to significantly enhance coverage, input diversity, and fault detection efficiency in automated API testing workflows [9].

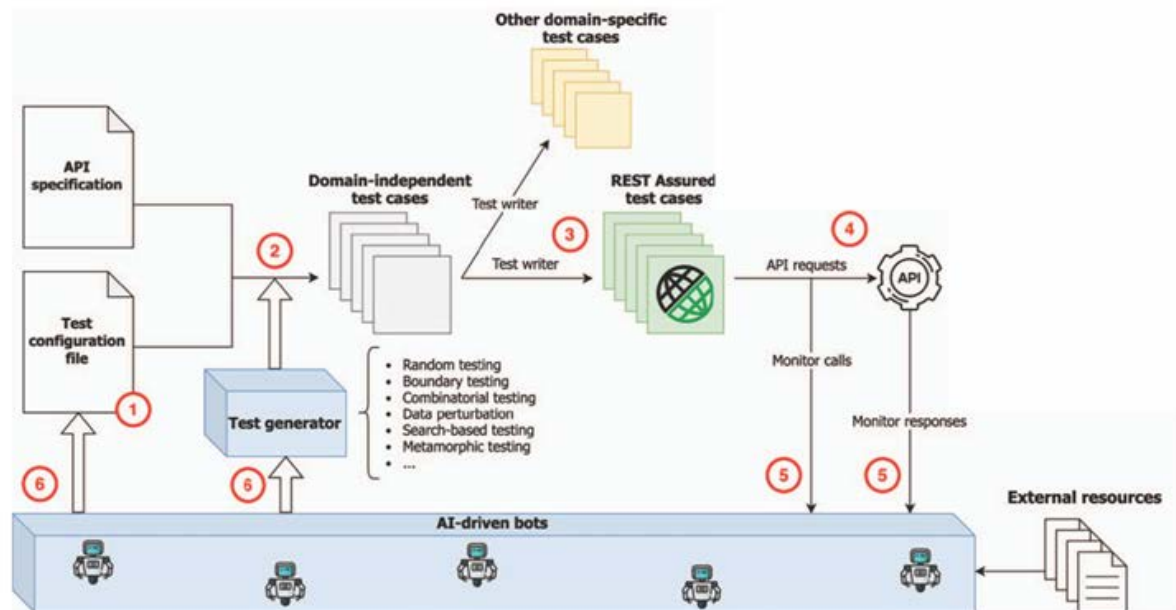


Figure 2. General architecture of the AI agent testing system [16].

The system can intelligently explore edge cases, uncover hidden defects, and identify vulnerabilities such as injection flaws, broken authentication mechanisms, and improper error handling. Additionally, AI improves response validation by learning the distinction between normal and abnormal behaviors—comparing real-time responses against historical baselines or detecting structural anomalies like unexpected status codes, irregular JSON/XML formats, and latency deviations.

Another significant advantage is the optimization and prioritization of test execution. AI-based impact analysis enables the test suite to adapt dynamically based on factors such as recent code changes, API usage patterns, or known high-risk areas. This results in faster, more focused feedback, particularly valuable in CI/CD environments where execution speed and accuracy are critical.

In summary, AI enhances API testing by: (1) automating the generation of meaningful test cases; (2) intelligently validating hypertext transfer protocol responses; (3) autonomously exploring boundary and error-prone scenarios; (4) expanding coverage while reducing manual effort; and (5) optimizing test execution in continuous delivery pipelines. These capabilities make AI an indispensable component for achieving scalable, adaptive, and high-quality API testing in modern software systems.

AI in performance testing. Traditional performance testing typically relies on pre-scripted scenarios and static heuristics, such as fixed warm-up durations. However, these approaches often fail to accurately capture real-world behavior—especially in Java applications, where just-in-time compilation introduces execution variability. AI offers a more adaptive and data-driven alternative, capable of dynamically analyzing performance signals in real time.

One of the most impactful AI techniques in this context is time series classification (TSC). Rather than relying on predefined warm-up thresholds, TSC [10] models analyze live

performance metrics (e.g., response times, central processing unit (CPU) utilization, memory usage) to automatically determine when the application reaches a steady state. This allows tests to start or terminate at precisely the right moment, minimizing execution time and improving measurement reliability.

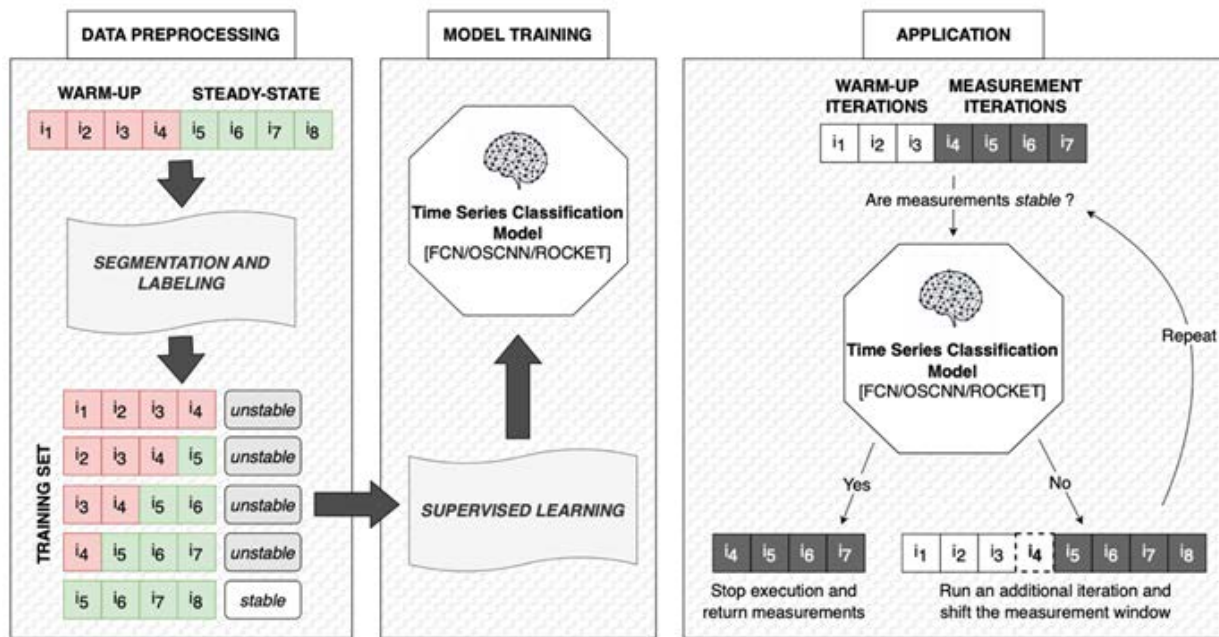


Figure 3. Overview of the main phases of the AI-assisted framework: Data Preprocessing, Model Training, and Application [8].

AI-powered tools can also adjust the testing window dynamically, optimizing when to stop or extend execution—contrasting with rigid traditional methods. In DevOps pipelines, this intelligence translates into faster feedback cycles, improved resource utilization, and greater alignment with production-like conditions.

Benefits and limitations of AI-assisted testing. The integration of AI into automated software testing has brought a paradigm shift in both functional and non-functional test processes. Unlike traditional frameworks that rely on static test generation, execution, and adaptation, AI-augmented frameworks introduce dynamic learning, semantic analysis, and intelligent decision-making [11,12].

While gTAA integrates project management, test management, and configuration pipelines using deterministic logic, AI frameworks adapt in real-time, adjusting test selection, input data generation, and validation based on system feedback and learned patterns.

In performance testing, AI-based systems extend traditional frameworks by incorporating TSC [13] for warm-up detection and measurement stability, reducing test duration without sacrificing accuracy. Similarly, in API testing, reinforcement learning and behavioral modeling allow for more diverse and meaningful test inputs, surpassing the limitations of scripted testing.

AI-enhanced frameworks offer several benefits: increased test coverage by autonomously exploring edge cases, reduced maintenance through self-healing mechanisms, and improved semantic alignment with business logic via AI-driven test case generation. Anomaly detection further enhances scalability, feedback speed, and resource efficiency, particularly in CI/CD environments [14].

However, AI-assisted testing faces challenges such as the lack of transparency in AI decisions, which raises issues in explainability and traceability—critical in regulated environments. AI models also depend on high-quality training data and remain in experimental stages for many testing tools, with limited production validation. Additionally, AI introduces complexity, requiring validation of the models themselves and integration into existing testing pipelines [15].

In summary, AI-enhanced frameworks add autonomy, resilience, and intelligence to traditional test automation, offering the potential to overcome current limitations and drive software testing toward greater efficiency and effectiveness.

3.3. Comparative Analysis: Traditional vs AI-assisted TAF

This paragraph presents a comparative documentary analysis aimed at introducing an AI-assisted testing approach for web applications. By synthesizing recent research on both traditional and AI-driven testing frameworks, the section proposes a practical methodology for integrating AI to enhance testing. This provides a roadmap for both researchers and practitioners to evaluate the current landscape and explore future improvements.

Table 1

Artificial Intelligence vs Traditional Automation Frameworks			
Criterion	Traditional testing	AI-assisted testing	Cases / System under test characteristics
Test generation	Manual, scripted	Automated, based on machine learning/ large language models	Dynamic or large applications
Maintenance	High, requires constant updates	Reduced, with self-healing mechanisms	Systems with frequent changes or user interface instability
Adaptability to changes	Low	High, through contextual learning	Agile, fast-paced environments
Anomaly detection	Limited to predefined assertions	Dynamic, using predictive models	Detecting unexpected issues and regressions
Validation	Based on fixed rules	Contextual and flexible	Context-sensitive validation (e.g., application user interfaces)
Scripting effort	High	Minimal or non-existent	Minimal scripting and automation
(CI/CD) Integration	Partial, high latency	Optimized for continuous execution	CI/CD pipelines for faster feedback
Exploration capacity	Limited, predefined	Extended, through autonomous input generation	Complex, variable inputs
Scalability	Resource-constrained	Optimized through impact analysis	Scaling tests across large systems with varied inputs

Strategic selection of testing frameworks. AI vs Traditional Approaches. Traditional testing frameworks like Selenium and JUnit offer stability and maturity, but they face significant challenges in rapidly evolving environments, requiring high maintenance and

struggling with adaptability to frequent UI and API changes. These frameworks are limited in their ability to detect anomalies, as they rely on predefined assertions.

AI-assisted testing frameworks introduce autonomy, dynamic adaptation, and self-healing mechanisms, making them ideal for environments with constant updates. AI-driven systems can autonomously generate test cases, adapt to changes, and detect anomalies without predefined rules.

However, the rise of AI tools requires careful selection, as improper integration could lead to inefficiency and unnecessary complexity. A balanced approach that leverages the strengths of both traditional and AI-driven methods is crucial for maximizing testing effectiveness.

Framework selection based on system under test (SUT) characteristics. To guide framework selection, this paper introduces a Framework Selection Guide based on SUT characteristics. Key factors such as UI stability, API complexity, performance requirements, and rate of change influence the choice between traditional or AI-assisted frameworks. AI tools should be used strategically, offering value for systems with high volatility, like frequent UI updates, while stable systems may continue benefiting traditional methods. The goal is to avoid a one-size-fits-all approach, favoring tailored automation strategies. By evaluating these factors, teams can select the most suitable testing framework aligned with the SUT's needs.

4. Conclusions

This paper explores the transformation occurring in web application testing, as AI-driven frameworks are integrated alongside traditional testing methods. Our comparative analysis reveals that while traditional frameworks provide stability, control, and transparency, they struggle with the increasing complexity and dynamic nature of modern applications. The challenges include high maintenance costs, brittle test scripts, and difficulty in adapting to frequent UI or API changes.

AI-assisted testing frameworks offer a paradigm shift, providing autonomy, adaptability, and continuous learning. These systems excel at automatic test case generation, contextual validation, anomaly detection, and self-healing, greatly enhancing scalability, resilience, and resource optimization. However, AI integration comes with methodological and technical challenges—such as lack of explainability, tool maturity, and data dependency—which prevent it from being a universal solution.

Recommendations for practitioners. QA engineers are recommended to analyze adopting a hybrid testing approach where AI supports repetitive tasks, while traditional methods remain in place for critical, regulated, or complex scenarios. It's also essential to continuously evaluate AI tools' performance and reliability in real business applications, while investing in training to ensure proper integration and use of AI.

Recommendations for researchers. For researchers, exploring AI model validation and meta-testing techniques for automated testing, along with contributions to the development of open-source frameworks, would support the continued evolution of AI-based testing solutions.

Future directions. As part of our working plan, we aim to develop a comprehensive guide for framework selection based on SUT characteristics. This long-term initiative will leverage AI-assisted testing frameworks, integrating industry feedback and practical experience into an adaptable guide for researchers and practitioners. The guide will focus on

selecting the appropriate testing framework based on factors like UI complexity, API reliability, and performance needs, ensuring customized solutions for real-world applications.

In the short term, we will continue gathering practical experience by working closely with industry partners, conducting pilot studies, and evaluating AI tools under various application scenarios. This hands-on experience will inform the framework guide and provide actionable insights for practitioners looking to integrate AI-enhanced testing into their workflows.

The continuous development of Large Language Models and their integration into DevOps pipelines, combined with emerging technologies such as RPA, AIOps, and autonomous testing, promises to modernize quality assurance processes. Soon, AI-based testing will not just be a competitive advantage but a necessity for sustaining software delivery in dynamic, scalable, and user-centric environments.

Conflicts of Interest: The authors declare no conflict of interest

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Citation: Darii, O.; Beldiga, M.; Bragaru, T. Automated web application testing based on artificial intelligence. *Journal of Engineering Science*. 2025, XXXII (4), pp. 41-53. [https://doi.org/10.52326/jes.utm.2025.32\(4\).04](https://doi.org/10.52326/jes.utm.2025.32(4).04).

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Submission of manuscripts:

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[https://doi.org/10.52326/jes.utm.2025.32\(4\).05](https://doi.org/10.52326/jes.utm.2025.32(4).05)

UDC 528.9:528.4:930.253(478-21)



APPLICATION OF 2D TRANSFORMATION MODELS FOR THE CONVERSION OF HISTORICAL TOPOGRAPHIC PLANS FROM SOROCA CITY, REPUBLIC OF MOLDOVA

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Received: 11. 18. 2025

Accepted: 12. 22. 2025

Abstract. The integration of historical geodetic data into the national spatial data infrastructure represents an essential step for valorizing cartographic heritage and ensuring the interoperability of modern reference systems. The present study has as its main objective the transformation of coordinates from the local Soroca system, historically used on the territory of the Republic of Moldova, into the national coordinate system MOLDREF99, compatible with European Terrestrial Reference System 1989 (ETRS89). The proposed methodology is based on the application of 2D mathematical transformation models – Helmert (4 parameters) and Affine (6 parameters) – for the conversion of topographic plans at scale 1:500 from Soroca city. Twelve common geodetic points were used, identified both on vectorized plans and in the national GeoData database, and the transformation parameters were calculated using the least squares method and validated on eight independent points. The comparative analysis demonstrated that both models offer similar precision, with mean square errors of approximately 0.002 m and RMSE (Root Mean Square Error) of 0.0226 m in the X direction and below 0.008 m in the Y direction, values that fall within acceptable limits for cadastral and topographic works at scale 1:500. The obtained results demonstrate the feasibility of coordinate transformation without direct field measurements, using exclusively existing cartographic resources and the national geodetic database, thus contributing to the development of the National Spatial Data Infrastructure (NSDI) and the valorization of cartographic heritage for cadastral applications, territorial planning, and urban development.

Keywords: *2D transformation, transformation parameters, least squares method, historical topographic plans, NSDI, Soroca local system, MOLDREF99.*

Rezumat. Integrarea datelor geodezice istorice în infrastructura națională de date spațiale reprezintă o etapă esențială pentru valorificarea patrimoniului cartografic și asigurarea interoperabilității sistemelor moderne de referință. Studiul de față are ca obiectiv principal transformarea coordonatelor din sistemul local Soroca, utilizat istoric pe teritoriul Republicii Moldova, în sistemul de coordonate național MOLDREF99, compatibil cu Sistemul European de Referință Terestră 1989 (ETRS89). Metodologia propusă se bazează pe aplicarea modelelor

matematice de transformare 2D – Helmert (4 parametri) și Afină (6 parametri) – pentru conversia planșelor topografice la scara 1:500 din orașul Soroca. Au fost utilizate 12 puncte geodezice comune, identificate atât pe planșele vectorizate, cât și în baza de date națională GeoData, iar parametrii de transformare au fost calculați prin metoda celor mai mici pătrate și validați pe opt puncte independente. Analiza comparativă a demonstrat că ambele modele oferă precizie similară, cu erori medii pătratice de aproximativ 0.002 m și RMSE de 0.0226 m în direcția X și sub 0.008 m în direcția Y, valori care se încadrează în limitele acceptabile pentru lucrările cadastrale și topografice la scara 1:500. Rezultatele obținute demonstrează fezabilitatea transformării coordonatelor fără măsurători directe în teren, utilizând exclusiv resurse cartografice existente și baza de date geodezică națională, contribuind astfel la dezvoltarea Infrastructurii Naționale pentru Date Spațiale (INDS) și valorificarea patrimoniului cartografic pentru aplicații cadastrale, planificare teritorială și dezvoltare urbană.

Cuvinte cheie: *transformare 2D, parametri de transformare, metoda celor mai mici pătrate, planuri topografice istorice, INDS, sistem local Soroca, MOLDREF99.*

1. Introduction

The transformation of coordinates between different geodetic systems represents a fundamental necessity in the context of modernizing geospatial infrastructure and integrating historical cartographic data into contemporary reference systems [1,2]. With the evolution of Global Navigation Satellite System (GNSS) positioning technologies and the adoption of geocentric reference frames at national and international levels, the need has emerged to ensure the compatibility and interoperability of spatial databases created in traditional local systems with modern geospatial infrastructures [3,4].

In the Republic of Moldova, the transition from local coordinate systems, derived from the Soviet reference framework, to the national MOLDREF99 system, compatible with the European standard ETRS89, has generated significant challenges in the field of geodesy and cadastre [5,6]. Most of the technical works carried out previously were executed in local coordinate systems, adapted to regional specificities, which led to the formation of substantial databases that require transformation to be integrated into current GIS platforms and smart infrastructure projects [7,8]. A considerable part of this cartographic heritage is represented by topographic plans at scale 1:500, elaborated for numerous localities and districts of the republic, which are preserved in archives in analog form [9]. These plans contain valuable geodetic and topographic information, but remain insufficiently valorized due to the incompatibility of the coordinate systems in which they were created with the current national spatial data infrastructure [10,11].

The digitization, georeferencing, and integration of these historical cartographic materials into the National Spatial Data Infrastructure (NSDI) database represent a strategic objective for consolidating the informational capacity of the national geodetic system [10,12]. The process involves not only the scanning of plans and vectorization of graphic elements, but also the rigorous transformation of coordinates from local systems into the MOLDREF99 system, thus ensuring the continuity and coherence of spatial data at the national level [13-15]. This approach allows the recovery and reuse of a significant volume of detailed topographic information, essential for cadastral projects, territorial planning, and urban development [16,17]. In this context, national methodological guidelines regarding the

analog-digital conversion of spatial data and their georeferencing offer a standardized procedural framework for processing historical cartographic materials [18,19].

The coordinate transformation process involves the application of rigorous two-dimensional mathematical models, capable of describing the geometric relationship between two reference systems through translation, rotation, and scaling parameters [20,21]. The 2D Helmert transformation method and Affine transformation, based on the least squares principle, have proven to be the most efficient and precise solutions for such conversions, allowing minimal deviations to be obtained and ensuring the spatial coherence of transformed data [22-24].

The present study aims to analyze the coordinate transformation process from the local Soroca system into the national MOLDREF99 system, using historical cartographic data and resources from the national geospatial database. Through the application of the Helmert method and Affine transformation in plane, as well as validation of results on a set of geodetic control points, the work demonstrates the feasibility and precision of 2D transformation models for the conversion of spatial data in the absence of direct field measurements [25]. The selected study area, Soroca city, located in the northeastern part of the Republic of Moldova, offers a representative framework for this analysis, due to its varied geomorphological characteristics, availability of topographic plans at scale 1:500 in the local system, and existence of data from the national geodetic infrastructure [26,27].

The results of this study contribute to the broader process of harmonizing geospatial data at the national level, while offering a methodology applicable in practical situations where verification of geodetic benchmarks is not possible, and the integration of historical data into modern systems must be carried out exclusively based on existing cartographic resources and official databases [28,29]. The proposed methodology can be extended and applied for the valorization of the entire fund of archived topographic plans, thus contributing to the completion and enrichment of the National Spatial Data Infrastructure of the Republic of Moldova.

2. Materials and Methods

2.1. Study Area

The study area selected for the present research is Soroca municipality with an area of approximately 13.11 km², located in the northeastern part of the Republic of Moldova, on the right bank of the Dniester River, near the border with Ukraine (Figure 1). The municipality represents an important urban and administrative center of the region, with varied geomorphological characteristics that include Dniester terraces, steep slopes, and hilly areas [26]. These topographic particularities, together with the historical context and availability of a dense network of historical geodetic points, make Soroca an adequate framework for the study of geodetic transformations between different reference systems.

From an administrative perspective, Soroca municipality has a geodetic infrastructure developed during the soviet period, consisting of a network of geodetic and topographic points that were used in multiple local technical projects. These points were registered in local coordinate systems, derived from soviet reference frames, generating a topographic and cadastral database specific to the region.

The local coordinate system used in Soroca city belongs to the category of plane reference systems, adapted to the national triangulation and leveling network from the USSR period. In this system, the terrestrial surface was projected onto a plane using a transverse

conformal projection, with an adjusted scale to reduce deformation errors at the regional level [3]. Although this system allowed efficient operation of local topographic projects in the long term, it presents significant limitations regarding integration with modern systems, especially concerning absolute positioning, interoperability, and precision [14].

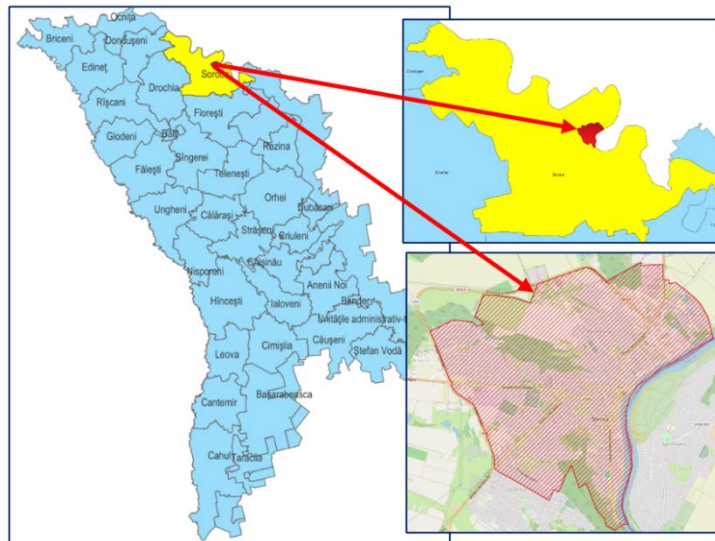


Figure 1. Study area – Soroca locality.

With the geodetic and cadastral reforms initiated in the Republic of Moldova starting in the 2000s, the MOLDREF99 system was officially adopted, which corresponds to the European framework ETRS89 [6]. The MOLDREF99 system allows precise three-dimensional positioning, and coordinates can be expressed in plane projection using a version of the Transverse Mercator projection, adapted to the national territory through the definition of an axial meridian at longitude $28^{\circ}24' E$ and a central scale factor of 0.9996 [6]. Thus, the system ensures optimal integration with European and international networks, offering centimeter-level precision when used together with modern GNSS technologies and permanent station networks. Therefore, it is necessary to perform the transformation of coordinates from the local Soroca system into MOLDREF99, which derives from the objective of harmonizing historical geodetic data with the national spatial data infrastructure (NSDI).

2.2. Data Sources

The basic cartographic material used in this study consisted of topographic plans at scale 1:500 on paper support, elaborated in the local Soroca system, originating from the local cadastral documentation archive. Some plans are missing, the cause is unknown. These plans contain detailed information about the geodetic point network, topographic elements, and urban infrastructure of Soroca municipality. The plans were scanned at a resolution of 300 dpi in TIFF format to ensure the necessary quality for the subsequent vectorization process.

To obtain coordinates in the MOLDREF99 system, the GeoData platform was used, which provides access to the national geodetic coordinate database [27]. This platform allows the querying and extraction of official coordinates of geodetic points from the national network.

For processing the scanned topographic plans, GeoniCS software compatible with AutoCAD software was used [30], a specialized geospatial extension for vectorization and

design work in the field of geodesy and cadastre [18,19]. This tool offers advanced functionalities for georeferencing rasters, vectorizing graphic elements, and extracting coordinates of geodetic points. For spatial analysis, results validation, and data visualization, open-source GIS applications were used, including QGIS [24], which allow the integration and processing of geospatial data from multiple sources and formats.

2.3. Methodology

2.3.1. Georeferencing and Unification of Topographic Plans

The processing of topographic plans followed a succession of standardized steps, according to national methodological guidelines regarding analog-digital conversion and georeferencing of spatial data [17,18]. The scanned plans were imported into the GeoniCS environment and preliminarily positioned on the local Soroca system grid, using grid intersections and corners of the graphic frame as references. To ensure precise positioning, the *Rubbersheet* function from GeoniCS was applied, which allows controlled deformation of the image through four control points, distributed at the corners of the plan's graphic frame, for which initial positions (source points) and desired positions (destination points) were defined according to the grid coordinate values.

Preliminary georeferencing was performed using the first-degree polynomial method. The georeferencing precision was evaluated through RMS error (root mean square), which was maintained below the limit of 0.05 m, according to technical standards for digital processing work of topographic plans. After georeferencing, the plans were clipped by eliminating exterior graphic elements and precise delimitation of the area of interest [31]. Relevant graphic elements (geodetic points, boundaries, infrastructure) were vectorized, and for each identified geodetic point, coordinates were extracted in the local Soroca system directly from the vectorized plan (Figure 2).

For each geodetic point identified in the vectorized plans, corresponding coordinates were searched in the MOLDREF99 system using the GeoData platform [27]. Identification criteria included the point name, identifier code, and approximate position on the map (Figure 3). Thus, a dataset was created consisting of coordinate pairs – one in the local Soroca system (x_1, y_1) and one in the MOLDREF99 system (x_2, y_2) – for each geodetic point.

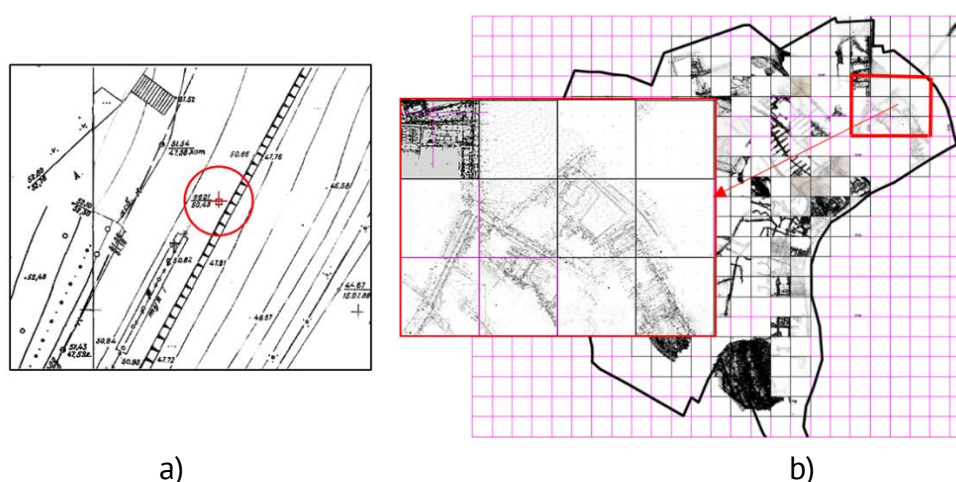


Figure 2. Topographic plans at scale 1:500: a) geodetic point; b) example of 12 unified aligned plans from the Soroca city area.

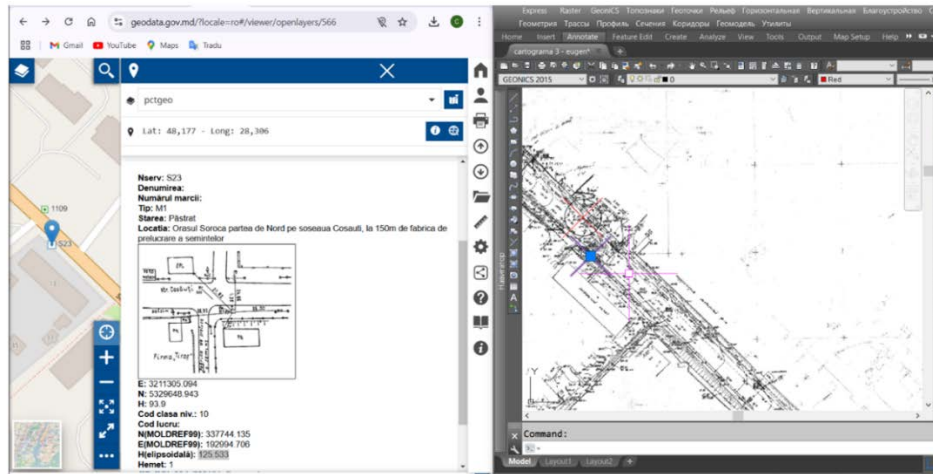


Figure 3. Correlation of points from the Local Soroca system to the MOLDREF99 system.

2.3.2. Mathematical Models of 2D Transformation

For the coordinate transformation between the local Soroca system and the national MOLDREF99 system, the 2D Helmert transformation with four parameters was applied, and for comparison, the 2D affine transformation with six parameters was used [22,23,32].

The 2D Helmert model is defined by the equation (1):

$$\begin{pmatrix} x_2 \\ y_2 \end{pmatrix} = \begin{pmatrix} x_0 \\ y_0 \end{pmatrix} + \begin{pmatrix} a & -b \\ b & a \end{pmatrix} \cdot \begin{pmatrix} x_1 \\ y_1 \end{pmatrix}, \quad (1)$$

where: (x_0, y_0) represent the translation components between the origins of the local Soroca and MOLDREF99 coordinate systems; the coefficients a and b were replaced by the relations $a = k \cdot \cos \alpha$ and $b = k \cdot \sin \alpha$ (k – scale factor, α – rotation angle between the coordinate systems); (x_1, y_1) and (x_2, y_2) are the coordinates of the point in the source system (local Soroca) and in the target system (MOLDREF99), respectively.

The determination of the parameters x_0 , y_0 , a , and b was carried out using the least squares method, based on common points with known coordinates in both systems [23,24]. This process requires at least two common points, but in practice, a larger number of tie points is recommended [32]. The adjustment process was performed by constructing a system of linear equations expressed in matrix form:

$$L + V = A \cdot X, \quad (2)$$

where:

$$A = \begin{pmatrix} 1 & 0 & x_1^{LOC} & -y_1^{LOC} \\ 0 & 1 & y_1^{LOC} & x_1^{LOC} \\ \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot \\ 1 & 0 & x_n^{LOC} & -y_n^{LOC} \\ 0 & 1 & y_n^{LOC} & x_n^{LOC} \end{pmatrix}; \quad X = \begin{pmatrix} x_0 \\ y_0 \\ a \\ b \end{pmatrix}; \quad L = \begin{pmatrix} x_2^{MOLDREF99} \\ y_2^{MOLDREF99} \\ \cdot \\ \cdot \\ x_2^{MOLDREF99} \\ y_2^{MOLDREF99} \end{pmatrix}; \quad V = \begin{pmatrix} v_{x1} \\ v_{y1} \\ \cdot \\ \cdot \\ v_{xn} \\ v_{yn} \end{pmatrix}. \quad (3)$$

n – the number of common points, with known coordinates in both systems.

The unknown transformation parameters X result from the relationship:

$$X = (A^T A)^{-1} A^T L. \quad (4)$$

The standard deviation of the Helmert transformation, σ_0 , which can be used to compare the geometry of the two coordinate systems, is obtained using the following formula:

$$\sigma_0 = \pm \sqrt{\frac{V^T V}{2n-4}}. \quad (5)$$

The 2D Affine transformation, unlike the 2D Helmert linear transformation, introduces separate corrections for each of the two coordinate axes and is based on a total of six parameters [22,32]. The coordinates of the points in the transformed system are determined in matrix form using the following equation:

$$\begin{pmatrix} x_2 \\ y_2 \end{pmatrix} = \begin{pmatrix} x_0 \\ y_0 \end{pmatrix} + \begin{pmatrix} a & b \\ c & d \end{pmatrix} \cdot \begin{pmatrix} x_1 \\ y_1 \end{pmatrix}. \quad (6)$$

In this case, a minimum of three common points is required to solve the system using the least squares method for determining the six unknowns (x_0, y_0, a, b, c, d). The same steps as in the 2D linear Helmert transformation are followed to apply the least squares principle, with only the following modifications in the matrix formation:

$$A = \begin{pmatrix} x_1^{LOC} & y_1^{LOC} & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & x_1^{LOC} & x_1^{LOC} & 1 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ x_n^{LOC} & y_n^{LOC} & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & x_n^{LOC} & y_n^{LOC} & 1 \end{pmatrix}; \quad X = \begin{pmatrix} a \\ b \\ x_0 \\ c \\ d \\ y_0 \end{pmatrix}. \quad (7)$$

The standard deviation of the transformation is calculated in a similar way, but with a modification in the denominator of the equation:

$$\sigma_0 = \pm \sqrt{\frac{V^T V}{2n-6}}. \quad (8)$$

In addition to the Helmert and Affine transformations, higher-order polynomial transformation models (degree 2, 3, or higher) are also described in the specialized literature [22]. However, the application of these methods to the available data from the Soroca area resulted in lower accuracy due to their increased sensitivity to the non-uniform distribution of control points.

3. Results and Discussion

For the determination of the transformation parameters, 12 common points (control points) were selected from the national geodetic network, where coordinates are known in both systems (the local Soroca system and the MOLDREF99 system) for the locality of Soroca (Figure 4).

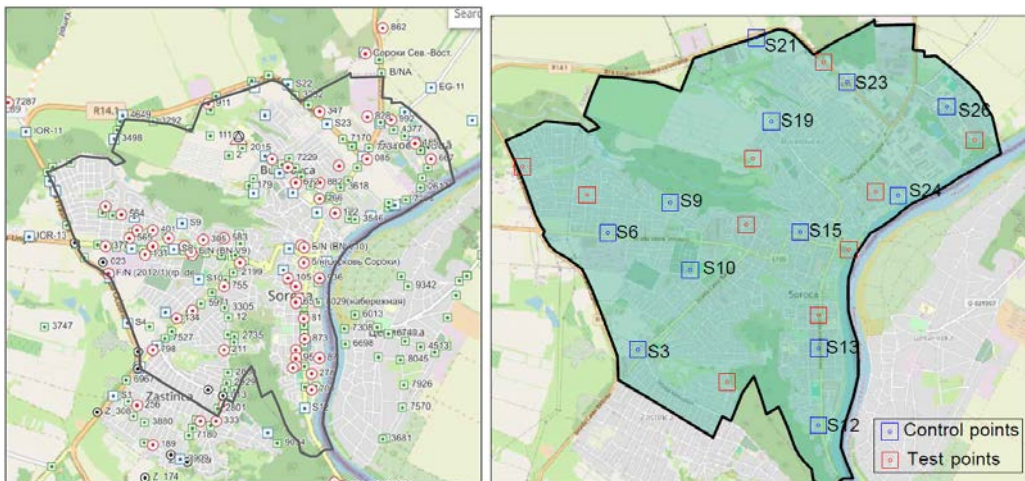


Figure 4. Selection of control and test points in both coordinate systems for the locality of Soroca.

The distribution of control and test points is presented in Figure 5, where blue squares indicate the control points (a total of 12), and red squares indicate the locations of the test points (a total of 8). Figure 5a shows the positions of the points in the local Soroca coordinate system, while Figure 5b shows their positions in the national MOLDREF99 coordinate system.

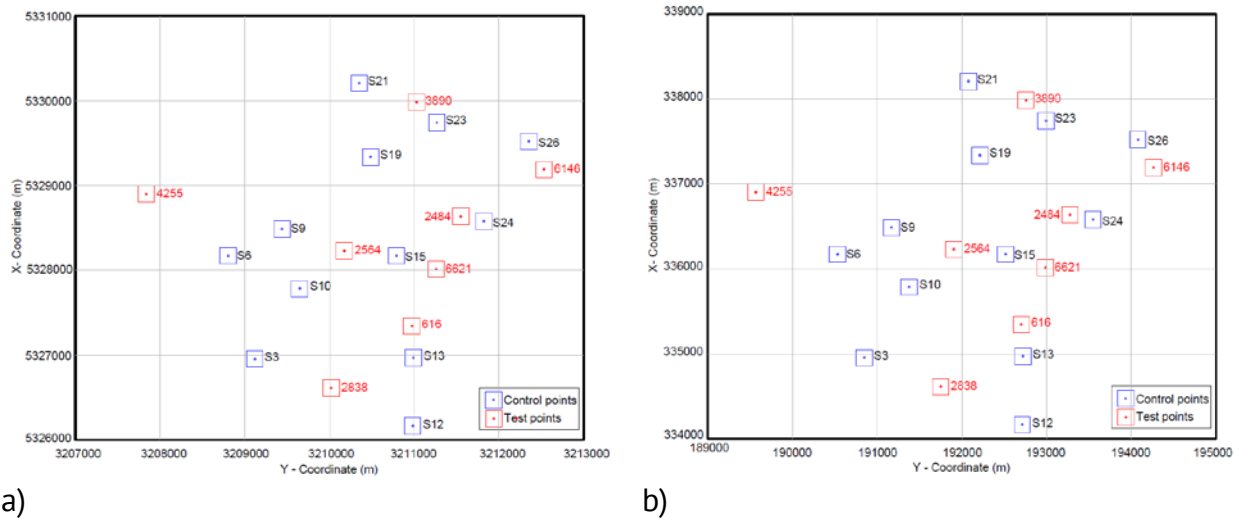


Figure 5. Distribution of control and test points in both coordinate systems:
a) Local Soroca, b) MOLDREF99.

The control points were used to estimate the transformation parameters, while the test points were used to verify the validity of the estimated parameters by analyzing whether the obtained results are close to the original coordinates (from the registry). The eight test points were transformed into the second reference system (MOLDREF99) using the transformation parameters calculated by the two models – Helmert (4 parameters) and Affine (6 parameters). Table 1 lists the transformation parameters for converting coordinates from the local Soroca reference system to the national MOLDREF99 system, obtained by the least squares method based on common (control) points in both systems.

Table 1

Estimated Transformation Parameters	
Helmert transformation parameters	Affine transformation parameters
$x_0, m = -5036471.608$	$x_0, m = -5036474.323$
$y_0, m = -2941065.247$	$y_0, m = -2941064.688$
$a = 0.999727971$	$a = 0.999728445$
$b = -0.014329569$	$b = 0.014329628$
	$c = -0.014329225$
	$d = 0.999727227$

The mean square error (σ_0), representing the accuracy of the transformation operation, and the average errors of the transformation parameters are presented in Table 2.

Table 2

The Mean Square Error of Transformation (σ_0) and Average Errors of Transformation Parameters (meter)

Helmert transformation parameters	Affine transformation parameters
$\sigma_0 = 0.002262$	$\sigma_0 = 0.002298$
$\sigma_{x0} = 0.005832$	$\sigma_{x0} = 0.007470$
$\sigma_{y0} = 0.005832$	$\sigma_{y0} = 0.007470$

Continuation Table 2

$\sigma_a = 9.37 \times 10^{-10}$	$\sigma_a = 1.34 \times 10^{-9}$
$\sigma_b = 9.37 \times 10^{-10}$	$\sigma_b = 1.52 \times 10^{-9}$
	$\sigma_c = 1.34 \times 10^{-9}$
	$\sigma_d = 1.52 \times 10^{-9}$

The coordinates of the test points from the local Soroca coordinate system were transformed into the national MOLDREF99 system using the transformation parameters determined by the two models. Subsequently, the differences between the transformed coordinates and the original coordinates (from the registry) in the MOLDREF99 system for the same test points were calculated (Table 3).

Table 3

Differences between Local Soroca to MOLDREF99 Transformed Coordinates and Register MOLDREF99 Coordinates in Test Points

Point ID	Helmert transformation		Affine transformation	
	ΔX (m)	ΔY (m)	ΔX (m)	ΔY (m)
2838	0.0014	-0.0020	0.0025	-0.0029
2564	-0.0012	0.0006	-0.0009	-0.0008
2484	0.0005	0.0014	0.0005	0.0009
616	-0.0019	-0.0001	-0.0012	-0.0005
4255	-0.0535	0.0221	-0.0535	0.0187
3890	0.0004	-0.0019	-0.0002	-0.0032
6146	0.0028	0.0038	0.0025	0.0038
6621	-0.0350	-0.0002	-0.0346	-0.0007

Based on these differences, statistical indicators were evaluated, including the minimum and maximum errors, the root mean square error (RMSE), and the mean absolute error (MAE) [23].

The mean absolute error (MAE) is determined using the following equation:

$$MAE = \frac{1}{n} \left[\sum_{i=1}^n X_{MOLDREF99(register)} - X_{MOLDREF99(transformed)} \right]. \quad (9)$$

The root mean square error (RMSE) is determined by the relationship:

$$RMSE = \sqrt{\frac{1}{n} \left(\sum_{i=1}^n (X_{MOLDREF99(register)} - X_{MOLDREF99(transformed)})^2 \right)}. \quad (10)$$

The statistical indicators are presented in Table 4 for the Helmert transformation case and for the Affine transformation case.

Table 4

Statistical Values for Differences between Local Soroca to MOLDREF99 Transformed Coordinates and Register MOLDREF99 Coordinates in Test Points

Statistical values	Helmert transformation		Affine transformation	
	X - direction errors (ΔX)	Y - direction errors (ΔY)	X - direction errors (ΔX)	Y - direction errors (ΔY)
Min, m	-0.0535	-0.0020	-0.0535	-0.0032
Max, m	0.0028	0.0221	0.0025	0.0187
MAE, m	0.0108	0.0030	0.0106	0.0019
RMSE, m	0.0226	0.0080	0.0226	0.0069

MAE - the mean absolute error, RMSE – the root mean square error.

If the coordinate differences (ΔX , ΔY) between the transformed coordinates and the existing coordinates in the MOLDREF99 system are represented separately for the eight test points, the results shown in Figure 6 for the Helmert transformation model and in Figure 7 for the Affine transformation model are obtained.

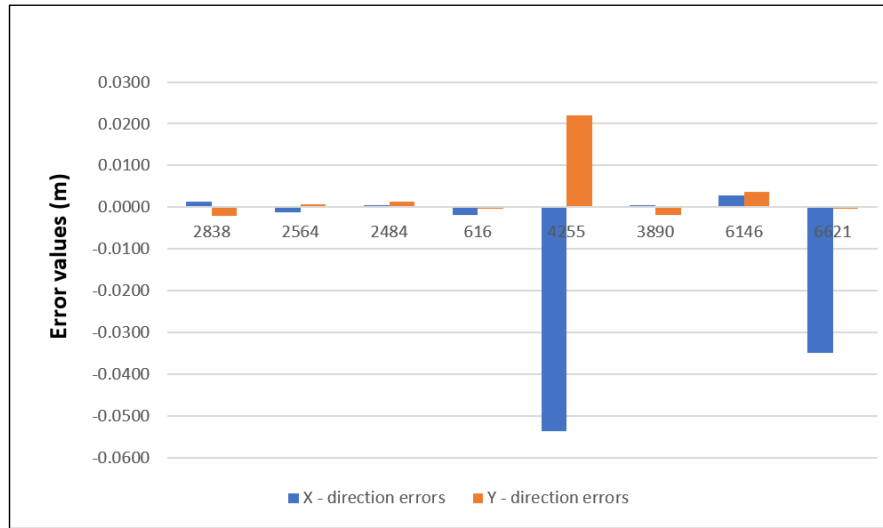


Figure 6. Coordinate differences of test points for the Helmert transformation model.

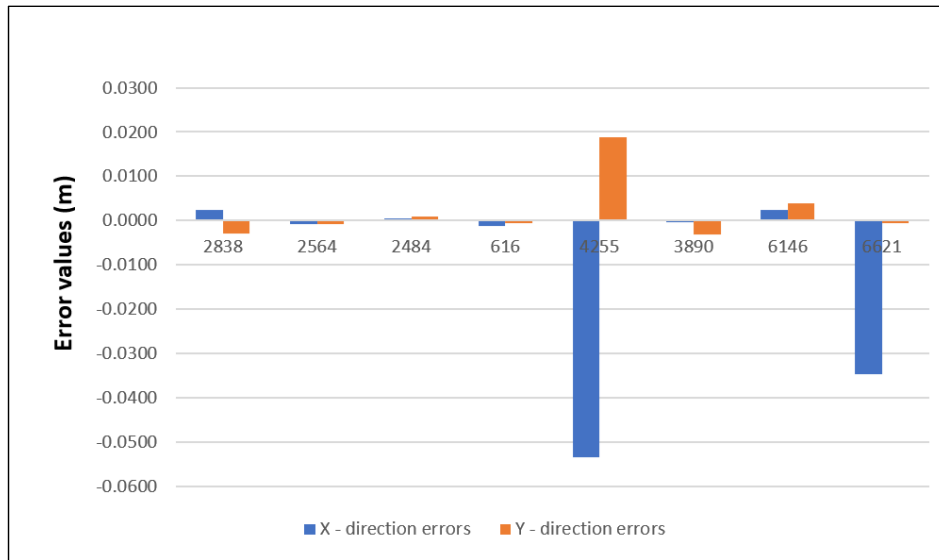


Figure 7. Coordinate differences of test points for the Affine transformation model.

If the deviations ΔS for each geodetic test point are represented for each transformation model, the situation shown in Figure 8 is obtained. The deviation ΔS represents the displacement vector at a point, which can be determined using the following equation:

$$\Delta S = \sqrt{\Delta X^2 + \Delta Y^2}, \quad (11)$$

where: $\Delta X = X_{MOLDREF99(register)} - X_{MOLDREF99(transformed)}$,

$\Delta Y = Y_{MOLDREF99(register)} - Y_{MOLDREF99(transformed)}$.

It can be observed that the histogram in Figure 6 is approximately identical to that in Figure 7, indicating that the coordinate differences of the points are the same, whether determined using the Helmert model or the Affine transformation model. The same situation can be seen in Figure 8 for the ΔS deviations of the test points.

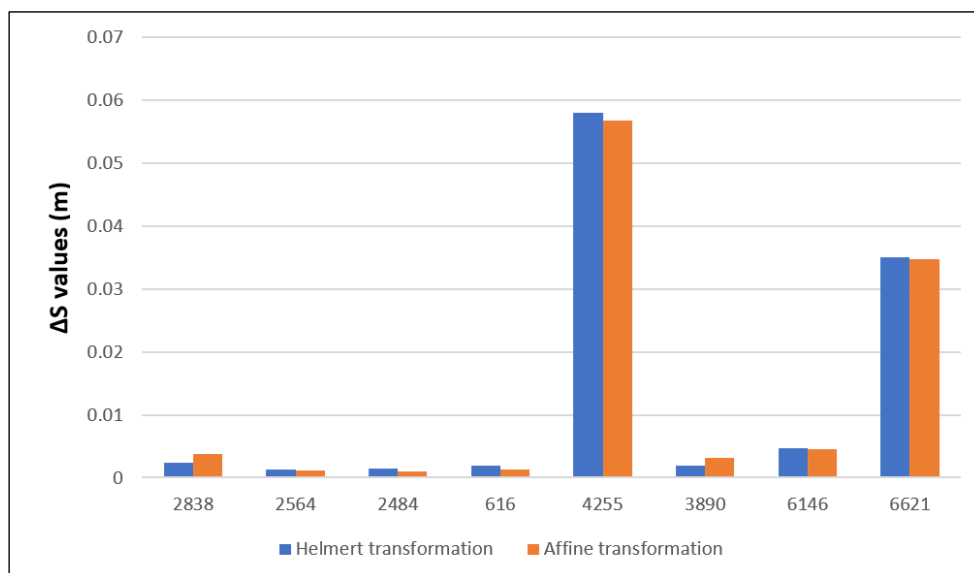


Figure 8. ΔS deviations of test points for the Helmert transformation and Affine transformation models.

It can be concluded that, for the 2D coordinate transformations from the local Soroca system to the MOLDREF99 system, both the Helmert and Affine transformation model parameters can be used, since the obtained error values are approximately equal in both cases.

4. Conclusions

The present study demonstrated the feasibility and efficiency of two-dimensional transformation methods for converting coordinates from the local Soroca system to the national MOLDREF99 system, using exclusively existing cartographic resources and the national geodetic database, without the need for direct field measurements.

The comparative analysis of the 2D Helmert transformation (with four parameters) and the 2D Affine transformation (with six parameters) highlighted that both mathematical models provide similar results in terms of accuracy. The mean square error obtained for both methods is approximately 0.002 m, confirming the reliability of the transformation process. Validation on the eight test points showed RMSE values of 0.0226 m in the X direction and 0.0080 m in the Y direction for the Helmert transformation, and 0.0226 m and 0.0069 m respectively for the Affine transformation. These results fall within the acceptable accuracy limits for cadastral and topographic works at the 1:500 scale.

The obtained results confirm that, in the absence of the possibility to directly verify geodetic benchmarks in the field, coordinate transformation can be successfully performed by utilizing archived topographic plans and the official GeoData database. The minimal differences between the results of the two transformation models indicate good geometric compatibility between the local Soroca system and the national MOLDREF99 system within the study area.

The methodology developed in this study can be extended and applied to process the entire collection of topographic plans preserved in national archives, thus contributing to the enhancement and enrichment of the National Spatial Data Infrastructure (NSDI) of the Republic of Moldova. The process of digitizing, georeferencing, and transforming historical cartographic materials represents a strategic investment in preserving and capitalizing on the

national geodetic heritage and ensuring the continuity of spatial data necessary for cadastral, territorial planning, and urban development projects.

Future research could focus on a comparative analysis of transformation accuracy across different geographic regions of the country, characterized by diverse relief conditions and varying geodetic network densities, as well as on the development of automated procedures for large-scale processing of archived topographic plans.

Conflicts of interest: The authors declare no conflict of interest.

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Citation: Vlasenco, A.; Şişcanu, C. Application of 2D transformation models for the conversion of historical topographic plans from Soroca city, Republic of Moldova. *Journal of Engineering Science*. 2025, XXXII (4), pp. 54-66. [https://doi.org/10.52326/jes.utm.2025.32\(4\).05](https://doi.org/10.52326/jes.utm.2025.32(4).05).

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Submission of manuscripts:

jes@meridian.utm.md

[https://doi.org/10.52326/jes.utm.2025.32\(4\).06](https://doi.org/10.52326/jes.utm.2025.32(4).06)

UDC 624.131.22: 624.15:624.042



THE ROLE OF STRUCTURAL COHESION IN CLAY SOILS IN THE EVALUATION OF SOIL BASES BEARING CAPACITY

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Received: 11. 04. 2025

Accepted: 12. 09. 2025

Abstract. The article examines the causes of bearing-capacity failures in foundations of buildings and structures situated on sloping areas in Republic of Moldova, focusing on the response of Sarmatian clay foundation soils, which exhibit time-dependent strength reduction and variations of shear resistance when subjected to prolonged loading. The experiments included tests on undisturbed monolithic samples using a direct shear apparatus at various levels of normal stress, as well as an evaluation of residual and long-term strength in accordance with the principles of the physico-technical theory of creep. The obtained data indicate that the investigated clays are characterized by a significant structural cohesion (average value of approximately 60kPa) and a relatively low internal friction angle of 7-12° (average around 9°). It was established that the application of long-term loads leads to a pronounced reduction in shear strength parameters: the long-term strength reaches approximately 40kPa, while the residual strength is on the order of 20kPa. Calculations of ultimate bearing capacity showed that, depending on the applied stress level, the permissible bearing capacity of the foundation may decrease by 2.5-3 times compared to the initially determined values. These findings highlight the need to account for time-dependent strength degradation when designing foundations on Sarmatian clays and emphasize the importance of accurately determining soil strength parameters, particularly the structural cohesion.

Keywords: *clay soils, stability, strength, structural cohesion, ultimate load.*

Rezumat. În articol sunt analizate cauzele pierderii capacității portante a terenurilor de fundare ale construcțiilor amplasate pe versanți sau zonele în pantă din Republica Moldova, cu un accent pe comportarea argilelor sarmatiene utilizate ca teren de fundare, care prezintă o reducere și o variație în timp a rezistenței la forfecare sub acțiunea sarcinilor permanente. Partea experimentală a inclus încercări pe probe monolitice neperturbate (stare naturală), utilizând aparatul de forfecare directă pentru diferite valori ale tensiunii normale, precum și evaluarea rezistenței reziduale și cea de lungă durată în conformitate cu principiile teoriei fizico-tehnice a fluajului. Datele obținute indică faptul că argilele studiate sunt caracterizate printr-o coeziune structurală ridicată (valoare medie de aproximativ 60 kPa) și un unghi de frecare internă relativ redus, cuprins între 7 – 12° (în medie circa 9°). S-a constatat că aplicarea sarcinilor de lungă durată conduce la o diminuare pronunțată a parametrilor de rezistență la forfecare: rezistența pe termen lung ajungând la aproximativ 40 kPa, iar rezistența reziduală

poate ajunge până la 20 kPa. Calculul capacității portante la starea limită ultimă a arătat că, în funcție de nivelul solicitării aplicate, capacitatea portantă admisibilă a fundației poate scădea de 2,5–3 ori în comparație cu valorile determinate inițial. Aceste rezultate subliniază necesitatea luării în considerare a degradării în timp a rezistenței pământului la proiectarea fundațiilor pe argile sarmatiene și evidențiază importanța determinării cât mai corecte a parametrilor de rezistență, în special a coeziunii structurale.

Cuvinte-cheie: *pământuri argiloase, stabilitate, rezistență, coeziune structurală, sarcină limită ultimă.*

1. Introduction

When designing buildings and engineering structures, a number of issues arise related to the quantitative assessment of overall stability and deformation of foundation soils.

The bearing capacity of the foundation is defined as the ultimate pressure exerted on the soil beneath the foundation at which shearing initiates and a failure/slip zone develops. Any further load increase leads to a loss of stability and, consequently, to catastrophic deformations (Figure 1).



Figure 1. Loss of stability of a residential building in Seoul, South Korea, 2014

Source: image taken from the website [1].

Terzaghi K. is rightfully regarded as the founder of theoretical research in this field [2, 3]. The theory developed by Terzaghi K. was later modified to take into account the shape and embedment depth of foundations, the magnitude of the applied load, and the eccentricity of loading [4-7].

Among the extensive body of scientific works in this area, notable contributions include those of Bartolomey [8], Berezantsev [9], Bogomolov [10], Vyalov [11], Goldshtein [12], Gorbunov-Posadov [13], Maslov [14], Ter-Martirosyan [15], Florin [16], Tsytoich [17], as well as Akai [18], Lundgren. and Mortensen [19], Meyerhof [20], Prandtl [21] and many others.

In particular, the studies conducted by Berezantsev [9], Bugrov [22], Malyshev [23,24], and other researchers have played a crucial role in ensuring the reliability and long-term performance of structures founded under complex engineering–geological conditions. Their contributions are grounded in systematic investigations and analytical developments within the fields of solid mechanics, as well as geology and hydrogeology.

A substantial contribution to the theory and practice of evaluating foundation bearing capacity and slope stability was made by the works of Maslov N.N. In particular, reference [14] presents the author's perspective on the potential loss of stability in foundations resulting from time-dependent reduction of soil strength.

With the development of an extensive experimental database and a robust mechanico-mathematical framework, it has become possible to address complex problems related to the interaction between structures and soil masses.

The studies of Kalaev [25], Ter-Martirosyan [15], Dobrov [26], Bogomolov [10], Karaulov [27], Dyba [28], and others are dedicated to modern numerical methods for analyzing the stress–strain state (SSS) of soil masses.

Within the framework of the present study, it is particularly important that these works not only enable the solution of specific practical problems but also make it possible to formulate and analyze mathematical experiments that account for the deformation and failure characteristics of the Sarmatian clays under investigation.

Shakirzyanov proposes employing a methodology for SSS analysis based on determining the ultimate load using the principles of limit equilibrium theory [29]. The method developed for assessing the bearing capacity of the “structure–soil” system incorporates a range of influencing factors, including the time-dependent reduction of soil strength parameters.

Based on an analysis of various factors affecting the stress state and bearing capacity of a two-layer foundation, Vaingolts [30] developed an engineering method that allows the determination of the design bearing resistance of foundations for the most commonly encountered geological conditions. The author's findings and the proposed method may be applied in Republic of Moldova, as most foundation soils in the region are represented by sandy–clayey sequences.

This brief review of the literature indicates that the established principles of soil mechanics inevitably introduce certain simplifications and idealizations of soil properties, whereas the actual behavior of soils in a foundation under structural loading is considerably more complex.

As a classical example of foundation instability, the case most frequently cited is the leaning tower in Pisa, Italy [31, 32]. The main causes of the excessive settlements and differential deformations are attributed to the presence of weak clay layers beneath the foundation and a high groundwater level. Stabilization was achieved by controlled soil excavation on the northern side of the structure.

However, analysis of the literature sources has shown that, in addition to this well-known case from Italy, several other examples convincingly demonstrate the necessity of a timely and properly executed assessment of foundation bearing capacity.

This can be illustrated by the construction of a high-rise building in San Francisco, USA [33, 34]. Since its completion in 2009, the Millennium Tower has settled more than 40cm. The deformations developed non-uniformly, resulting in a lateral tilt of approximately 35cm. The tower has consequently been nicknamed the “Leaning Tower of San Francisco.” Unfortunately, even after repair and remediation works amounting to 100 million USD, the deformation process has continued.

The reasons for the observed behavior of the tower can be attributed to the following factors:

1. **Geological conditions classifying the foundation as “weak”.** The tower was built in an area underlain by former marine deposits known as Bay Mud - soft, water-saturated clays and silts capable of undergoing significant deformation under long-term loading (in this case, from the building's own weight).

2. **Incorrectly selected foundation type.** Instead of pile foundations resting on rock formations and functioning as end-bearing piles, the designers employed friction piles (“floating piles”) resting on sandy-clayey soils at depths of 20–30 m. Although this choice reduced construction costs, but made the building vulnerable to slow, weakly attenuating deformations of the underlying soft sediments.

3. **The exceptionally large loads transmitted to the “weak foundation”.** The tower consists of 58 stories, with a height of nearly 200 m and a total weight reaching approximately 700,000 t. Naturally, such loading induced consolidation of the water-saturated clays, resulting in excessive settlement.

4. **The influence of the nearby foundations of the Salesforce Transit Center.** During its construction, deep excavations and groundwater pumping were carried out. The sharp change of hydrogeological conditions accelerated the settlement of the tower and contributed to its lateral tilting.

5. **Improperly selected foundation strengthening the foundation and foundation soils.** In 2020–2022, a project was implemented to install additional piles designed to transfer loads directly to bedrock. However, during the drilling operations and installation of these additional piles the foundation soils were compacted again. The settlement continued and, according to the most recent data, has reached approximately 45 cm.

As a result, increasing the number of piles, by installing additional ones bearing on the rock, did not stop the settlement process. Currently, engineers are developing another project that should stop the settlement of the existing complex foundation and contribute to the equalization of the pressure transmitted to the foundation soils.

In the given examples, plastic clays served as the foundation soils. As shown by the analysis of the literature sources and by the results of previously performed studies by the authors of the present paper [35–38], most slopes in the central and northern parts of Republic of Moldova are composed of Sarmatian clays in a stiff to semi-stiff state. Despite this circumstance, it often creates difficulties for designers when selecting the design strength parameters for evaluating the bearing capacity of the foundation soils. For example, during construction on clayey soils, creep processes and strength reduction under long-term loading are very often not taken into account.

In most cases, a certain schematization of soil behavior under loading, in accordance with the applicable design standards, provides quite acceptable results. However, there are numerous examples where ignoring the mentioned soil-specific characteristics in calculations has led to irreversible consequences. Some of these include the deformations of several private buildings in the Codru town, Chişinău municipality; the deformation of an embankment section on the Ialoveni bypass; the loss of stability of a wind turbine foundation in the Varzăreşti village; and the overturning of a retaining wall near the “Valea Morilor” lake in Chişinău.

There are also other examples which, along with those mentioned above, indicate the need for closer attention to studying the nature of strength of clay soils and, in particular, the role of structural cohesion in assessing long-term strength and the bearing capacity of the foundation soils.

2. Theoretical Principles

The methodological framework of the theoretical investigation is formed by contemporary principles of soil mechanics for determining stresses in soil masses, as well as in conceptual approaches addressing the potential reduction of soil strength over time.

In solving various geotechnical and structural engineering tasks, it is essential to determine the magnitude of load that can be safely transmitted to the foundation soil. The applied bearing pressure depends on the loaded area. If the soil is unable to sustain the required load, structural safety must be ensured by increasing the bearing area of the foundation. Naturally, the size of the foundation should be increased within reasonable limits. The ultimate objective of the designer is to specify the minimum feasible foundation dimensions while simultaneously ensuring the strength and stability of the foundation soil and limiting potential settlements.

Upon application of an external load, a complex stress state develops almost instantaneously within the soil mass. Under the action of normal stress P_n a compaction process is initiated, which results in the settlement of the structure (Figures 2 and 3). This process has an attenuation nature.

As the applied load increases to value P_2 further soil compaction and settlement occur, but at a lower intensity (the soil progressively compacts).

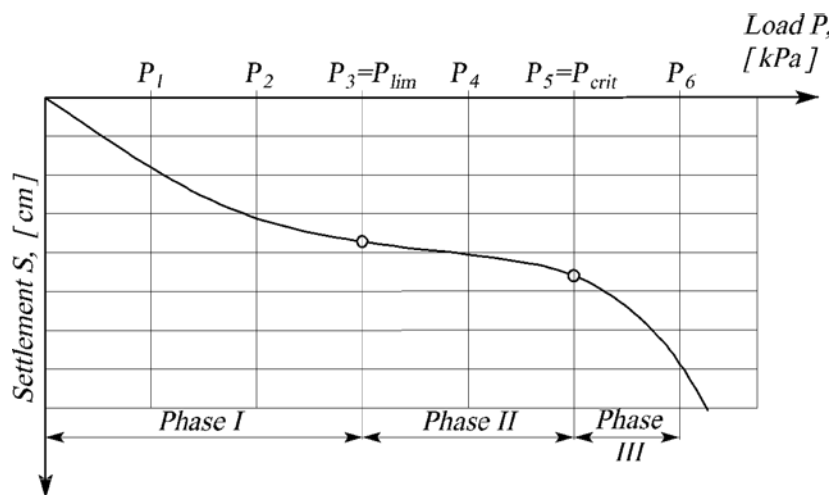


Figure 2. Three phases of loading and deformation of a structure's foundation [26, p. 120].

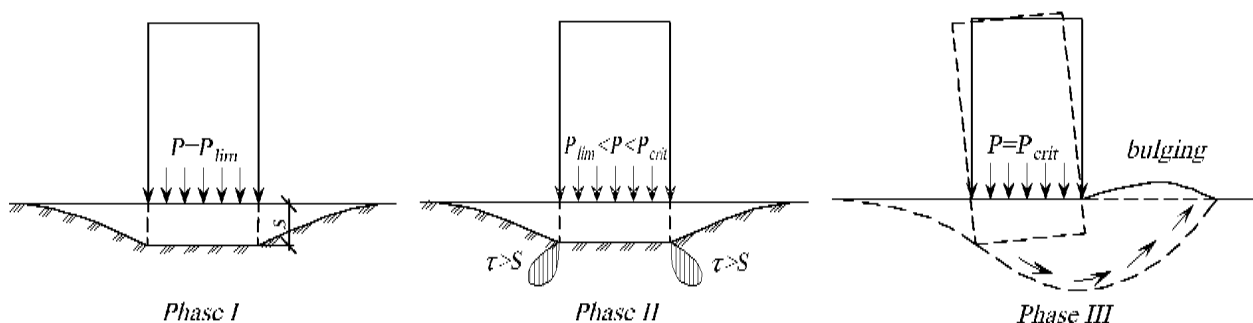


Figure 3. Three phases of soil behavior in a foundation: compaction, local shear, and deepbulging [26, p. 120].

Within the pressure interval $0 < P < P_{lim}$, the contribution of shear stresses is almost reduced to zero. These conditions define the first phase of soil behavior at the base of the structure. In this phase – commonly referred to as the soil compaction (or consolidation)

phase under structural loading – the soil strength and the stability of the foundation system are fully ensured.

With a further increase in load ($P > P_3$) progressive settlement of the structure begins, associated not only with soil compaction but also with local strength failures. Zones appear where $\tau > S$ (shear stress τ exceeds the soil strength S). The load corresponding to the initial stage of development of these local shear zones is called the limit load ($P_3 = P_{lim}$). The corresponding soil behavior conditions define the Phase II, known as the strength failure phase or local shearing phase. Such soil conditions in the building foundation are not acceptable in all cases. The resulting settlement is limited to certain limits that ensure the normal operational performance of the structure ($S < S_u$).

With a further increase in load, the progressive development of local failure zones (shear zones) occurs. These zones extend to increasing depths and, finally, at $P_5 = P_{crit}$, a usually sudden and catastrophic failure of overall foundation stability takes place, associated with soil uplift. This load is called critical load. The process corresponds to the Phase III of soil behavior in the foundation, called phase of general failure of the foundation or the phase of global shear and soil uplift. It is evident that Phase III must be excluded under all circumstances.

Returning to Phase II, under certain conditions, local shear zones develop in the soil. Their growth must be limited, which is achieved by reducing the pressure transmitted to the foundation soil. In foundation design, it is assumed that the depth of local shear zones should not exceed 1/4 of the foundation width ($h \leq b/4$). The pressure at which the local failure zones extend to a depth $h \leq b/4$ is called the design bearing capacity of the foundation soil R . The fundamental inequality that must be satisfied in calculations is therefore:

$$P \leq R, \quad (1)$$

where: P – average pressure under the foundation base, kPa;

R – design bearing capacity of the foundation soil, kPa.

The design bearing capacity R is determined based on the equations proposed by Berezantsev, according to cl. 2.41 [39]:

$$R = \frac{\gamma_{c1} \cdot \gamma_{c2}}{k} (M_\gamma \cdot k_z \cdot b \cdot \gamma_{II} + M_q \cdot d \cdot \gamma'_{II} + M_c \cdot C_{II}), \quad (2)$$

where: γ_{c1}, γ_{c2} – coefficients of working conditions;

M_γ, M_q, M_c – coefficients depending on the value of the angle of internal friction φ of the soil under foundation base;

k_z – coefficient taken as $k_z = 1$ for $b < 10$ m, and $k_z = 8/b + 0.2$ for $b \geq 10$ m;

b – width of the foundation base, m;

γ_{II} – design value of the unit weight of the soil lying below the foundation base (considering the buoyant effect of groundwater, if present), kN/m³;

γ'_{II} – average unit weight of the soil layers above the foundation base, kN/m³;

d – foundation depth embedment from the ground surface, m;

C_{II} – design value of the cohesion of the soil directly below the foundation base, kPa;

k – coefficient equal to 1.0 if the soil parameters φ_{II} and C_{II} are determined by direct testing, and $k = 1.1$ if they are adopted approximately from reference tables.

As seen from Equation (2), the design bearing capacity depends on a number of factors: the structural system of the building, the foundation embedment depth, the foundation base width, the unit weight of the soil, and its strength parameters.

A preliminary analysis showed that, in most cases, the role of cohesion is decisive.

It should be emphasized that incorrect estimation of this strength parameter has often led to loss of bearing capacity and excessive deformations of buildings and structures.

The main design error consisted in underestimating the potential decrease of cohesion (and overall strength) over time due to various factors such as:

- changes in the moisture regime;
- creep deformations;
- the presence of weakened zones within the soil thickness, etc.

In light of the above, this study investigates the role of soil cohesion in determining the safe, permissible, and critical load transmitted to the foundation soil.

For this purpose, analytical relationships given in sources [15,25,40] were applied.

The safe load P_{safe} , determined with a significant margin of safety and therefore unquestionably acceptable for the structure, was established for the case of a uniformly distributed load according to the following formula:

$$P_{safe} \approx \pi \cdot C_w \approx 3C_w. \quad (3)$$

The permissible load P_{perm} was limited by the inequality:

$$P_{perm} \leq R, \quad (4)$$

where: R – is the design bearing capacity of the foundation soil, kPa.

To determine the critical load P_{crit} , approximate relationships were used as proposed by Prandtl, Terzaghi, Shieldt and Pauker, and presented by Maslov [40], which can be expressed as follows:

$$P_{crit} = (\pi + 2.0) \cdot C = 5.14 \cdot C, \quad (5)$$

where $C = \Sigma \omega$ – hydro-colloidal cohesion, kPa.

$$P_{crit} = 5.7 \cdot C, \quad (6)$$

$$P_{crit} = (5.14 + b/l) \cdot C, \quad (7)$$

where b – half the width of the loaded area, m; l – length, m.

$$P_{crit} = \gamma \cdot (d + h_c) \cdot tg^4 \left(45 + \frac{\varphi}{2} \right), \quad (8)$$

where γ – weight density of soil, kN/m³; d – foundation embedment depth, m; φ – angle of shearing resistance, degrees; C – cohesion intercept, kPa.

$$h_c = \frac{C}{\gamma \cdot tg \varphi}. \quad (9)$$

The assessment of soil strength was carried out based on the premise that, depending on the operating conditions of the foundation, their strength parameters may vary. At the same time, shear stresses in all cases adversely affect the working conditions of the soils.

For the calculations, the constitutive framework of the Physico-Technical Theory of Creep (PTTC) [11] was used.

The shear strength was determined using the following expression:

$$S_{pw} = P_n \cdot tg \varphi_w + C_w, \quad (10)$$

where: P_n – normal stress, kPa; φ_w – angle of shearing resistance corresponding to the “density–moisture” state; C_w – total cohesion intercept, kPa.

$$C_w = C_c + \Sigma_w, \quad (11)$$

where: C_c – rigid structural cohesion, kPa; Σ_w – hydro-colloidal soil cohesion, kPa.

The strength of clay soils may vary under the influence of various factors. To identify the role of structural cohesion and moisture content, a series of tests were performed: direct shear tests on samples in their natural state, with a prepared and water-moistened failure surface, as well as on samples pre-moistened in consolidation rings.

The tests conducted simulated the behavior of the soil under conditions of existing cracks and slip surfaces within the soil mass, as well as potential reductions of cohesion due to the degradation of structural cohesion C_c and hydro-colloidal cohesion Σ_w caused by changes in moisture content within the shear zone.

The long-term strength of quasi-plastic clays was determined from the following expression:

$$S_\infty = \sigma \cdot \operatorname{tg} \varphi_w + \Sigma_w. \quad (12)$$

The residual strength values were determined from shear tests performed on samples with a prepared and moistened shear surface:

$$S_{res} = \sigma \cdot \operatorname{tg} \varphi_{w,res} + \Sigma_{w,res}. \quad (13)$$

3. Results and Discussion

The soil property investigations were carried out in the “Ingeotech-Grup” laboratory. Within the objectives of the study, the primary task consisted in determining the strength parameters of clay soils and demonstrating the possibility of their reduction due to the degradation of structural cohesion. The direct shear tests were performed using standard direct shear apparatus under various normal consolidation pressures. Undisturbed block samples (monoliths) were collected from a site located in Codru town.

Analysis of the obtained results showed the following:

1. The strength of the investigated samples in their natural state is characterized by high values:

- total cohesion $C_w=70-90$ kPa, with an average value - $C_{w,avg}=83$ kPa;
- angle of shearing resistance $\varphi=7-12^\circ$, with an average value - $\varphi_{avg}=9^\circ$.

2. The average value of structural cohesion is $C_c=60$ kPa; the hydro-colloidal cohesion component $\Sigma_w=23$ kPa.

3. Under conditions where structural cohesion may be reduced due to creep-induced deformations on the slope, the long-term shear strength can be described by the following relationship:

$$S_\infty = \sigma \cdot \operatorname{tg} 9^\circ + 23, kPa \quad (14)$$

4. Further reduction is possible due to a significant increase in moisture content within the shear zone, along the slip surface. The residual strength equation is expressed as:

$$S_\infty = \sigma \cdot \operatorname{tg} 5^\circ + 10, kPa \quad (15)$$

At the design pressure under the foundation base specified by the designers, $\sigma = 100$ kPa (while in practice the contact pressure commonly ranges between 150–250 kPa), using

the equations presented above, was obtained that the average strength of $S_{avg}=100$ kPa. At values of shear stress exceeding the creep threshold (in this case $\tau_{lim}=76$ kPa), and under conditions of complete destruction of structural cohesion, the long-term shear strength becomes $S_{\infty}=39$ kPa, and the minimum residual strength is $S_{res}=19$ kPa.

The obtained strength values for the investigated clays characterize the foundation soil as unreliable.

The conducted investigations convincingly confirm that the total cohesion in clay soils, in this case Sarmatian clays, is represented by a rigid, irreversible component of structural cohesion C_c and hydro-colloidal cohesion Σ_w . Structural cohesion may be partially or completely destroyed under the action of shear stresses, while hydro-colloidal cohesion may decrease to residual values as a result of increased moisture.

The possible strength reduction inevitably affects the permissible bearing pressures considered when determining the dimensions of foundation footings. Tables 1-4 present the final results of determining the safe, permissible and critical bearing pressures corresponding to different cohesion values.

The calculations were performed using the following input parameters:

- foundation footing width: $B=2b=2.0$ m;
- foundation embedment depth: $d=2.5$ m;
- average contact pressure under the footing: $P=250$ kPa;
- angle of shearing resistance: $\varphi=9^\circ$;
- cohesion: $C=10-100$ kPa.

Table 1

Results of Determining the Critical Load P_{crit}							
Nr.	Method (formula)	Values of the critical load for cohesion C , kPa					
		100	80	60	40	20	10
1*	Prandtl–Tsytovic method (plane strain problem) for a purely cohesive soil $P_{crit} = (\pi + 2.0) \cdot C + \gamma_w \cdot d = 5.14C + \gamma_w \cdot d$	563	460	357	255	152	100
2*	Terzaghi method (plane strain problem) taking into account the roughness of the foundation base $P_{crit} = 5.7C + \gamma_w \cdot d$	619	505	391	277	163	106
3*	Ishlinsky method (for foundations with a square footing) $P_{crit} = 5.71C + \gamma_w \cdot d$	620	506	392	277	163	106
4*	Hencky method (under a rigid circular stamp) – “generalized form” $P_{crit} = 5.64C + \gamma_w \cdot d$	613	500	387	275	162	105
5*	Schildt method (for rectangular foundations) and $l \rightarrow \infty$ (plane strain problem) $P_{crit} = \left(5.14C + \frac{b}{l}\right) \cdot C + \gamma_w \cdot d$	563	460	357	255	152	100
6*	Prandtl method (plane strain problem) for soils with $\phi \neq 0$ $P_{crit} = \frac{C}{tg\varphi} \left(tg^2 \left(45^\circ + \frac{\varphi}{2} \right) \cdot e^{\pi \cdot tg\varphi} - 1 \right) + \gamma_w \cdot d$	841	683	524	366	207	128

Continuation Table 1

7	Pauker–Maslov method		1279	1041	804	567	329	211
	$P_{crit} = \gamma_w \left(d + \frac{C}{\gamma_w \cdot tg\varphi} \right) \cdot tg^4 \left(45^\circ + \frac{\varphi}{2} \right)$							
8**	Terzaghi K. method		1037	855	674	492	310	219
	$P_{crit} = \gamma_w \cdot b \cdot N_\gamma + \gamma_w \cdot d \cdot N_p + C \cdot N_c$							

Note: The formulas presented in the Table can be found in [40, pp. 340–342, 350, 355]; * – changes made: Since the formulas presented for determining the critical load were developed for the case of direct surface loading ($d=0$), the authors considered it necessary, in accordance with the recommendations of TsytovichN.A., to introduce corrections to these expressions by including the pressure due to the self-weight of the soil located above the foundation base; ** – the values of the coefficients N_γ , N_p , and N_c were selected from [41] for $\varphi=9^\circ$.

Table 2

Results of Determining the Design Bearing Resistance of the Foundation Soil R

Nr.	Method (formula)	Values of the design bearing resistance of the foundation soil for cohesion C , kPa					
		100	80	60	40	20	10
1	According to [30]						
	$R = \frac{\gamma_{c1} \cdot \gamma_{c2}}{k} (M_\gamma \cdot k_z \cdot 2b \cdot \gamma_{II} + M_q \cdot d \cdot \gamma'_{II} + M_c \cdot C_{II})$	615	513	412	311	210	159

Note: The formula used in the calculations is given in cl. 2.41 [39]; for $l_c < 0.25$, the values of the coefficients are: $\gamma_{c1}=1.25$ and $\gamma_{c2}=1.0$, for $\varphi=9^\circ$ the values of the coefficients are: $M_\gamma=0.16$, $M_q=1.64$, $M_c=4.05$.

Table 3

Results of Determining the Safe Load P_{safe}

Nr.	Method (formula)	Values of the safe load for cohesion C , kPa					
		100	80	60	40	20	10
1*	Case of uniformly distributed load						
	$P_{safe} = \pi C_w + \gamma_w d = 3.14 C_w + \gamma_w d$	349	289	229	169	109	79
2*	Case of triangular distributed load						
	$P_{safe} = 4 C_w + \gamma_w d$	449	369	289	209	129	89
3*	Puzyrevsky formula						
	$P_{safe} = \frac{\pi \gamma_w \left(d + \frac{C}{\gamma_w \cdot tg\varphi} \right)}{ctg\varphi + \varphi - \frac{\pi}{2}} + \gamma_w d$	485	404	323	242	161	121

Note: The formulas presented in the table are given in [40, pp. 336, 341]. * – changes made: pressure from the weight of soil above the foundation base has been included.

Table 4

Results of Determining the Permissible Load P_{perm}

Nr.	Method (formula)	Values of the permissible load for cohesion C , kPa					
		100	80	60	40	20	10
1	Maslov method						
	$P_{perm} = \frac{\pi \cdot \gamma_w \cdot \left(2b \cdot tg\varphi + d + \frac{C}{\gamma_w \cdot tg\varphi} \right)}{ctg\varphi + \varphi - \frac{\pi}{2}} + \gamma_w d$	489	408	327	246	165	125

Continuation Table 4

2	Pauker–Maslov method						
	$P_{perm} = \frac{1}{k_{safe}} \cdot \gamma_w \left(d + \frac{C}{\gamma_w \cdot tg\varphi} \right) \cdot tg^4 \left(45^\circ + \frac{\varphi}{2} \right)$	639	521	402	283	165	105
	for $k_{safe} = 2.0 \in (1.5 \dots 2.0)$						
3	Terzaghi method						
	$P_{perm} = \frac{1}{k_{safe}} (\gamma_w \cdot b \cdot N_\gamma + \gamma_w \cdot d \cdot N_p + C \cdot N_c)$	519	428	337	246	155	110
	for $k_{safe} = 2.0 \in (2.0 \dots 3.0)$						

Note: The 1st and the 2nd formulas presented in the table are given in [40, p. 336, 350], the 3rd is determined by dividing the 8th formula from Table 1 by the safety coefficient k_{safe} , according to provides from [40, p. 356];

Comparison of the obtained values (see Tables 3 and 4) shows that determining the design soil resistance R according to clause 2.41 [39] allows an increase of the safe load calculated using Puzyrevsky's formula by a factor of 1.25.

It should be recalled that the behavior of the foundation is considered in Phase II.

It is noteworthy that the value of the design resistance corresponding to the long-term strength of the soil is approximately 225 kPa, which is 2.3 times lower than the value obtained at the average cohesion in the natural state ($C_{w,avg}=83$ kPa, $R=528$ kPa).

Thus, the commonly adopted value for the average pressure under the foundation base, equal to 200kPa, can be considered fully justified.

For preliminary calculations of foundation base dimensions, ensuring the bearing capacity of the soil, Puzyrevsky's formula may be used with cohesion values corresponding to the long-term strength of the soil (in presented case, $\Sigma_w=23$ kPa)

Analysis of the results showed that the pressure transmitted to the foundation, taking into account the reduction of soil strength over time, should be reduced by a factor of 2.5–3.0 relative to the value obtained at the average strength at the design stage.

This circumstance indicates the necessity, when calculating foundation dimensions and evaluating the bearing capacity of the soil, to account for the presence of weakened zones with disrupted structural cohesion within the Sarmatian clay layer.

4. Conclusions

1. In the design of buildings and structures located on sloped terrains in Republic of Moldova, the calculation of the ultimate load transmitted from foundations to the soil is a fundamental task. The reliability of this value determines both the safe operation of the structures and the economic efficiency of the adopted design solutions.

2. In engineering practice, methods based on the theory of ultimate soil equilibrium are still widely used to calculate bearing capacity. Calculations performed using this theory rely on strength characteristics such as structural cohesion, hydro-colloidal cohesion component, and internal friction forces.

3. When determining the ultimate load, it is necessary to account for the potential reduction in the strength of clay soils (Sarmatian clays) due to the disruption of structural cohesion under creep deformation and the decrease in hydro-colloidal cohesion caused by additional soil moisture.

4. To identify the causes of structural cohesion reduction (manifestation of seismo-gravitational processes, development of creep deformations on slopes, excessive vertical loads, etc.), specialized studies should be conducted at the stage of technical justification.

Due to the anticipated increase in loads on the soil caused by the construction of high-rise buildings (more than 20 floors), it is advisable in the future to consider determining soil strength as a function of the actual external load acting on the foundation base.

Acknowledgments: The present research was conducted as part of the scientific research project “020406. *Models, systems, and technologies for energy efficiency, decarbonization, and digitalization of processes in energy, industry, construction, and transport*” under the supervision of Dr. Ilie Nucă, with participation of the Department of Civil Engineering and Geodesy at the Technical University of Moldova. The authors express their sincere gratitude to Dr. Ceban O. for his assistance in conducting experimental research at the geotechnical laboratory “Ingeoteh-Grup” SRL.

Conflicts of Interest: The authors declare no conflict of interest.

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Citation: Polcanov V.; Cîrlan A. The role of structural cohesion in clay soils in the evaluation of foundation bearing capacity. *Journal of Engineering Science*. 2025, XXXII (4), pp. 67-79. [https://doi.org/10.52326/jes.utm.2025.32\(4\).06](https://doi.org/10.52326/jes.utm.2025.32(4).06).

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Submission of manuscripts:

jes@meridian.utm.md

[https://doi.org/10.52326/jes.utm.2025.32\(4\).07](https://doi.org/10.52326/jes.utm.2025.32(4).07)

UDC 664:577.112:633:612.392.7



PLANT-BASED PROTEINS: SOURCES, EXTRACTION METHODS AND FUNCTIONAL PERSPECTIVES FOR THE SUSTAINABLE FOOD INDUSTRY

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Received: 11. 09. 2025

Accepted: 12. 05. 2025

Abstract. Current research in the food industry highlights the importance of alternative protein sources in the context of global population growth and the need to reduce the negative impact of raising animals, producing animal proteins, on the environment. Plant proteins represent a sustainable, affordable and beneficial option for health, with high potential to replace animal proteins in the food industry. Plant sources rich in protein include legumes, cereals, pseudocereals, oilseeds, green leaves, algae and, more recently, insects. They differ considerably in protein content and amino acid profile, and their strategic combination can ensure a balanced intake of essential nutrients. Legumes offer high protein content, but are deficient in sulfur amino acids. Cereals, although lower in protein, bring technological benefits and can complement the amino acids missing from legumes. Pseudocereals have a higher nutritional value than conventional cereals, while green leaves and algae offer a high proteins intake and compounds with antioxidant potential, although they require advanced technologies to overcome extraction limitations. Extraction methods play a decisive role in the yield and quality of plant proteins. Conventional techniques are efficient but can cause denaturation and provide low yields. On the other hand, emerging methods allow the recovery of proteins in larger quantities, preserving their functional and technological properties.

Keywords: *cereals, green leaves, green technologies, legumes, pseudocereals, techno-functional characteristics.*

Rezumat. Cercetările actuale în domeniul industriei alimentare evidențiază importanța surselor alternative de proteine în contextul creșterii populației globale și al necesității de a reduce impactul negativ al creșterii animalelor, producătoare de proteine de origine animală, asupra mediului. Proteinele vegetale reprezintă o opțiune sustenabilă, accesibilă și benefică pentru sănătate, cu potențial înalt de a înlocui proteinele animale în industria alimentară. Sursele vegetale bogate în proteine includ leguminoasele, cerealele, pseudocerealele, oleaginoasele, frunzele verzi, algele și, mai recent, insectele. Acestea diferă considerabil prin conținutul proteic și profilul de aminoacizi, iar combinarea lor strategică poate asigura un

aport echilibrat de nutrienți esențiali. Leguminoasele oferă un conținut proteic ridicat, dar sunt deficitare în aminoacizi sulfurați. Cerealele, deși mai sărace în proteine, aduc beneficii tehnologice și pot completa aminoacizii lipsă din leguminoase. Pseudocerealele prezintă o valoare nutritivă superioară cerealelor convenționale, în timp ce frunzele verzi și algele oferă un aportului înalt de proteine și compuși cu potențial antioxidant, deși necesită tehnologii avansate pentru a depăși limitările de extracție. Metodele de extracție au un rol determinant asupra randamentului și calității proteinelor vegetale. Tehnicile convenționale sunt eficiente dar pot cauza denaturări și oferi randamente scăzute. Pe de altă parte, metodele emergente permit recuperarea proteinelor în cantități mai mari, păstrarea proprietățile lor funcționale și tehnologice.

1. Introduction

Plant-based proteins offer a sustainable, economical and health-conscious alternative to animal proteins. They can be consumed directly as a source of protein or incorporated into various food products to balance amino acid profiles and improve nutritional values. Due to their rich nutritional content, including their more affordable price, plant-based protein sources have managed to become an attractive and innovative resource in the food industry [1-3]. Research aimed at meeting the rising demand for protein underscores the importance of alternative protein sources. Protein is increasingly recognized by consumers as a valuable nutrient, driving growing demand for both plant- and animal-based proteins [4]. According to Machovina et al., meat production is an extremely intensive and unsustainable activity that led to the deterioration of the planet's environment, leading to deforestation, pollution, negative impact on hydrogeological reserves and the disappearance of biodiversity [5].

Recently, there has been growing interest in developing food products that offer both physiological and nutritional benefits. The source of protein significantly influences nutrient availability: animal proteins generally exhibit higher digestibility and provide all essential amino acids, whereas plant-based proteins often contain anti-nutritional compounds that limit digestibility and are deficient in certain amino acids, such as lysine, tryptophan, cysteine, and methionine [6].

Health-promoting potential of plant-based meat alternatives is linked to dietary shifts, population growth, and the state of natural resources and biodiversity. In 2017, the global population was approximately 7.5 billion, and the United Nations projects it will reach 8.5 billion by 2030. To meet the protein demands of an estimated 9 billion people by 2050, meat production is expected to increase by 50–73%, highlighting the need for sustainable alternatives such as plant-based meat products [7]. According to Gerber et al. the livestock sector accounts for 14.5% of human-caused greenhouse gas emissions and consumes approximately 30% of the world's freshwater resources [8]. Population growth drives increased food production, placing significant strain on the sustainability of the current food system. The combination of rapid population expansion and the inefficiency of existing food patterns create a critical challenge: ensuring adequate nutrition for a growing global population.

Scarborough et al. reported that diets high in meat are associated with greater carbon dioxide emissions, averaging 7.19 kg CO₂ equivalent per day—more than twice the emissions from a vegan diet, which averages 2.89 kg CO₂ equivalent per day. The study concluded that reducing the consumption of meat and other animal products is an effective strategy to mitigate climate change [9].

Umerous factors contribute to the decline in meat consumption at both individual and global levels. A key concern is the rising global production of protein, particularly high-quality animal protein, which poses challenges for environmental sustainability. Nevertheless, despite growing consumer awareness, only a small proportion of the population has adopted a meat-free diet, with approximately 5% following a vegetarian diet and 3% adhering to a vegan diet [10-12].

One strategy to support the reduction of meat consumption is to offer meat substitutes. According of Ulhas et al. and Akyuz et al. where due to their rich nutritional content, affordable price, plant-based protein sources have become an attractive source in the food industry. Research efforts addressing ways to meet the growing demand for protein highlight the importance of alternative protein sources [1,2].

The study realized by Bouchard et al. highlighted the impact of the biological properties of plant proteins on overall health and well-being. These effects include antidiabetic, anticancer, antioxidant, and nephroprotective activities, as well as the ability to reduce risk factors for cardio-metabolic diseases, regulate appetite and lipid metabolism, and modulate the intestinal microbiome [13].

Despite all the benefits offered, the use of plant-based proteins in the food industry remains a challenge due to their technological properties, such as solubility, emulsification and gelling properties, and water retention capacity, which are lower than those of animal proteins. To overcome these disadvantages, various methods have been applied to modify and improve the techno-functional characteristics, thus enhancing their use as nutritional ingredients.

The purpose of this study was to identify and characterize protein-rich plant sources, conventional and unconventional extraction methods, and describe the physicochemical and functional properties of plant-based proteins.

2. Plant Protein Sources

Globally, approximately 80% of dietary energy and 65% of dietary protein are provided by foods of plant origin [14]. Plant-based proteins are complex and differ in quality characteristics.

2.1 Legumes

Legumes (such as beans, peas, chickpeas, and lentils) are a primary source of dietary protein and play a vital role in the food supply, particularly in developing countries [15-17]. Legumes contain approximately 21-25% protein. These values can vary slightly depending on genetic factors, the maturity of the plant, and environmental conditions [4]. Legumes are rich in lysine, leucine, aspartic acid, glutamic acid and arginine, but poor in sulfur-containing amino acids (cysteine, methionine and tryptophan). When consumed together with cereals, which are rich in sulfur-containing amino acids, they provide a balanced profile of essential amino acids to meet human nutritional needs [18].

Legumes are rich in bioactive compounds such as enzyme inhibitors, lectins, phytates, and phenolic compounds, which serve as part of the seed's defense system and may influence various biological functions when consumed. Certain substances, including enzyme inhibitors and lectins, are regarded as antinutritional factors due to their ability to decrease protein digestibility and impair nutrient absorption [19]. However, this effect can be reduced by cooking food before consumption [20]. On the other hand, health benefits have been associated with the same bioactive compounds. Regular consumption of legumes is

associated with reduced cholesterol levels and a reduced risk of cardiovascular disease, cancer, and diabetes [16,21-23]. Given these characteristics, there is increasing interest in incorporating legumes into the development of health-oriented ingredients, such as flour blends and extruded snacks, with the goal of producing functional and nutritious foods. Soy and pea proteins are among the most widely utilized plant-based sources, valued for their favorable functional properties, including water retention, gelation, fat absorption, and emulsification capacity. Other legume proteins—such as those from lentils, lupins, chickpeas, peas, mung beans, and fava beans—are also being investigated for their physicochemical properties, particularly foam stability, emulsification capacity, and gel formation [24]. Soy protein is historically the best-known alternative to animal proteins [25]. The study by Zhou et al. showed that soybean seeds contain approximately 42.95 to 46.32% of proteins which include several amino acids (g/100 g protein), such as alanine (3.59), arginine (6.67), aspartic acid (10.2), cystine (1.46), glutamic acid (17.45), glycine (3.6), histidine (2.3), isoleucine (4.25), leucine (6.78), lysine (5.33), methionine (1.13), phenylalanine (4.59), proline (4.96), serine (4.59), threonine (3.14), tryptophan (1.12), tyrosine (3.22) and valine (4.1) [26].

2.2 Cereals

Cereals (wheat, corn, rice, barley, sorghum, etc.) provide 62% of the world's food supply, being the main staple foods consumed globally, especially in developing countries, where the average protein intake is below recommended values [27]. Compared to legumes, cereal grains contain little protein, averaging about 10–12% on a dry weight basis [28], however, they are widely used in the food industry due to their functional properties. More than 50% of the total protein content of mature cereal seeds consists of storage proteins (prolamins and glutelins), which are rich in glutamine, proline, leucine and alanine. On the other hand, the essential amino acids lysine, tryptophan, methionine and histidine are limiting [29,30]. Wheat is the largest source of plant-based protein in the diets of western consumers and is widely used in the manufacture of everyday food products. Wheat protein is not utilized as efficiently as animal protein, and when consumed as the sole protein source, relatively large amounts are required to meet human physiological demands for both total protein and specific essential amino acids. In particular, lysine, threonine, and tryptophan are frequently limiting in wheat-based foods. Nevertheless, when combined with other dietary proteins such as those from legumes, oilseeds, or animal products, wheat proteins demonstrate excellent nutritional complementarity [31]. The digestibility of wheat proteins is reported to be approximately 90% [32]. Another study conducted by Juliano et al. [33] showed that the protein content of different varieties of brown rice ranges from 6.8 to 11.2%. According to Kalman [34] the predominant amino acids in brown rice include glutamic acid (13.9 g/100g), aspartic acid (6.94 g/100g), leucine (6.41 g/100g), arginine (6.32 g/100g), valine (4.56 g/100g), alanine (4.47 g/100g), phenylalanine (4.41 g/100g), and tyrosine (4.26 g/100g).

2.3 Pseudocereals

Pseudocereals are dicotyledonous species rich in starch and exhibiting properties similar to those of true cereals. Their protein content generally ranges from 10% to 20%, and compared with cereals and other legumes, their protein quality is relatively high. The most commonly consumed pseudocereals include buckwheat, amaranth, quinoa, and chia. They serve as valuable sources of essential amino acids, essential fatty acids, phenolic compounds, vitamins, flavonoids, and minerals.

Buckwheat, a dicotyledonous crop belonging to the Polygonaceae family, is cultivated and adapted to withstand extreme climatic conditions in marginal areas. Native to China and Central Asia, it is an annual herbaceous plant with protein profile rich in diverse amino acids. Notably, buckwheat is the only pseudocereal known to contain rutin [35]. Buckwheat contains a wide range of proteins that are of high quality. Buckwheat proteins are significantly rich in lysine, aspartic acid and arginine and have lower levels of glutamic acid and proline than cereal proteins [36]. The protein content is 12.0–18.9% for buckwheat [37]. Furthermore, buckwheat also contains essential amino acids like lysine and arginine that ensure the full range of amino acids required for the proper functioning of the human body [38]. Buckwheat is the pseudocereal with the highest phenylalanine content (862 mg/100 g). Buckwheat proteins are gluten-free. Buckwheat proteins have a strong additional impact on other proteins to improve nutritional and amino acid balance. With special bioactivities such as cholesterol-lowering effects and constipation improvement, antihypertensive effects, as well as obesity situations, acting similarly to dietary fibers and interfering with metabolic processes in vivo [39]. Due to its protein composition, buckwheat is considered a healthier option compared to cereals.

Quinoa seeds have an average protein content ranging from 12 to 23%, which is higher than conventional cereals, although lower than oilseeds and legumes [40]. Previous research on quinoa protein has confirmed that the main proteins in quinoa are globulins, which account for approximately 37% of total protein, and albumins account for approximately 35%. Quinoa has a low prolamin content (0.5-7.0% of total protein) [41]. The protein content of quinoa is considered highly valuable, owing to its well-balanced composition of essential amino acids. These proteins contribute not only to the nutritional quality but also to the functional and structural properties of quinoa, including solubility, gel network formation, emulsification, and foaming capacity [42]. Quinoa has almost all the essential amino acids, as suggested by standard-setting bodies such as Food Agriculture Organisation (FAO) and World Health Organisation (WHO) [43]. Quinoa has a high concentration of lysine, which ranges mainly from 2.4 to 7.8 g/100 g of protein. The methionine content of quinoa ranges approximately from 0.3 to 9.1 g/100 g of protein. The threonine content ranges from 2.1 to 8.9 g/100 g of protein [44]. Analysis of the composition of quinoa has shown that its proteins are mainly deposited in the embryonic tissue, which also contains lipids, fiber and saponins, while the perisperm contains a high starch content [45].

Quinoa seed flour has a higher total protein content than grains such as barley (11%), rice (7.5%), or corn (13.4%). Additionally, the protein content of quinoa is higher than the protein content of peanuts (8.8-11.6%) and cowpeas (8.8-12.1%) [42,46]. The amino acid composition of quinoa protein isolate includes histidine (2.76), leucine (4.60), isoleucine (1.30), lysine (17.13), methionine with cystine (1.70), phenylalanine with tyrosine (9.34), threonine (1.47) and valine (2.03) as essential amino acids, while the non-essential amino acids include alanine (5.34), glycine (9.60), proline (0.10), serine (2.57), tyrosine (2.88), glutamic acid (12.80), aspartic acid (8.54) and arginine (0.03) (g/100 g protein), as reported by Elsohaimy et al. [42]

Amaranth and its varieties fall into the class of traditional crops with high nutritional and medicinal value, but their true potential has not yet been realized. The nutritional and bioactive components found in these plants contribute significantly to their ability to provide health benefits [47]. The protein content in amaranth varies and reaches maximum accumulation during the blossoming phase, ranging from 17.2 to 32.6% on a dry weight basis

for various samples. Amaranth has twice as much lysine as cereals, such as wheat, and three times the lysine concentration of corn [48]. According to the literature, amaranth protein fractions contain albumin, globulin, prolamin and glutelin which are approximately 65%, 17%, 11% and 7% respectively. The amount of distinct fractions in the separated protein as well as its functional and nutritional qualities depends on the extraction process used [49]. The primary storage proteins in amaranth are albumins and globulins. Albumins are abundant in sulfur-containing amino acids, as well as lysine and valine, whereas globulins are rich in valine, leucine, glutelin, and histidine, but remain limited in lysine. Amaranth is a notable source of essential amino acids, particularly lysine, containing approximately twice the amount found in wheat and three times the concentration present in corn [48]. In addition, it contains significant amounts of sulfur amino acids that are limiting in legumes. Amaranth is a gluten-free pseudocereal, making it an effective choice for a gluten-free diet. The nutritional characteristics of amaranth have attracted the attention of researchers investigating the use of amaranth as a functional ingredient. Previous studies have shown that there is a significant difference in nutritional attributes between amaranth species, which includes amino acid composition, fatty acid composition, and mineral content [50].

2.4 Oilseeds

Oilseeds are the seeds of plants primarily used for oil extraction and include varieties such as peanuts, rapeseed, sesame, and sunflower. The protein content of oilseeds typically ranges from 6% to over 60%, with specific types like soybean meal containing high protein levels (e.g., 33-56%), while canola/rapeseed meal and sesame meal also offer valuable proteins. The oil extraction process generates a by-product known as meal, which is rich in protein and other valuable nutritional and bioactive components. Although traditionally used for animal feed, the growing demand for plant-based proteins has led to an increased utilization of these by-products for human nutrition. Oilseeds can contain up to 40% protein and possess a relatively well-balanced amino acid profile, particularly in sulfur-containing amino acids, though they are generally deficient in lysine. Additionally, bioactive peptides in oilseeds may offer health benefits, including the prevention and management of diabetes, obesity, and cardiovascular diseases [51].

Rapeseed protein exhibits a balanced amino acid composition except for methionine. Sesame protein is composed primarily of globulin (67.3%), albumin (8.6%), glutelin (6.7%), and prolamin (1.3%). It is nutritionally notable for its high content of methionine and tryptophan but is limited in lysine, threonine, isoleucine, and valine. Sesame is widely processed into protein-rich products, including flakes, flour, concentrates, and protein isolates, with the latter containing up to 80% protein [52].

2.5 Green Leafy Crops

Leafy greens occupy a prominent position in this industry due to their nutritional value, abundance, sustainability and are renowned for their high protein content, essential vitamins, minerals and phytochemicals. Furthermore, leafy greens are characterized by their low environmental impact, requiring minimal inputs of land, water and energy compared to conventional protein sources. The reason for focusing on leafy greens as a promising solution lies in their multiple benefits and their potential to address key challenges facing the global food system. By harnessing the nutritional potency and sustainability attributes of leafy greens, stakeholders can promote environmental stewardship, public health and food security [53].

Green leaf biomass represents one of the largest underutilized nutrient sources globally. Whether deliberately cultivated or recovered as a by-product from large-scale

agricultural crops, it can serve as a viable alternative source of plant protein for food and feed formulations. The nutrient composition of green leaf biomass differs markedly from that of plant seeds, particularly in terms of protein quality, vitamin and mineral content, and fatty acid profiles, including omega-6 and omega-3 fatty acids [54]. In contrast to cereal proteins, which are often deficient in lysine and/or tryptophan, and legume seed proteins, which are limited in methionine and/or cysteine, leafy green proteins nearly meet the FAO criteria for a complete protein. Additionally, unlike edible seeds that are typically rich in omega-6 fatty acids, leafy greens are a significant source of omega-3 fatty acids, particularly α -linolenic acid, a key precursor of the metabolites eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [55].

Alfalfa (*Medicago sativa* L.) is recognized as a versatile and nutrient-rich source in modern food systems, offering a wide range of benefits and applications for human health. The health benefits of alfalfa, including antioxidant, anti-inflammatory, cholesterol-lowering and blood sugar-regulating properties, are highlighted along with its potential to combat chronic diseases and oxidative stress [56]. Alfalfa is rich in valuable nutrients, including proteins with a high content of essential amino acids, minerals, vitamins and dietary fiber. The proteins contained in alfalfa have a major impact on techno-functional properties, including water retention, emulsification, foaming and gelling properties, useful for obtaining next-generation products. Alfalfa is beneficial in alleviating some health disorders, including diabetes, atherosclerosis and hypercholesterolemia, due to its antioxidant properties. Chloroplasts in plant leaves contain almost 80% protein, with most of the remaining protein being located in the stroma and thylakoid membranes [57]. About half of the leaf proteins are white and hydrophilic, while the other half are green and hydrophobic. The hydrophobic green fraction comprises chloroplast lamellar proteins, which are associated with lipids, fat-soluble vitamins, and chlorophyll [58]. The crude protein content of alfalfa is typically between 15 and 22% and is mainly distributed in the leaves. Crude protein is divided into two major categories: protein proper and non-protein nitrogen (NPN), based on chemical properties. NPN includes free amino acids, amides, purines, pyrimidines, and alkaloids, accounting for about one-third of the total nitrogen content of alfalfa. L-glutamic acid and glutamine are the structural units of protein. They play a vital role in nitrogen metabolism and are biosynthetic precursors of a variety of amino acids, purines, and pyrimidines in the body [59,60].

2.6 Algae

Algae biomass has low water consumption (including seawater growth), no competition for arable land, and carbon neutral emissions, and has become an alternative to global food security issues. Macroalgae and microalgae species have protein amounts comparable to protein sources such as milk, eggs, and dairy products. Microalgae, such as *Chlorella* and *Spirulina*, have high potential for functional food processing [61]. Over 30% of the world's microalgae biomass is derived from spirulina (*Arthrospira platensis* and *Arthrospira maxima*), which contains approximately 60% protein. Among *Chlorella* species, the most commonly used are *Chlorella pyrenoidosa*, *Chlorella sorokiniana*, *Chondrus crispus*, *Scenedesmus acutus*, and *Chlorella vulgaris*, with protein contents ranging from 51% to 58%. This high protein content makes microalgae a valuable nutrient source and an attractive option for enhancing the nutritional quality of foods. Moreover, various cultivation and extraction techniques have been developed to maximize the recovery of beneficial compounds from microalgae, thereby exploiting their full nutritional and functional potential [62].

The main factors limiting the development of microalgae as a protein alternative are pigmentation, difficulty in harvesting and production. Pigmentation and the fishy smell of microalgae make the product unpalatable. This is one of the strongest barriers to the use of microalgae as a food ingredient. In addition, microalgae protein contains less methionine, cysteine, lysine and tryptophan than meat protein and has a lower degree of digestibility. Nevertheless, microalgae protein remains a useful ingredient for meat alternatives and has great potential to improve the quality of plant-based food products [63].

2.7 Insects

Insects are gaining recognition as a promising food source due to their considerable nutritional value [64]. Insect protein, also called entomophagy, involves consuming insects as a food source [65]. It is nutritious, sustainable and offers an alternative to traditional protein sources. Insects are rich in protein, vitamins and minerals and can be consumed whole or processed into various food products. Queiroz et al. also reported that edible insects offer a promising solution as an alternative source of protein. In particular, they meet all the criteria necessary to promote the main sustainable development goals highlighted by the WHO, because unlike conventional primary protein sources used for human food and animal feed, insects present a high protein content and well-balanced amino acids. Furthermore, the integration of insect farming into traditional food practices heralds a more sustainable and efficient food system. Research by de Castro et al. [65] elucidated that insect peptides are potential antihypertensive, antimicrobial, and antioxidant agents, demonstrating their broad utility. Research has indicated that insect protein hydrolysates possess bioactive characteristics, such as anti-inflammatory, antihypertensive, antidiabetic, and antioxidant effects [66]. Lipxygenase and cyclooxygenase inhibitors exhibit anti-inflammatory properties [67]. However, these protein sources remain consumed more in tropical and subtropical regions, and less in Western countries [68]. However, insects have significant potential as a sustainable solution to future nutritional demands, as they are renewable natural resources available year-round and have a low environmental impact, with increased food security [69,70]. The protein content in different sources of plant origin is presented in Table 1.

Table 1

Protein Content of Different Plant Protein Sources			
Protein type	Protein resource	Protein content (%)	References
Legumes	Lentils	23-31	[71]
	Chickpea	17-22	[72]
	Lupin	32.0-55.3	[73]
	Faba bean	26.4-39.7	[74]
	Cowpea	23.6-33.0	[75]
	Black bean	22.9-23.2	[76]
	Pea	14-31	[18]
	Mung bean	25.8-39.7	[74]
	Soybean	42.95-46.32	[26]
Cereals	Wheat	7-22	[77]
	Rice	6.6-8.4	[78]
	Oat	8.7-16.0	[79]
	Sorghum	6-20	[80]
Pseudo-cereals	Hemp seeds	20.17-25.06	[81]

Continuation Table 1

	Amaranth	11.7-18.4	[79]
	Quinoa	13.0-14.0	[44]
	Chia	19.0-23.0	[82]
	Buckwheat	21.6-25.3	[83]
Oilseed protein	Groundnut	18.92-30.53	[84]
	Sun flower	10-25	
	Mustard	18-24	[85]
	Almonds	10-35	[86]
	Sesame	12-16	[84]
	Rapeseed	34-40	[87]
Green leaves	Alfafa leaves	15.3–32.27	[88]
	Spinach leaves	30	[53]
	Cabbage leaves	39.76–53.33	[89]
	Broccoli leaves	12	[90]
	Cassava leaves	33.6	[91]
Algae	Spirulina platensis	50-65	[92]
	Chlorella sp.	51-58	
	Scendesmus sp.	50-56	
	Spirogyra	6-20	
	Spirulina maxima	60-71	
Insects	Tenebrio molitor	46-69	[67, 93-95]
	Hermetia illucens	40.0-60.8	[96-99]
	Gryllodes sigillatus	61.3-70.0	[67,100]

3. Established Methods of Plant Protein Extraction

Extraction of plant-based proteins with increased quality indices are obtained from ecological and sustainable protein sources with the use of emerging technologies for their extraction, cost-effectiveness and incorporation as food supplements in the manufacture of food products.

The most important phase in obtaining plant-based protein is the appropriate extraction technology. Pojić et al. [101] reported the results of a study that reviewed the techniques for extracting proteins from plant sources. The most important ones remain conventional methods using different solvents and unconventional methods using microwaves, high-pressure processing, pulsed electric field and ultrasound (Figure 1).

3.1 Conventional Methods of Plant-Based Protein Extraction

Conventional methods refer to commonly used techniques, which can sometimes result in lower extraction yields due to protein degradation. This decrease in protein yield is governed by a number of factors, including extraction time, solvent type, pH, and temperature. Conventional methods used for extracting plant proteins include wet extraction methods and dry fractionation.

Wet extraction refers to the recovery of proteins using aqueous solvents or chemical agents such as alkalis, acids, or salts, followed by protein precipitation or isolation. Among conventional techniques, alkaline extraction is the most widely applied due to its simplicity, cost-effectiveness, and efficiency. Under alkaline conditions, proteins acquire a negative charge, and their solubility increases when the pH rises above the isoelectric point; insoluble

residues are subsequently removed by centrifugation [102]. In acid extraction, solutions containing butanol, pentanol, hexane, or acetone are typically employed. The addition of acid gradually lowers the pH below the isoelectric point, resulting in the generation of a positive charge and enhanced protein solubility. The pH is then adjusted back to the isoelectric point, leading to the aggregation of soluble proteins, which are further separated by precipitation, centrifugation, or filtration. Salt extraction generally employs neutral pH saline solutions, such as sodium, calcium, or potassium chloride.

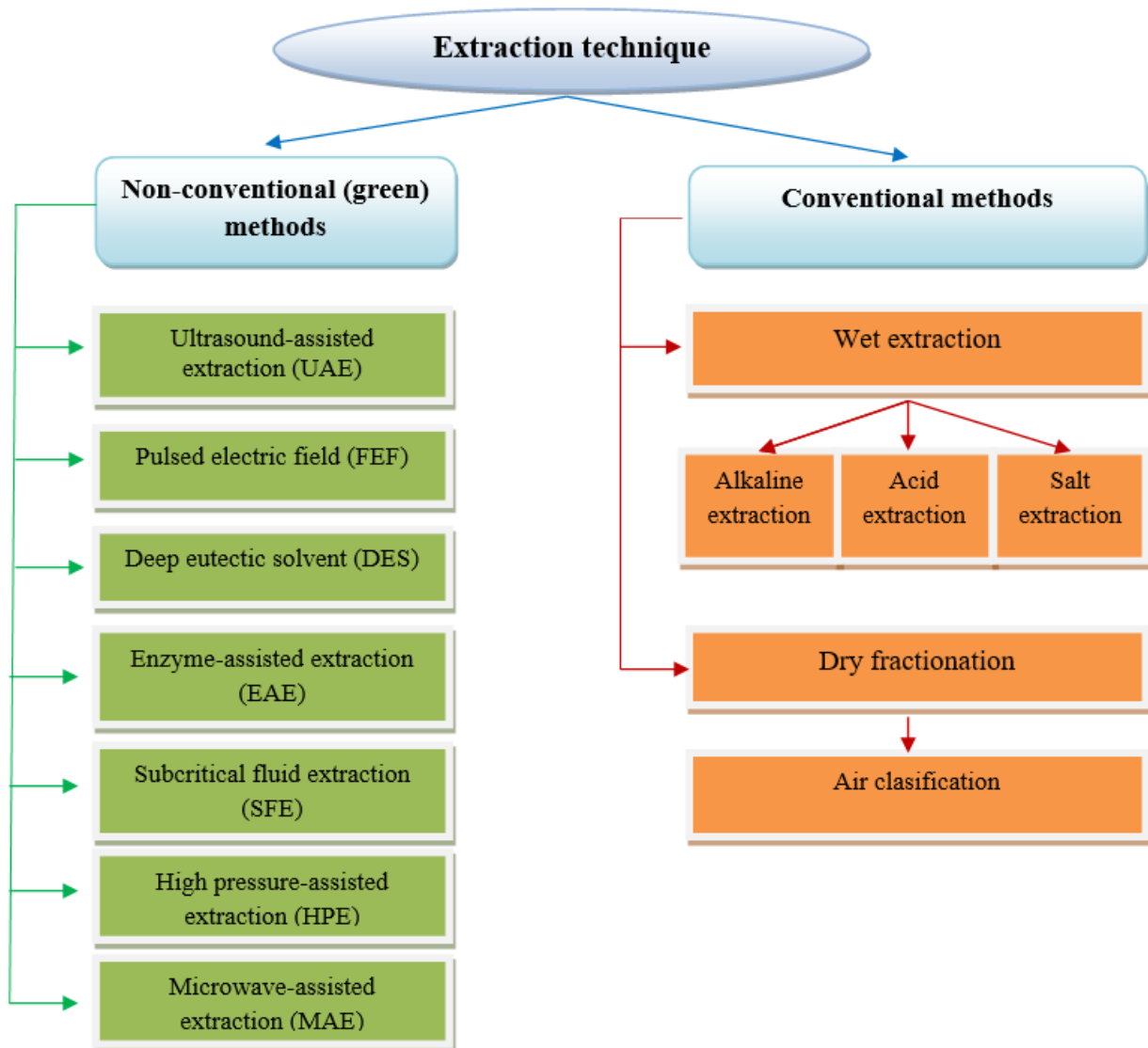


Figure 1. Methods for extracting proteins from plant-based raw materials.

This method relies on protein precipitation through salting-out and protein destabilization, followed by the removal of insoluble fractions by sedimentation, decantation, sieving, and centrifugation. The supernatant is subsequently desalted and dried to obtain the protein fraction. Compared to other methods, proteins extracted with saline solutions exhibit lower degrees of denaturation and aggregation, thereby retaining higher functional activity in terms of emulsifying, foaming, and gelling properties [103].

3.2 Non-Conventional Extraction Methods for Plant-Based Protein Sources

Nowadays, researchers are currently focusing more on non-thermal or non-conventional green technologies with the aim of improving extraction efficiency and

reducing protein degradation during extraction. These methods do not have any harmful effects on the environment, and the proteins obtained are also safe for consumption due to the minimal use of harmful chemicals and solvents. Exploiting innovative protein extraction techniques can improve protein yield, nutritional and techno-functional properties.

Ultrasound-assisted extraction, enzymatic extraction, supercritical fluid extraction, pulsed electric field, deep eutectic solvent extraction, high pressure-assisted extraction and microwave-assisted extraction are modern processing techniques developed [24].

3.2.1 Ultrasonic Asisted Extraction (UAE)

UAE represents an emerging technique for the extraction of plant-derived proteins, as it is capable of disrupting the cellular matrix, thereby enhancing extractability and tailoring the functional properties of these biomolecules. The efficiency of protein recovery and the overall extraction yield are strongly influenced by operational parameters such as sonic energy density, duration of treatment, and the substrate-to-agitation ratio. Beyond extraction, UAE is also employed to induce modifications in the physical, structural, and functional characteristics of protein ingredients. Notable alterations in protein physical properties induced by sonication include particle size reduction, changes in rheological behavior, electrical conductivity, and zeta potential. These modifications are attributed to cavitation-induced shear forces, which provoke alterations in secondary and tertiary structures, such as protein aggregation and oxidative crosslinking. Consequently, both the functionality of the protein ingredients and their nutritional quality may be affected. Changes in functional properties—particularly hydrophobicity, solubility, emulsification, and foaming capacity—are closely dependent on the intensity of ultrasound energy applied. A key challenge in this context lies in optimizing ultrasonic parameters, including frequency, power, and treatment time, to maximize protein extraction efficiency while minimizing structural degradation that may impair functionality. The beneficial effects of high-power ultrasound in enhancing protein recovery are primarily ascribed to acoustic cavitation, a phenomenon involving the nucleation, growth, and implosive collapse of microbubbles in a liquid medium exposed to ultrasound irradiation at frequencies above 20 kHz [104,105].

3.2.2 Pulsed Electric-Field Assisted Extraction (PEF)

PEF technology is a non-thermal approach, the effectiveness of which depends on several parameters, including electric field strength, total specific energy input and treatment temperature. PEF uses an electric field (electrical pulses of very short time of 10^{-4} to 10^{-2} s and high amplitude of 0.1–80 kV/cm) to create irreversible pores in the cell membrane, increasing membrane permeability. The PEF system works by cellular electroporation, whereby the application of electric fields induces the formation of positive and negative poles in the lipid bilayer of the cell membrane. Exceeding the critical electric field strength results in the irreversible breakdown of the cell membrane. Consequently, the disruption of the cell membrane improves the extraction efficiency [106]. This technique enhances protein extraction by inducing a critical transmembrane potential in plant cells. The application of PEF alters membrane permeability characteristics, thereby facilitating mass transfer processes and improving protein extractability [107]. The advantages of this technique consist of improved mass transfer, high extraction yields, with some authors showing an increase in protein extraction yield of over 28% compared to conventional extraction [108], short processing times, minimal protein degradation and reduced energy costs [109].

3.2.3 Deep Eutectic Solvents (DES)

DES are a mixture of hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) molecules that include natural plant metabolites (sugars, carboxylic acids, amino acids) and ionic molecules, usually represented by ammonium salts. DES represent versatile and sustainable media that can be specifically applied to the extraction of bioactive compounds, owing to their favorable physicochemical characteristics (recyclable, non-toxic, non-inflammable, thermally and chemically stable, easy to prepare, highly pure, highly biodegradable, low cost, and availability). The concept of natural deep eutectic solvents was first introduced by Choi et al. [110], where primary plant metabolites were employed to form DES that may naturally occur in biological systems. In addition, they serve as alternative solvents for protein extraction, offering a more environmentally friendly option due to their natural origin [111]. DES-assisted protein extraction can be carried out through both solid–liquid and liquid–liquid extraction methods. In solid–liquid extraction, solid materials are dispersed in a liquid medium, while liquid–liquid extraction entails the separation or partitioning of two compounds within an immiscible solvent system [112]. However, not all DESs are compatible with the protein extraction process, considering that the viscosity and conductivity of DES are temperature dependent. The temperature dependence poses significant challenges for the recovery and isolation of proteins from the DES extraction medium, thereby limiting the scalability and industrial applicability of this method. However, proteins extracted by this method may exhibit improved solubility, emulsification, foaming and gelation [109].

3.2.4 Enzyme-Assisted Extraction (EAE)

EAE is regarded as an environmentally sustainable technique for isolating proteins from diverse plant sources, owing to its mild processing conditions, reduced energy requirements, and lower waste generation compared to conventional chemical and physical extraction methods. Various food-grade carbohydrases and proteases have been applied to promote protein solubilization and release by hydrolyzing structural components of the plant cell wall as well as associated proteins [113,114]. The efficiency of EAE is determined by several factors, including incubation temperature, pH, enzyme-to-substrate ratio, extraction time and the solvent system employed. Beyond enhancing protein recovery, EAE can also improve nutritional quality (e.g., digestibility), as well as the techno-functional and bio-functional properties of the recovered proteins, particularly when protease-assisted strategies are used. To further enhance protein extraction efficiency, EAE has been increasingly integrated with complementary technologies to improve overall protein yield. One of the primary advantages of enzymatic treatment lies in its high selectivity and efficiency, as enzymes specifically target protein–cell wall interactions or intracellular structures without requiring harsh chemicals or extreme processing conditions. This approach allows for better preservation of protein functionality and structural integrity, while also representing a more sustainable and environmentally benign alternative, generating fewer undesirable by-products [115]. Moreover, enzymatic degradation of cell wall components facilitates the release of endogenous proteases, which may further promote protein hydrolysis, conformational modification, and the formation of short-chain bioactive peptides with potential physiological activity [116]. Proteins obtained through EAE are typically characterized by higher purity and improved nutritional quality, making them particularly suitable for human consumption. Despite its relatively higher cost and longer processing time compared with conventional alkaline extraction, enzyme-assisted extraction remains a

promising, eco-efficient approach for producing high-quality plant proteins with superior functional and nutritional properties.

3.2.5 Supercritical Fluid Extraction (SCF)

SCF is a viable alternative to conventional solvent extraction systems. Supercritical fluids are substances for which both pressure and temperature are higher than their critical values, assuming physical characteristics intermediate between those of a liquid and a gas [117,118]. Therefore, the synergism between density, low viscosity, diffusivity, near-zero surface tension, and pressure and temperature dependence allows supercritical fluids to easily penetrate a microporous matrix to extract intracellular compounds [119].

One of the most widely used supercritical fluids is carbon dioxide (CO₂), it is an excellent solvent that has received special attention in SFE because it is chemically inactive, economical, easily accessible, separable from extracts, non-toxic and is an approved food grade solvent. [120,121]. However, subcritical water extraction is widely used in the protein extraction industry. Subcritical water (SCW) serves as an effective medium for the extraction of less polar compounds within relatively short processing times (approximately 30 min). SCW is defined as water maintained in a liquid state at temperatures between 100 and 374 °C and pressures below 22.064 MPa. At elevated temperatures, the dielectric constant of water decreases and hydrogen bonding is weakened, rendering SCW similar in polarity to organic solvents such as ethanol and methanol. The efficiency of subcritical water extraction is governed by several factors, including extraction temperature, processing time, water-to-solid ratio, physical and particle characteristics of the feed material, water flow rate, and the type and concentration of catalyst employed. Given the non-toxic nature of water, subcritical water extraction is considered a green technology for the recovery of plant proteins, offering a rapid, clean, and cost-efficient alternative to conventional extraction methods. Previous studies have reported extraction yields ranging from 70% to 78% using this approach [122].

3.2.6 High pressure-Assisted Extraction (HPAE)

HPAE is a non-thermal technique for protein recovery, in which the feedstock is exposed to hydrostatic pressures ranging from 100 to 1000 MPa under controlled temperature and time conditions. The process facilitates protein release primarily through the disruption of cell walls induced by increased pressure [113]. HPAE has shown a faster extraction process for plant-based proteins, a high extraction yield (82% at 100 MPa) [123] compared to other methods, and the production of pure proteins with improved functional properties and digestibility [124].

3.2.7 Microwave-Assisted Extraction (MAE)

MAE offers simultaneous, rapid, and uniform energy transfer compared to conventional methods. This accelerated heating causes water to evaporate, generating internal pressure on the cell wall, which eventually ruptures and releases intracellular organic compounds into the solvent. However, precise temperature control is essential, as excessive heating may lead to protein degradation and altered functional properties. Therefore, defining the optimal processing conditions is critical when applying microwave-assisted extraction [125]. MAE demonstrates uniform heat distribution in the extraction feedstock, rapid extraction rates, lower solvent consumption, shorter extraction times, higher extraction yield, and proteins with improved quality properties [126]. According to Chao et al. the extraction yield for pumpkin proteins was over 90% [127].

4. Quality Characteristics of Plant-Based Proteins

Proteins are essential nutrients that support human growth, contribute to the repair of damaged tissues and cells, and help maintain muscle mass. Beyond their physiological and nutritional roles, the techno-functional properties of proteins play a key role in determining the appearance, texture, and stability of food products. Plant-derived proteins represent a sustainable, cost-effective, and health-conscious alternative, owing to their high nutritional value, affordability, and wide availability.

Plant protein sources have emerged as valuable resources in the food industry, largely due to their functional attributes. These include biofunctionality, which encompasses their nutritional and physiological roles, and technofunctionality, which relates to properties such as solubility, water- and fat-binding capacity, gel formation, rheological behavior, emulsification, foaming, and whipping abilities [128,129]. These properties play a crucial role in the development of innovative functional food products. Consequently, understanding and evaluating the factors that influence them, whether positively or negatively, is essential for optimizing extraction processes and producing suitable protein-based ingredients.

However, studies by Gentil et al., Chen and Campanella have concluded that the formation of protein complex systems with other food components (polysaccharides, lipids, polyphenols, etc.) can affect the behavior of proteins in food systems during processing, manufacturing, storage and preparation, e.g., absorption, solubility, gelation, surfactant, ligand binding and film formation [130-132].

The physicochemical and functional properties of food proteins can be influenced by several environmental factors, such as pH, temperature, pressure, and ionic strength, as well as processing conditions. In addition, protein structure-function relationships govern how proteins interact with each other and with other substances in complex food systems [132]. For example, soy protein isolate showed the lowest emulsifying activity at pH 5.8 and the highest emulsifying activity at pH 8.0. The results indicated that the emulsifying activity increases with increasing pH, which may be attributed to enhanced protein solubility at higher pH values [133]. At pH values near the isoelectric point (pH 5.0), pea protein exhibited the lowest emulsifying capacity, making the resulting emulsions more susceptible to coalescence and foaming. Interestingly, its emulsifying ability at pH 3.0 was superior to that observed at neutral (pH 7.0) or alkaline (pH 9.0) conditions. Furthermore, surface hydrophobicity—a key physicochemical factor influencing the surface functional properties of proteins—was nearly twice as high for purified legumin at pH 7.0 compared with purified vicilin, suggesting a markedly greater tendency for hydrophobic cluster formation.

Amaranth protein isolates showed high solubility at pH 2.0 (76–84%) but minimal solubility near the isoelectric point (pH 5.5–10%). The lowest emulsifying activity occurred at pH 5.0, while the highest was observed at pH 9.0. Foaming capacity was also lowest at pH 5.0 and peaked at pH 7.0. The lower solubility, emulsifying activity index, and foaming capacity observed at pH 5.0 can be attributed to the isoelectric point of amaranth proteins. At this point, the proteins carry no net charge, resulting in minimal repulsive interactions and promoting protein–protein interactions that are unfavorable for solubility [134].

The solubility of protein isolates from *Amygdalus pedunculata* seeds initially increased and then decreased with rising sodium chloride concentration, peaking at 0.4 mol/L. This behavior may be explained by the effect of ionic strength: at low salt concentrations, solubility is enhanced due to the salt's solubilizing effect, whereas at higher concentrations,

increased ionic strength diminishes the repulsive electrostatic interactions between charged protein molecules, thereby reducing solubility [13].

However, some technological treatments applied to proteins, such as high pressure and high-intensity ultrasound, can improve the functional properties of the protein. Although soy protein isolate exhibited low surface hydrophobicity, it demonstrated the highest emulsifying activity index following treatment at 400 MPa. This effect was likely due to the high pressure inducing partial or complete denaturation of the protein into monomers, thereby enhancing its emulsifying capacity [136].

The hydrophobicity of Bambara bean protein was significantly higher at pH 4.0 and lower at pH 9.0. At pH 4.0, the protein carried little net charge, favoring hydrophobic protein–protein interactions, whereas at pH 9.0, the protein was negatively charged, promoting protein–water interactions. Hydrophobicity decreased with increasing temperature up to 70 °C at pH 4.0, and heat treatment also reduced hydrophobicity at pH 9.0. These changes were likely due to protein aggregation through hydrophobic contacts (decrease) or partial unfolding that exposed previously buried regions (increase). The emulsifying capacity of the protein isolate peaked at 80 °C and pH 9.0, while emulsion stability was highest at pH 4. Protein solubility decreased with rising temperature at pH 4.0 and 7 but increased at pH 9.0 at 100 °C [137].

The sprouting process enhanced the physical and functional properties of faba bean protein isolates, including a water absorption index of 2.97 g/g and foaming stability of 140.13 mL/100 mL. Additionally, pressure-cooked faba beans exhibited superior functional properties compared to those subjected to conventional thermal processing, with higher water solubility (2.12 g/100 g) and water absorption capacity (2.02 g/g) [138]. Another study also showed that higher functional properties were observed in germinated amaranth flour than in raw amaranth flour. The improvement in the functional properties of proteins during germination is due to the activity of proteases that can form compounds (proteins) with lower molecular weight [139].

In addition, Yao et al. [140] examined the effects of γ -irradiation on the physicochemical and functional properties of rice protein. They reported higher physicochemical index values at lower irradiation doses compared to higher doses. This effect was likely due to the disruption of intermolecular bonds at low doses, resulting in smaller protein particles. At higher doses, however, protein aggregation occurred through cross-linking, electrostatic and hydrophobic interactions, and disulfide bond formation, leading to increased particle size and higher molecular weight. [141,142]. Rice protein exhibited the highest surface hydrophobicity at a gamma irradiation dose of 2 kGy, whereas increasing the dose to 5 kGy resulted in a decrease in surface hydrophobicity [140]. This could be because radiation treatment exposed hydrophobic residues in the protein, increased the number of hydrophobic sites, and improved the ability to contact the external environment, thus enhancing surface hydrophobicity [143]. On the other hand, when the γ -irradiation dose was excessively high, the hydrophobic sites of the protein were reduced due to the increase in the number of β -sheets in the protein secondary structure, thus reducing the surface hydrophobicity [144]. Also, the solubility of rice protein increased after γ irradiation treatment, as the irradiation dose increased, the solubility of rice protein initially increased and then decreased. The solubility of rice protein was 69.18% when the γ irradiation dose is 2 kGy. This could be a result of the γ -irradiation treatment, which contributes to opening the protein structure, exposing internal hydrophilic groups and facilitating the binding of water

molecules, increasing the protein solubility [145]. At the same time, when the irradiation dose increased to 5 kGy, protein molecules either aggregated or protein residues were oxidized to form disulfide bonds, which decreases protein hydration and rice protein solubility [140,146]. Furthermore, the highest water and oil retention capacities of rice protein were 5.89 g/g and 3.45 g/g, respectively, at an irradiation dose of 2 kGy. [146]. This could be due to the fact that gamma irradiation treatment exposed internal groups and opened the protein structure, which led to increased contact with water molecules and, consequently, improved water-holding capacity of the protein.

Plant-based proteins exhibit excellent emulsifying and foaming properties, making them valuable for use in various food formulations. Emulsification is the process of dispersing one liquid phase into another, usually oil in water, creating a stable and homogeneous mixture. Foaming, on the other hand, involves incorporating air into a liquid to form a stable foam [147]. They contain both hydrophilic (water-attracting) and hydrophobic (oil-attracting) regions in their structure. This unique composition allows the protein to interact with both the aqueous and oily phases, facilitating the formation and stabilization of emulsions [148]. When plant-based protein is added to an oil-water mixture and stirred, it forms a stable emulsion by surrounding the oil droplets with a protein layer, preventing coalescence and separation of the two phases. This property is particularly useful in salad dressings, mayonnaise, and various emulsified sauces. Plant-based proteins can also generate stable foams by trapping air bubbles in a protein network. [149]. When proteins are whipped or stirred, they form a stable foam with high volume and good stability. The protein film surrounding the air bubbles ensures the structural integrity of the foam, preventing the bubbles from coalescing and collapsing. Protein foams find applications in meringues, whipped toppings, and aerated desserts. [150]. The emulsifying and foaming properties of plant-based protein contribute to the texture, mouthfeel and stability of food products, improving their sensory characteristics and overall quality [151].

Proteins are capable of gel formation and can thicken solutions, making them valuable texturizing agents in a variety of food products. Chickpea protein, in particular, can form gels upon heat treatment or when subjected to changes in pH [152]. Protein molecules undergo conformational changes, leading to aggregation and the formation of a three-dimensional protein network. The resulting gel has a solid structure that traps water and other components, creating a firm and cohesive texture [153]. Chickpea protein gels can be commonly used in meat analogues, vegetarian sausages, and plant-based burgers to mimic the texture of meat. The protein can also act as a thickening agent in liquid systems. When added to a liquid, such as soups, sauces, or gravies, chickpea protein interacts with water molecules and forms a viscous solution [154]. The thickened solution improves the consistency and mouthfeel of the product, enhancing overall sensory attributes. The gelling and thickening properties of the protein contribute to the structural integrity and texture of food products, enabling the development of a wide range of plant-based food applications [155].

Proteins have high water and oil absorption capacities, essential in food applications where moisture retention and fat binding are crucial. They can absorb and retain large amounts of water [156]. When incorporated into food formulations, proteins can bind water, reducing its loss during cooking or processing. This property enhances the juiciness and tenderness of meat analogues and other plant-based products, while also improving moisture retention in baked goods. Likewise, chickpea protein can absorb and bind oil, which helps control oil migration, prevent separation, and improve emulsion stability. This oil-binding

capacity is particularly valuable in formulations where fat replacement or imitation is desired [157]. Overall, the water- and oil-binding properties of proteins enhance texture, juiciness, and stability across a variety of food products, highlighting their value as functional ingredients in the food industry.

5. Conclusions

The integration of plant proteins into the food industry and the diet of the global population is an essential direction for the development of a sustainable food system. Given that the demand for protein is constantly expanding, and meat production involves a considerable ecological impact, plant sources, along with algae and insects, represent viable solutions for the future. The quality and functionality of plant proteins depend on multiple variables: plant variety, cultivation conditions, and extraction and processing methods. Adapting technological parameters to the characteristics of the raw material is crucial for obtaining an optimal yield and maintaining functional properties. Emerging technologies, such as microwave, ultrasound or electric field-assisted extraction, open promising perspectives by increasing efficiency and reducing energy consumption, while maintaining the nutritional integrity of proteins. In terms of nutrition, the strategic combination of different plant sources can counteract deficiencies in essential amino acids, providing an intake comparable to that of animal proteins. In addition, their bioactivity – including antioxidant, antidiabetic or cardioprotective effects – amplifies their value for consumer health. The integration of proteins from green leaves and microalgae requires innovative solutions to overcome technological and sensory barriers, so that they are widely accepted by consumers. The successful implementation of plant proteins in the food industry depends on the balance between sustainability, functionality and acceptability. Plant proteins can become the foundation of resilient food systems, able to respond to demographic and environmental challenges.

Acknowledgments: The authors would like to thank the National Project Stimulating Excellence in Research of National Agency for Research and Development of Moldova 25.80012.5107.21SE “Optimization of the extraction technology of protein concentrates from alfalfa (*Medicago Sativa*) for the manufacture of new food products”, being implemented at the Technical University of Moldova.

Conflicts of Interest: The authors declare no conflict of interest.

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Submission of manuscripts:

jes@meridian.utm.md

[https://doi.org/10.52326/jes.utm.2025.32\(4\).08](https://doi.org/10.52326/jes.utm.2025.32(4).08)
UDC 637.146:634.8:663.26



EFFECT OF GRAPE SKIN INCORPORATION ON THE QUALITY OF A DAIRY-BASED FROZEN DESSERT

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Received: 12. 02. 2025

Accepted: 12. 28. 2025

Abstract. The valorisation of winemaking by-products is an important approach for the development of functional foods. This study investigated the effect of grape skin powder (GSP, 0–10%, *m/m*) incorporation on the quality of a laboratory-scale dairy-based frozen dessert prototype. Five formulations (0, 2.5, 5.0, 7.5 and 10% GSP) were evaluated in terms of physicochemical properties, oxidative stability, colour parameters, microbiological quality and sensory acceptability. Increasing GSP levels led to a progressive decrease in pH (6.72–5.62) and a slight increase in fat content (26.25–27.66%), while dry matter remained above 54%. Lipid oxidation during frozen storage was reduced in GSP-fortified samples, with peroxide values decreasing from 1.70 meq O₂/kg fat in the control to 1.36 meq O₂/kg fat at 10% GSP after 28 days. Pronounced colour changes were observed ($\Delta E^* = 37–55$), associated with grape skin anthocyanins. Functional enrichment was confirmed by increased total polyphenol content (up to 139 mg GAE/100 g) and antioxidant activity (up to 33.6%). Moderate GSP addition (2.5–5.0%) was associated with lower microbial counts, while higher levels introduced additional indigenous microflora. Sensory evaluation indicated good overall acceptability, with the 5% GSP formulation providing the most balanced profile. Overall, grape skin powder shows strong potential as a functional ingredient for dairy-based frozen desserts.

Keywords: *antioxidant activity, grape skin, dairy-based frozen dessert, microbiological quality.*

Rezumat. Valorificarea subproduselor din vinificație reprezintă o direcție importantă pentru dezvoltarea alimentelor funcționale. Prezentul studiu a investigat efectul incorporării pulberii de pieleță de struguri (GSP, 0–10%, *m/m*) asupra calității unui desert lactat congelat, obținut la scară de laborator. Cinci formulări (0, 2,5, 5,0, 7,5 și 10% GSP) au fost evaluate din punct de vedere al proprietăților fizico-chimice, stabilității oxidative, parametrilor de culoare, calității microbiologice și acceptabilității senzoriale. Creșterea nivelului de GSP a determinat o scădere progresivă a pH-ului (6,72–5,62) și o ușoară creștere a conținutului de grăsime (26,25–27,66%), în timp ce substanța uscată a rămas peste 54% pentru toate probele.

Oxidarea lipidică în timpul depozitării la congelare a fost redusă în probele fortificate cu GSP, valoarea peroxidică scăzând de la 1,70 meq O₂/kg grăsime în proba martor la 1,36 meq O₂/kg grăsime pentru 10% GSP după 28 de zile. S-au observat modificări cromatice pronunțate ($\Delta E^* = 37-55$), asociate cu antocianinele din pielea de struguri. Îmbogățirea funcțională a fost confirmată prin creșterea conținutului total de polifenoli (până la 139 mg GAE/100 g) și a activității antioxidante (până la 33,6%). Nivelurile moderate de GSP (2,5–5,0%) au fost asociate cu încărcături microbiene mai reduse, în timp ce adaosurile mai mari au introdus microfloră indigenă suplimentară. Evaluarea senzorială a indicat o bună acceptabilitate generală, formularea cu 5% GSP prezentând cel mai echilibrat profil. În ansamblu, pulberea de piele de struguri prezintă un potențial ridicat ca ingredient funcțional pentru deserturile lactate congelate.

Cuvinte cheie: *activitate antioxidantă, piele de struguri, desert lactat congelat, calitate microbiologică.*

1. Introduction

Until the end of the 20th century, increasing food production without improving the efficiency of food systems was the prevailing policy throughout the world. Nowadays, increasing demands for processed food has required certain strategies to minimize energy demands and economic costs, as well as to reduce food losses and waste [1]. The quantities of the latter are huge, and the figures are shocking. The Food and Agriculture Organization published a report in 2011 listing food losses and wastage around the world and reporting that the amount of food lost or wasted produced for human consumption amounts to 1.3 million tonnes globally each year [2]. The European Commission has also mentioned this significant problem, as in 2010 it indicated that 90 million tonnes of food waste is generated annually in the EU [3].

Grapes are recognized as a valuable plant-derived source of antioxidants, notably resveratrol, anthocyanins, catechins, and quercetin. The grapes contain skin, pulp, seeds and stems and all of the presented components may contain antioxidants. Mostly antioxidants are found in the skin, as are coloring substances [4]. Grapes also contain the polyphenol resveratrol, which is well known for its therapeutic effects. This substance is located in the skin of grapes [5]. It is known for its antioxidant properties and was found in red grapes in large amounts, which is why red grapes have more antioxidants than white grapes [6].

Most of the grapes harvested worldwide are used to make wine, while winemaking has been one of the most important industries in many countries [7]. Along with the growing global trend for wine consumption comes an increasing volume of accompanying by-products such as grape marc, seeds, skins, stems, leaves and wine lees. These by-products are an interesting source for further exploitation as they are rich in polyphenols, tannins, procyanidins, anthocyanins, stilbenes, dietary fibres, tartrates, essential oils, proteins, tocopherols, etc. [8]. In this context, they have the potential to be used as a source of ingredients that could be used in different applications in functional foods, dietary supplements, pharmaceuticals and cosmetics, as well as for agricultural and oenological purposes [9].

Due to the fact that grapes have valuable components that can be used to enrich any food product, this topic remains very relevant in our time and in our territory [10]. However, despite the well-known functional potential of grape skins, their application in frozen dairy products remains insufficiently explored, especially with regard to their technological impact,

oxidative stability, microbiological behaviour and sensory acceptance. Dairy-based frozen desserts represent one of the most popular dessert categories, widely consumed by individuals across all age groups and demographic segments. Various artificial colorants are used in their manufacture, such as tartrazine (E102), sunset yellow FCF (E110), Allura Red AC (E129), Brilliant Blue FCF (E133) and Fast Green FCF (E143), which are among the most commonly used colorants in dessert production. The use of natural colorants is a viable alternative to synthetic colorants, as they have superior biocompatibility, are free of cumulative toxic effects, and contribute to safer food products from a nutritional and functional standpoint. Therefore, the aim of this study was to evaluate the impact of grape skin powder (GSP) addition on the quality and functional properties of dairy-based frozen dessert, and to assess its potential as a natural ingredient for valorising winemaking by-products.

2. Materials and Methods

2.1 Materials

Grape pomace (Feteasca Neagră variety, Republic of Moldova) was obtained as a secondary by-product from a local wine producer. Prior to the experimental procedures, the pomace was stored at -20 ± 1 °C to preserve its chemical stability. Before processing, the grape skins were manually separated from seeds and remaining stem fragments. The grape skins were dried by convection at 60 ± 1 °C until reaching a final moisture content of $6.80 \pm 0.12\%$. The dried material was then ground using a coffee grinder (Bosch TSM6A013B, Bosch GmbH, Germany) and subsequently sieved through a 150 µm sieve to obtain a fine grape skin powder (GSP) suitable for incorporation into various food matrices. The resulting GSP was stored in airtight plastic bags at 4 ± 1 °C in a light-protected environment until further use.

Ingredients used for the dessert production included sweet cream (35% fat, JLC Ltd., Republic of Moldova), sugar and grape skin powder.

2.2 Method of Dairy-Based Frozen Dessert Prototype Preparation

The product developed in this study represents a laboratory-scale dairy-based frozen dessert prototype designed to evaluate the feasibility of grape skin powder incorporation in frozen dairy systems. The basic dairy-based frozen dessert formulation was adjusted to allow its enrichment with grape skin powder (GSP). Five variants were prepared by supplementing the formulation with different levels of GSP: 0% (control), 2.5%, 5.0%, 7.5% and 10.0%. The supplementation was performed by adding the corresponding amounts of GSP to the base recipe, while keeping the quantities of the base ingredients constant across all formulations, in order to avoid alterations in sweetness intensity and freezing point depression, which are key factors governing texture development and ice crystal formation in frozen dairy systems. Since no compensatory sweetening or structuring agents were applied, maintaining a constant sucrose level ensured that the observed effects could be primarily attributed to GSP incorporation.

Prior to processing, the bowl of the ice cream maker was placed in the freezer for at least 24 h, and all raw materials were kept under refrigerated conditions to ensure optimal freezing performance. Table 1 presents the ingredients and their respective proportions used in the preparation of the dairy-based frozen dessert formulations.

The liquid phase, consisting of sweet cream (35% fat) and sucrose (representing one-third of the total formulation mass), was transferred into the pre-chilled bowl of the ice cream machine (Cuisinart ICE31GE, USA).

Table 1

Formulation of Dairy-Based Frozen Dessert Samples with Constant Base Ingredients and Variable Grape Skin Powder Addition

Sample	Sweet cream, g	Sugar, g	Grape skin powder*, g
Control sample	75.0	25.0	0.0
2.5 % GSP	75.0	25.0	2.6
5.0 % GSP	75.0	25.0	5.3
7.5 % GSP	75.0	25.0	8.1
10.0 % GSP	75.0	25.0	11.1

*The amount of grape skin powder (GSP) was calculated to represent 2.5, 5.0, 7.5 and 10.0% (*m/m*) of the final product mass.

After gentle mixing, the required amount of GSP powder was incorporated, and homogenization was initiated using the electric ice cream maker for 40–50 min, until a soft cream consistency was achieved. Following production, all samples were stored in a freezer at -27 ± 1 °C until further physicochemical, microbiological and sensory analyses were carried out.

2.3 Physicochemical Analysis

The total solids content (DM) of grape skin and frozen dessert samples were determined gravimetrically by measuring the mass remaining after drying, according to AOAC Method 941.08. The moisture content was calculated as the complement of DM content and expressed as percentage of total sample mass. The pH of the samples was measured using a calibrated pH meter (TESTO 206-pH2, Pruszków, Poland), employing buffer solutions of pH 4.0 and 7.0. Measurements were performed by immersing the electrode directly into a homogenised sample previously macerated with distilled water, following AOAC (Method 945.10) recommendations. The total fat content (FC) of the frozen dessert samples was determined according to AOAC Method 952.06, using an ether extraction procedure. The method is based on solvent extraction of lipids with petroleum ether, followed by gravimetric determination of the extracted fat.

2.4. Acidity Index and Peroxide Value

Given the high fat content of the dairy-based frozen dessert prototype and its susceptibility to oxidation, both the acidity index (AI) and peroxide value (PV) were determined using AOAC (1997) official methods [11]. Milk fat was extracted from fresh and stored samples of the frozen dairy dessert prototype according to AOAC Method 960.32 in order to obtain the lipid fraction for oxidative stability analysis. Primary oxidation products were quantified by determining the peroxide value using AOAC Method Cd 8-53. The content of free fatty acids was assessed following AOAC Method Ca 5a-40. Analyses were carried out at different stages of processing and storage: (i) immediately after homogenization of the frozen dairy dessert mixture (day 0), and (ii) after freezing and packaging. During frozen storage, peroxide value and free fatty acid content were determined in all experimental replicates at 7, 14, 21, and 28 days to evaluate the evolution of lipid oxidation over time.

2.5. Colour Parameters

Colour parameters L^* , a^* , and b^* were determined using a chromameter (Minolta CR-400, Osaka, Japan), where L^* indicates lightness, a^* represents red–green values and b^* represents yellow–blue values. For all samples, the total colour difference (ΔE^*) was calculated according to Clydesdale [12].

2.6. Total Polyphenol Content (TPC)

2.6.1. Determination of Total Polyphenol Content in Grape Skin Powder

TPC of GSP was quantified using the Folin–Ciocalteu method, as described by Waterman and Mole, with minor adaptations [13]. Polyphenolic compounds were extracted using 50% (v/v) ethanol as solvent, at a solid-to-solvent ratio of 1:3 (w/v). Due to the high concentration of phenolic compounds in grape skin powder, the resulting extract was further diluted 1:10 prior to analysis. Absorbance was measured spectrophotometrically, and results were calculated based on a gallic acid calibration curve (0–10 mg/L; $y = 0.5056x + 0.0650$; $R^2 = 0.9977$). TPC was expressed as mg gallic acid equivalents per g of sample (mg GAE/g).

2.6.2. Determination of Total Polyphenol Content in Dessert Samples

For dairy-based frozen dessert prototype samples, a separate extraction procedure was applied in order to account for the complex food matrix and the high fat content. Samples were homogenized and polyphenolic compounds were extracted using 50% (v/v) ethanol, at a sample-to-solvent ratio of 1:3 (w/v). The mixture was subjected to centrifugation (≈ 5000 rpm, 10 min) to promote phase separation, and only the ethanolic supernatant was collected for subsequent analysis. This approach minimized lipid interference and ensured reliable spectrophotometric determination of phenolic compounds, in accordance with procedures reported for dairy and high-fat food matrices. The TPC of all samples was determined using the Folin–Ciocalteu method, based on the gallic acid calibration curve, and results were expressed as mg GAE/100 g of sample.

2.7. DPPH Antioxidant Activity (AA)

AA was evaluated using the DPPH radical scavenging assay and expressed as percentage of DPPH inhibition (%), in accordance with the method of Brand-Williams et al. [14]. The analysis was performed using the ethanolic extracts prepared for TPC determination, as described in Section 2.6.1 and 2.6.2, and results were expressed as percentage of DPPH radical inhibition.

2.8. Sensory Evaluation

Sensory evaluation of dairy-based frozen dessert prototype samples was performed by 37 non-smoking assessors aged 23–70 years. Panelists received preliminary training to familiarise themselves with the descriptive sensory attributes of frozen desserts and to ensure consistent evaluation, in accordance with ISO 6658:2017. A 9-point hedonic scale was used to assess consumer acceptability, where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely. Each assessor evaluated the samples individually and rated the following sensory quality indicators: appearance, colour, aroma, flavour, texture, melting behaviour, and overall acceptability. Samples were coded and served in a randomised order under controlled laboratory conditions.

2.9. Microbiological Analysis

Microbiological analyses of the dairy-based frozen dessert prototype fortified with grape skin powder (GSP) were performed in accordance with internationally recognized ISO standard methods and relevant European legislation [15, 16]. Samples were collected aseptically at defined storage intervals (0 and 28 days) and analyzed in triplicate. Prior to analysis, frozen samples stored at -27 ± 1 °C were thawed under refrigerated conditions (4 ± 1 °C).

The total viable count (TVC) was determined according to ISO 4833-1 [17], using plate count agar and incubation at 30 °C for 72 h, with results expressed as CFU/g. Yeasts and moulds were enumerated following ISO 21527-1 [18], using acidified yeast extract glucose chloramphenicol (YGC) agar, with incubation at 25 °C for 3–5 days.

The presence of *Listeria monocytogenes* was investigated according to ISO 11290-1 [19], and *Salmonella spp.* detection was carried out following ISO 6579-1 [20]. Results for pathogens were reported as presence or absence in 25 g of product.

Identification of microorganisms was supported by Gram staining, following the procedure described by Vijayakumar et al. [21].

2.10. Statistical Analysis

Statistical analysis was carried out using XLStat software (version 7.5.2, Addinsoft, Paris, France) integrated into Microsoft Excel. Differences between samples were assessed through one-way ANOVA, and mean separation was performed using Tukey's HSD post hoc test at a significance level of $p < 0.05$.

3. Results and Discussion

Grape skin powder obtained after convective drying preserved a high level of bioactive compounds, confirming its potential use as a functional food ingredient. The initial moisture content of fresh grape skins was $24.36 \pm 0.15\%$, which decreased to $6.80 \pm 0.12\%$ following standard drying procedures [22]. The dried grape skin powder exhibited an antioxidant activity of 87.9% and a TPC of 274.0 mg GAE/g, values consistent with those reported for polyphenol-rich grape by-products (Table 2).

Comparable values for TPC and antioxidant activity have been reported for grape skin and grape pomace derived from different *Vitis vinifera* varieties. Ky and Teissedre reported total polyphenol contents in grape skin extracts ranging from approximately 110 to 225 mg GAE/g DW, depending on grape variety and extraction solvent, with higher values obtained using hydroalcoholic extraction systems [23]. Similar trends were observed by Mostafa et al., who reported total phenolic contents of approximately 41.6 mg GAE/g DW and antioxidant activity of 10.9% for dried red grape skin powder obtained by convective drying, highlighting the strong influence of processing conditions and extraction efficiency on the measured bioactive levels [24].

Particularly relevant to the present study, Brezoiu et al. investigated polyphenolic extracts obtained from grape pomace and grape skin powder derived from *Feteasca Neagră* grapes, reporting high polyphenol concentrations and pronounced antioxidant potential in ethanolic extracts. In their study, total polyphenol contents reached up to 279.64 ± 4.52 mg GAE/g extract for grape pomace and approximately 212.21 ± 0.85 mg GAE/g extract for grape skin powder, accompanied by high DPPH radical scavenging capacity expressed as 105–137 mg TE/g vacuum dried extract [25].

In contrast, in the present study antioxidant activity was expressed as percentage of DPPH inhibition, as this unit is commonly used for comparative evaluation within a single experimental set.

The values obtained in the present study fall within or close to these reported ranges, confirming that convective drying followed by incorporation into dairy-based frozen desserts formulations preserved the bioactive potential of *Feteasca Neagră* grape skins.

Table 2

Physicochemical Characteristics and Bioactive Properties of Grape Skin Powder	
Parameter	Value
Moisture content of fresh grape skins, %	24.36 ± 0.15
Moisture content of GSP, %	6.80 ± 0.12
Antioxidant activity (DPPH inhibition, %)	87.9 ± 0.6
Total polyphenol content, mg GAE/g	274.0 ± 1.3

GSP – grape skin powder; GAE - gallic acid equivalents; DPPH - 2,2-diphenyl-1-picrylhydrazyl.

The dairy-based frozen dessert prototype developed in this study was designed as a model system to evaluate the impact of grape skin powder (GSP) incorporation on composition-related quality parameters. Accordingly, the discussion that follows focuses on physicochemical indicators directly determined in the experimental samples, including dry matter, fat content and pH, which are essential for understanding the technological behaviour of the fortified frozen dessert.

3.1. Effect of GSP Incorporation on the Physicochemical Characteristics

The addition of grape skin powder (GSP) led to several coordinated modifications in the physicochemical attributes of the dairy-based frozen dessert prototype formulations, particularly in terms of dry matter, lipid content and acidity (Table 3). These parameters are closely interrelated and reflect both the composition of the ingredients and the functional behavior of grape-derived bioactive compounds in dairy matrices.

Table 3

Physicochemical Indicators of the Dairy-Based Frozen Dessert Prototypes Fortified with Grape Skin Powder

Examples	Dry matter, %	Fat content, %	pH
Control sample	55.38±0.35 ^a	26.25±0.01 ^a	6.72±0.12 ^b
2.5% GSP	55.23±0.16 ^a	26.61±0.09 ^a	6.66±0.06 ^b
5.0% GSP	55.09±0.09 ^a	26.83±0.03 ^{ab}	6.32±0.11 ^{ab}
7.5% GSP	54.94±0.18 ^a	27.02±0.06 ^{ab}	6.02±0.09 ^{ab}
10.0% GSP	54.80±0.11 ^a	27.66±0.08 ^b	5.62±0.01 ^a

GSP – grape skin powder. Values are expressed as mean ± standard deviation (n = 3). Values in the same column followed by different superscript letters (^{a-b}) are significantly different ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's post hoc test.

Across all dairy-based frozen dessert prototype samples, the DM content exceeded 50%, values substantially higher than those typically reported for milk-based ice creams (28–39%) [26]. This is consistent with the high-fat raw material used (35% sweet cream), which naturally increases total solids. A slight gradual decrease in DM (55.38% - 54.80%) was observed with increasing GSP levels, likely due to the replacement of sucrose with fibre-rich plant material. The structural polysaccharides present in grape skins—known for their water-binding capacity—may influence the distribution of free vs. bound water, thereby subtly affecting total solids.

Fat content followed a similar pattern of moderate variation. Although GSP itself contains negligible lipid fractions, its incorporation modifies the solids balance and proportionally increases the relative contribution of cream fat in the mixture (26.25% - 27.66%).

The pH values decreased progressively with increasing amounts of GSP (from 6.72 in the control sample to 5.62 at 10% GSP), reflecting the intrinsic acidity of grape skins, which are rich in organic acids such as tartaric and malic acids. This reduction in pH was directly related to the level of GSP incorporation and contributed to perceptible changes in taste. At lower addition levels (2.5–5.0%), the moderate decrease in pH enhanced freshness and imparted a slightly acidic taste that was well accepted by panelists. In contrast, higher GSP concentrations (7.5–10.0%) intensified acidic and phenolic sensations, leading to more pronounced astringent notes that were reflected in slightly lower taste scores.

Overall, the results presented in Table 3 indicate that GSP can be incorporated into dairy-based frozen dessert prototype formulations without compromising physicochemical stability. Instead, its presence induces predictable and technologically manageable shifts related to the fibre composition and organic acids intrinsic to grape by-products. These observations are consistent with other studies incorporating plant-based powders into frozen dairy systems, supporting the feasibility of using grape skin powder as a functional fortifying ingredient.

3.2. Influence of GSP Addition on the Acidity Index and Peroxide Value

Given the high fat content of the sweet cream used (35%), the acidity index (AI) and peroxide value (PV) were monitored throughout frozen storage as indicators of hydrolytic and oxidative lipid degradation. Analyses were performed at 0, 7, 14, and 28 days of storage, and the results are summarized in Table 4.

Table 4

Evolution of Acidity Index and Peroxid Value Dairy-Based Frozen Dessert Prototype During Storage

Sample	Storage period, days				
	0	7	14	21	28
AI, mg KOH/g fat					
Control sample	0.10 ± 0.04 ^a	0.16 ± 0.08 ^a	0.17 ± 0.10 ^a	0.20 ± 0.09 ^a	0.21 ± 0.02 ^a
2.5% GSP	0.10 ± 0.02 ^a	0.14 ± 0.04 ^{ab}	0.16 ± 0.06 ^{ab}	0.17 ± 0.03 ^b	0.19 ± 0.03 ^b
5.0% GSP	0.10 ± 0.04 ^a	0.13 ± 0.06 ^b	0.13 ± 0.04 ^b	0.14 ± 0.01 ^c	0.16 ± 0.05 ^c
7.5% GSP	0.10 ± 0.03 ^a	0.11 ± 0.05 ^b	0.11 ± 0.06 ^c	0.13 ± 0.04 ^c	0.13 ± 0.03 ^c
10.0% GSP	0.10 ± 0.06 ^a	0.10 ± 0.02 ^b	0.10 ± 0.03 ^c	0.11 ± 0.06 ^c	0.12 ± 0.04 ^c
PV, meq O ₂ /kg fat					
Control sample	0.80 ± 0.02 ^a	1.30 ± 0.09 ^a	1.40 ± 0.06 ^a	1.60 ± 0.11 ^a	1.70 ± 0.07 ^a
2.5% GSP	0.80 ± 0.06 ^a	1.20 ± 0.08 ^b	1.25 ± 0.05 ^b	1.34 ± 0.07 ^b	1.49 ± 0.08 ^b
5.0% GSP	0.80 ± 0.03 ^a	1.20 ± 0.05 ^b	1.22 ± 0.03 ^b	1.30 ± 0.03 ^b	1.46 ± 0.02 ^b
7.5% GSP	0.80 ± 0.02 ^a	1.18 ± 0.04 ^b	1.39 ± 0.08 ^{ab}	1.40 ± 0.06 ^{ab}	1.41 ± 0.04 ^c
10.0% GSP	0.80 ± 0.03 ^a	1.11 ± 0.07 ^c	1.32 ± 0.10 ^b	1.36 ± 0.08 ^b	1.36 ± 0.09 ^c

Values are expressed as mean ± standard deviation (n = 3). Values in the same column followed by different superscript letters (^{a-c}) are significantly different ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's post hoc test. GSP – grape skin powder; AI – acidity index; PV – peroxide value.

During frozen storage, both acidity index (AI) and peroxide value (PV) exhibited a time-dependent evolution; however, the magnitude and pattern of change differed among samples (Table 4). In the control sample, a continuous increase in AI (from 0.10 to 0.21 mg KOH/g fat) and PV (from 0.80 to 1.70 meq O₂/kg fat) was observed throughout the entire storage period, indicating progressive hydrolytic and oxidative lipid degradation.

In contrast, samples fortified with grape skin powder (GSP) showed a slower increase and, in some cases, a tendency toward stabilization after 7–14 days of storage. At higher GSP addition levels (7.5–10.0%), both AI and PV values exhibited only minor variations during prolonged storage and remained consistently lower than those of the control sample. This behaviour suggests that the incorporation of GSP mitigated lipid oxidation processes, which can be attributed to the presence of grape skin polyphenols with radical-scavenging and metal-chelating activity, as reported in the literature [27]. These results indicate that although AI and PV tended to increase during storage, the rate and extent of these changes were strongly dependent on GSP concentration, with higher incorporation levels contributing to improved oxidative stability of the dairy-based frozen dessert.

3.3. Influence of GSP Addition on Colour Parameters

The colour parameters of the dairy-based frozen dessert prototype samples fortified with GSP are presented in **Table 5**. The data clearly show that the addition of GSP had a strong and concentration-dependent effect on the chromatic attributes of the formulations.

Table 5

CIELab Colour Parameters of Dairy-Based Frozen Dessert Prototype Fortified with GSP

Sample	CIELab colour parameters			
	L*	a*	b*	ΔE^*
Control	79.59 ± 0.42 ^a	-0.45 ± 0.03 ^d	23.29 ± 0.28 ^a	-
2.5% GSP	49.51 ± 0.36 ^b	4.54 ± 0.05 ^b	1.68 ± 0.09 ^b	37.37 ± 0.51 ^d
5.0% GSP	34.04 ± 0.27 ^c	5.79 ± 0.07 ^a	-0.52 ± 0.06 ^c	51.77 ± 0.62 ^c
7.5% GSP	31.56 ± 0.22 ^d	4.39 ± 0.04 ^b	-0.42 ± 0.05 ^c	53.78 ± 0.58 ^b
10.0% GSP	31.50 ± 0.25 ^d	2.56 ± 0.06 ^c	-3.13 ± 0.08 ^d	54.94 ± 0.67 ^a

GSP - grape skin powder; L* – lightness (0 = black, 100 = white); a* – colour coordinate ranging from green (-) to red (+); b* – colour coordinate ranging from blue (-) to yellow (+); ΔE^* – total colour difference compared to the control sample. Values are expressed as mean ± standard deviation (n = 3). Values in the same column followed by different superscript letters (a–d) are significantly different ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's post hoc test.

As observed, increasing the level of GSP resulted in a marked reduction of lightness (L*), with values dropping from 79.59 in the control sample to 31.50 in the 10% GSP frozen dessert. This effect is directly related to the high anthocyanin content of grape skins, which impart intense red–purple coloration to dairy matrices. Similar decreases in L values accompanied by shifts toward red/violet tones have been consistently reported in frozen dairy products fortified with grape skin or grape pomace derivatives rich in anthocyanins. Salem et al. (2014) demonstrated that the addition of red grape pomace extract to ice cream significantly reduced lightness and yellowness while increasing redness (a*), confirming the strong colouring capacity of grape-derived anthocyanins [28]. Comparable colour modifications were also reported by Nascimento et al., who observed statistically significant changes in CIELAB parameters following the incorporation of grape agro-industrial waste flour into ice cream formulations, without adversely affecting visual acceptability [29].

Further support for the anthocyanin-driven colour changes was provided by Rațu et al., who reported pronounced reductions in lightness and increased chromatic intensity in dairy-based frozen products enriched with grape pomace or grape skin derivatives [30]. In addition, studies on fermented dairy matrices, such as yogurt fortified with grape skin powder, have shown similar decreases in L values and increases in redness attributed to pH-

dependent anthocyanin stability and protein–polyphenol interactions [31]. The absence of a linear trend for *a* and *b* values in the present study can be explained by the pH sensitivity of anthocyanins and their interactions with milk proteins and water activity, which may affect pigment expression depending on the level of GSP incorporation.

By comparison, the grape skin powder used in the present study generated an equally strong and dose-dependent chromatic effect, with ΔE^* values exceeding 37–54 units, indicating visually perceptible and substantial differences from the control, comparable in magnitude to colour shifts reported in functional ice creams enriched with black carrot anthocyanins, grape marc or pomegranate peel. Taken together, these findings reinforce that grape skin powder operates effectively as a natural colourant, capable of imparting deep red–purple tones to both dairy and non-dairy frozen desserts.

3.4. Influence of Added GSP on the TPC and AA of Frozen Dessert Samples

The plant-based foods in our diet consist of primary and secondary ingredients produced by plants. Plants synthesize secondary metabolites such as terpenes, saponins, glycosides and polyphenolic compounds that are not present in all living things, as well as primary metabolites such as carbohydrates, proteins and fats to maintain their growth and vitality [32]. Most secondary metabolites are formed from polyphenols. Plants produce polyphenols as secondary metabolites to protect themselves and interact with other plant [33]. Although milk-based products are not considered major sources of polyphenols, previous studies have demonstrated the presence of measurable phenolic compounds in milk and dairy matrices, originating from animal feed, Maillard reaction products, and interactions with proteins [34]. Therefore, the detection of low baseline TPC values in control sample is consistent with literature data and does not invalidate the comparative assessment of GSP-fortified formulations [35,36]. The polyphenols in the studied samples were determined by applying the Folin Ciocalteu method, and based on the calibration curve for gallic acid. Thus, as a result, the data shown in Figure 1 were obtained.

Figure 1 shows a directly proportional relationship between the amount of added GSP and the TPC of the processed samples.

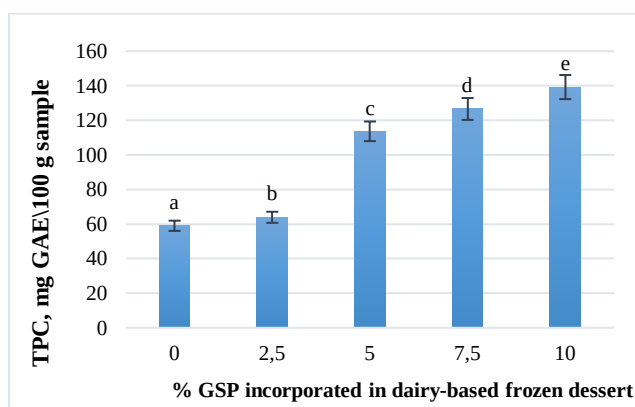


Figure 1. Total polyphenol content of dairy-based frozen dessert prototypes, mg GAE/100 g sample.

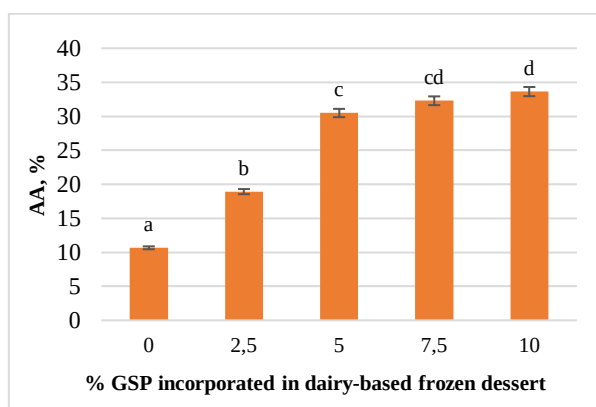


Figure 2. Antioxidant activity (% DPPH inhibition) of dairy-based frozen dessert prototypes.

Different letters above the bars indicate statistically significant differences ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's post hoc test.

Thus, the highest polyphenol content was found in the samples with 7.5 and 10.0% GSP (126.61 and 139.29 mg GAE/100 g respectively). In the same context, based on the results

obtained from the data in Figure 2, we can say that, as a rule, the desserts with a higher amount of addition showed higher AA, % values than the other samples. The antioxidant activity of GSP dairy-based frozen dessert prototypes ranged from 18.92 to 33.63%, showing a directly proportional relationship with total phenolic content (TPC, mg GAE/100 g).

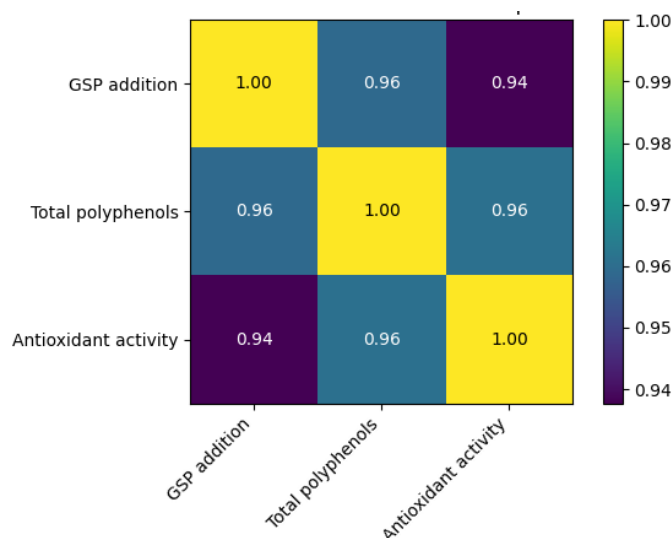


Figure 3. Pearson correlation heatmap illustrating the relationships between grape skin powder (GSP) addition level, total polyphenol content and antioxidant activity.

A very strong positive correlation was observed between total polyphenol content and antioxidant activity ($r = 0.96$), indicating that the increase in antioxidant capacity is closely associated with the higher polyphenol levels contributed by grape skin powder. In addition, GSP addition showed a strong positive correlation with both total polyphenol content ($r = 0.96$) and antioxidant activity ($r = 0.94$), confirming the dose-dependent functional contribution of grape skin powder (Figure 3). Comparable relationships have been reported for grape skin extracts, where Katalinić et al. observed a very strong linear association between total phenolic content and antioxidant capacity assessed by FRAP ($R^2 = 0.9456$, corresponding to $r \approx 0.97$), confirming that polyphenols are the principal contributors to the antioxidant potential of grape skin-derived materials [37].

3.5. Microbiological Quality of the Dairy-Based Frozen Dessert Prototype Fortified with Grape Skin Powder

The GSP incorporated into the dairy-based frozen dessert prototype was obtained through mild convective drying and grinding, without the application of sterilization treatments. As a result, this ingredient may act as a carrier of indigenous microflora typically associated with plant-derived raw materials, including bacteria, yeasts and moulds. Such microflora can survive processing and become detectable when favorable conditions, such as moisture availability during thawing and refrigerated storage, are present. Mould contamination is of particular relevance in plant-based ingredients due to the potential presence of mycotoxigenic species; therefore, monitoring microbial dynamics in fortified products represents an important aspect of product quality evaluation [38].

The microbiological quality of the dairy-based frozen dessert prototype fortified with different levels of GSP was evaluated during storage by determining total viable count, yeasts and moulds, Enterobacteriaceae, and the presence of selected pathogens, as presented in Table 6.

Microbiological analyses were performed after controlled thawing and refrigerated storage, as frozen storage at -27 ± 1 °C is not expected to support microbial growth but only microbial survival. On day 0, all formulations exhibited low total viable counts, ranging between approximately 1.2×10^2 and 2.3×10^2 CFU/g, indicating good initial hygienic quality of both the base formulation and the added grape skin powder. Yeasts and moulds were either below the detection limit or detected at very low levels, while *Listeria monocytogenes* and *Salmonella spp.* were absent in 25 g of product in all formulations.

Table 6

Qualitative Screening of Microbial Presence in the Dairy-Based Frozen Dessert Prototype Fortified with Grape Skin Powder During Refrigerated Storage after Thawing

Sample	Storage, days	Total viable count, CFU/g	Yeasts & moulds, CFU/g	<i>Listeria monocytogenes</i> (25 g)	<i>Salmonella spp.</i> (25 g)
Control	0	1.2×10^2	$<1.0 \times 10^1$	Absent	Absent
Control	28	4.5×10^3	2.1×10^2	Absent	Absent
2.5% GSP	0	1.4×10^2	$<1.0 \times 10^1$	Absent	Absent
2.5% GSP	28	3.2×10^3	1.6×10^2	Absent	Absent
5.0% GSP	0	1.6×10^2	$<1.0 \times 10^1$	Absent	Absent
5.0% GSP	28	2.8×10^3	1.3×10^2	Absent	Absent
7.5% GSP	0	1.9×10^2	1.1×10^1	Absent	Absent
7.5% GSP	28	5.8×10^3	3.6×10^2	Absent	Absent
10.0% GSP	0	2.3×10^2	1.5×10^1	Absent	Absent
10.0% GSP	28	7.4×10^3	4.8×10^2	Absent	Absent

During refrigerated storage after thawing, a gradual increase in total viable count and fungal populations was observed in all samples (Table 6). After 28 days, total viable counts increased to values ranging from approximately 2.8×10^3 CFU/g in the 5.0% GSP formulation to 7.4×10^3 CFU/g in the 10.0% GSP formulation. A similar trend was observed for yeasts and moulds, with counts remaining below 5.0×10^2 CFU/g even at the highest GSP addition level. Pathogenic microorganisms were not detected at any storage point. Formulations containing 2.5% and 5.0% GSP showed slightly lower microbial counts compared to the control during storage, particularly in terms of total viable count and yeasts and moulds. This behavior may be associated with the presence of grape skin polyphenols, which are known to exert antimicrobial effects against a broad range of microorganisms. In contrast, formulations containing 7.5% and 10.0% GSP exhibited higher microbial counts during prolonged storage, which may be attributed to the increased introduction of indigenous microflora and nutrients associated with higher amounts of grape skin powder, partially counterbalancing its antimicrobial potential.

3.6. Sensory Analysis

Sensory evaluation is one of the methods used to identify market acceptability especially in food or beverage products. It is useful for product development and improvement as the most important factor for a given market can be identified and improved. Influencing factors are essential for consumers to get the best product and for producers to develop and sell the best product [39].

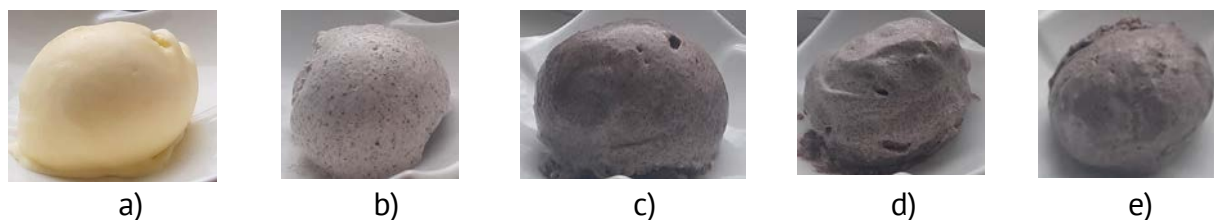


Figure 4. Visual appearance of dairy-based frozen dessert prototype samples fortified with different levels of grape skin powder (GSP): a) control; b) 2.5% GSP; c) 5.0% GSP; d) 7.5% GSP; e) 10.0% GSP.

Sensory attributes (Figure 5) in terms of colour, aroma, taste and texture and overall acceptability for dairy-based frozen dessert prototypes were determined after a period of 2 weeks after preparation. Sensory evaluation performed using the 9-point hedonic scale revealed that all samples, including those fortified with grape skin powder (GSP), obtained mean scores above 6.3, corresponding to the category “like slightly”. The distributions shown in the boxplots demonstrate that panellists generally evaluated the products favourably, with only a few lower ratings, all of which corresponded to the neutral point of the scale (5 = neither like nor dislike). This indicates that even for samples perceived as less optimal, the response was neutral rather than negative, suggesting room for further technological refinement but no sensory rejection.

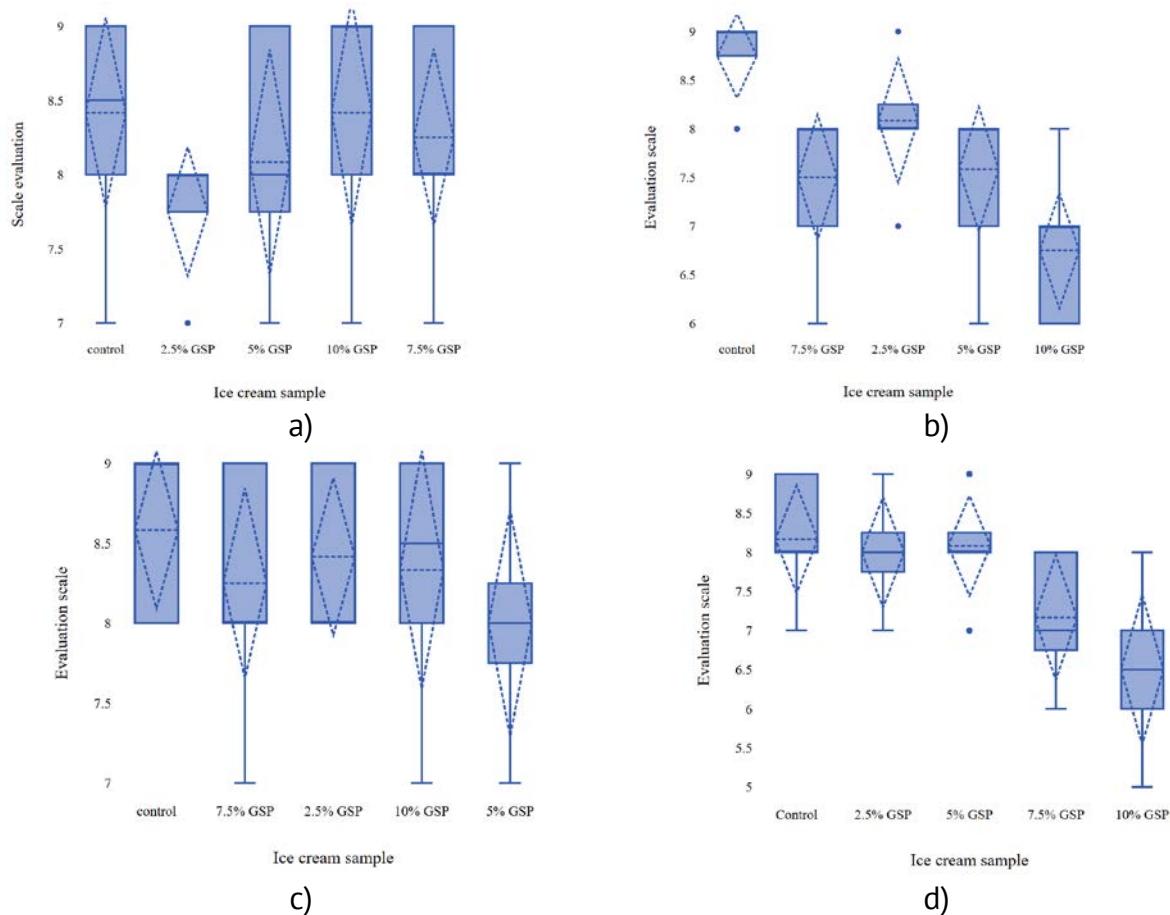


Figure 5. Sensory profile of dairy-based frozen dessert prototype samples: a) colour evaluation; b) texture evaluation; c) aroma evaluation; d) taste evaluation.

Across attributes, colour showed a clear upward trend in acceptability with increasing GSP concentration, reflecting the positive perception of the naturally intensified pigmentation provided by grape skin compounds. In contrast, texture exhibited the opposite tendency, with slightly lower and more dispersed scores at higher GSP levels, likely due to the perceptibility of fine grape skin particles. Still, even the 10% GSP sample maintained mean values around 6.3–6.5, confirming that texture remained within an acceptable range.

Aroma was consistently well appreciated across all samples, with compact distributions and high medians, indicating that the volatile compounds from grape skin integrated harmoniously into the frozen dessert matrix. Taste followed a similar pattern, with a slight decrease at higher GSP concentrations due to mild astringent or phenolic notes, yet maintaining mean values above 6 for all formulations.

Among all formulations, the 5% GSP sample clearly stood out as the most successful. It achieved the highest balance across attributes, with mean scores of 8 for colour, 7.6 for texture, 8 for aroma, and 8 for taste, corresponding to the categories “like very much” (score 8) and “like moderately” (score 7). These values highlight an optimal integration of GSP into the dairy-based frozen dessert prototype matrix, enhancing flavour and aroma while maintaining a pleasant texture and an appealing colour. Taken together, the results confirm that a 5% addition level offers the best compromise between functional enhancement and sensory acceptance.

4. Conclusions

This study demonstrates that grape skin powder, obtained from winemaking by-products, can be effectively incorporated into a dairy-based frozen dessert prototype, contributing to functional enrichment and improved oxidative stability. Increasing GSP levels induced predictable changes in product composition, including a moderate decrease in pH (6.72–5.62) and a slight increase in fat content (26.25–27.66%), without compromising overall physicochemical stability.

The addition of GSP significantly enhanced the functional profile of the product, as evidenced by higher total polyphenol content (up to 139 mg GAE/100 g) and increased antioxidant activity (up to 33.6%). These improvements were accompanied by enhanced resistance to lipid oxidation during frozen storage, with lower peroxide values compared to the control sample. Colour analysis confirmed a strong and dose-dependent chromatic effect ($\Delta E^* > 37$), highlighting the effectiveness of grape skin anthocyanins as natural colourants for frozen dairy systems.

Microbiological results indicated that moderate GSP incorporation levels (2.5–5.0%) were associated with lower microbial counts during storage, whereas higher levels (7.5–10.0%) introduced additional indigenous microflora from the plant material. Sensory evaluation revealed good consumer acceptance across all formulations, with the 5% GSP sample achieving the most favourable balance between colour, flavour, texture and overall acceptability.

Acknowledgments: The research was funded by State Project subprogram 020405 “Optimization of food processing technologies in the context of the circular bioeconomy and climate change”, running at Technical University of Moldova.

Conflicts of Interest: The authors declare no conflict of interest.

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Citation: Ruseva, O.; Covaliov, E.; Suhodol, N.; Deseatnicova, O.; Resitca, V. Effect of grape skin incorporation on the quality of a dairy-based frozen dessert. *Journal of Engineering Science*. 2025, XXXII (4), pp. 104–119. [https://doi.org/10.52326/jes.utm.2025.32\(4\).08](https://doi.org/10.52326/jes.utm.2025.32(4).08).

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Submission of manuscripts:

jes@meridian.utm.md

[https://doi.org/10.52326/jes.utm.2025.32\(4\).09](https://doi.org/10.52326/jes.utm.2025.32(4).09)

UDC 663.221:579.67:663.12(478)



THE USE OF BIOPROTECTIVE YEASTS TO REDUCE SULFITE CONSUMPTION IN WINEMAKING

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Received: 12. 02.2025

Accepted: 12. 29. 2025

Abstract. EU Regulation 2019/934 and consumer demand for "low-sulfite" wines necessitate alternative microbiological preservation strategies. Non-Saccharomyces yeasts—*Metschnikowia pulcherrima* and *Torulaspora delbrueckii*—suppress spoilage organisms through multi-pathway bioprotection (iron chelation, nutrient competition, oxygen depletion, cell-contact inhibition) without compromising fermentation or wine composition. This study presents the first Moldavian field trial validating bioprotective yeast efficacy. White wine must from Codru region (Glera and Fetească Regală cultivars) was inoculated with Zymaflore Égide TDMP and subsequently fermented with *Saccharomyces cerevisiae*. Analysis was performed by ISO 17025-accredited laboratory (LTPA). Results demonstrated 32–50% SO₂ reduction vs. traditional baseline. Volatile acidity (0.43–0.44 g/L) remained at 40% of EU threshold, confirming acetic acid bacteria suppression. Heavy metals and sodium levels remained significantly below regulatory limits, confirming market compliance. Bioprotective yeasts represent a validated, commercially proven alternative for Moldavian white wine production, aligning with global sustainability trends and enabling premium market positioning.

Keywords: *acetic acid bacteria, bioprotection, low-sulfite wines, Metschnikowia pulcherrima, non-Saccharomyces yeasts, pulcherrimin, sulfur dioxide reduction, Torulaspora delbrueckii,*

Rezumat. Regulamentul UE 2019/934 și cererea consumatorilor pentru vinuri „cu conținut scăzut de sulfiți” impun strategii alternative de conservare microbiologică. Drojdiile non-Saccharomyces (*Metschnikowia pulcherrima* și *Torulaspora delbrueckii*) suprimă organismele de alterare prin bioprotecție (chelarea fierului, competiția pentru nutrienți, epuizarea oxigenului) fără a compromite fermentația. Acest studiu raportează rezultatele primelor teste de teren efectuate în condițiile Republicii Moldova, care validează eficacitatea drojdiilor bioprotectoare pe musturi de struguri albi din regiunea Codru (soiurile Glera și Fetească Regală). Rezultatele au demonstrat o reducere de sulfiți 32–50% față de metodele tradiționale, menținând aciditatea volatilă la doar 40% din pragul UE. Nivelurile de metale grele și sodiu au rămas mult sub limitele de reglementare, confirmând corespunderea vinului cerințelor pieței și potențialul de poziționare premium a vinurilor moldovenești cu conținut redus de sulfiți.

Cuvinte-cheie: bacterii acetice, bioprotecție, vinuri cu conținut scăzut de sulfiți, *Metschnikowia pulcherrima*, drojdii non-*Saccharomyces*, reducerea dioxidului de sulf, *Torulaspora delbrueckii*.

Abbreviations

AAB: Acetic acid bacteria; LAB: Lactic acid bacteria; NS: Non-*Saccharomyces*; SO₂: Sulfur dioxide; CFU: Colony-forming units; TDMP: *Torulaspora delbrueckii*/*Metschnikowia pulcherrima*; EU: European Union; OIV: International Organization of Vine and Wine; ADY: Active dry yeast; LTPA: Laboratory for Testing Alcoholic Products; GMO: Genetically modified organism; IGP: Indication of Geographic Production; EFSA: European Food Safety Authority; WSET: Wine & Spirit Education Trust; FAAS: Flame atomic absorption spectrometry; GF-AAS: Graphite furnace atomic absorption spectrometry.

1. Introduction

1.1 Historical Context and Contemporary Challenge

Sulfur dioxide has served as the principal antimicrobial and antioxidant agent in winemaking for more than two millennia, with systematic documentation of use from the 17th century onward [1]. Its exceptional versatility—simultaneously inhibiting spoilage yeasts and bacteria, preventing enzymatic browning through polyphenol oxidase inhibition, and providing broad-spectrum antioxidant protection against oxidative degradation—has made SO₂ indispensable across virtually all viticulture regions and wine styles globally [2].

However, contemporary regulatory frameworks and evolving consumer preferences have fundamentally shifted this paradigm [3]. The European Union Regulation (EU) 2019/934, adopted in June 2019, imposes progressively stringent limits on SO₂ concentrations: maximum 150 mg/L for red wines and 200 mg/L for white wines, representing a 25–50% reduction from previous regulatory thresholds [4]. These restrictions are scientifically grounded in legitimate health considerations—SO₂ is classified as an allergen affecting approximately 1–5% of sulfite-sensitive individuals, particularly asthmatics—and reflect broader societal demand for minimally processed, "natural" food and beverage products [1, 13].

Concurrently, the global market for "low-sulfite" and "natural" wines has expanded dramatically, valued at €12–15 billion in 2025 [13] and growing at approximately 15–20% annually [14], with premium segments commanding 15–25% price premiums over conventional wines [14].

1.2 The Moldavian Context and Strategic Opportunity

In the Republic of Moldova, a nation with 120,000 hectares of vineyards [6] producing 1.4–2.0 million hectoliters annually, with white wines comprising 55% of exports valued at approximately €120 million annually [6], this regulatory and market environment, presents both significant challenge and unprecedented opportunity. [6]. Moldavian wine exports to demanding markets—European Union, North America, Japan—increasingly depend on compliance with restrictive SO₂ thresholds and on market positioning aligned with sustainability narratives valued by contemporary premium consumers [6].

The Codru wine region, historically celebrated for quality white wines, experiences particular oxidative vulnerability due to continental climate conditions and harvest timing (September, 13–18°C ambient temperature) [15]. Complete elimination of SO₂ from winemaking remains oenologically hazardous: without microbiological protection, wines become vulnerable to oxidative degradation, volatile acidity spoilage from acetic acid

bacteria (*Acetobacter* and *Gluconobacter* spp.), microbial instability, and sensory quality deterioration [4,5].

1.3 Bioprotection: An Ecological and Scientific Solution

Non-*Saccharomyces* yeasts have been used for many years due to their technological potential in mixed fermentations with *Saccharomyces cerevisiae* [6,7]. Recently, a new application has emerged, bioprotection, which consists of colonizing the environment in the context of reducing sulfites in wines. The use of yeasts with bioprotective activity is presented as an alternative for wine producers, with the aim of limiting the development of spoilage microorganisms and preserving the sensory quality of the products [8,9].

In the context of chemical constraint and quality imperative, bioprotection—the strategic use of beneficial microorganisms to suppress spoilage organisms—has emerged as the most scientifically validated and commercially proven alternative to classical SO₂ treatment [10,11]. There are strategies for reducing or replacing added sulfites using chitosan and low doses of lysozyme [12]. Non-*Saccharomyces* yeasts, particularly *Metschnikowia pulcherrima* and *Torulaspora delbrueckii*, have been intensively studied over the past decade and are now commercially available in standardized, inoculum-ready formulations certified for winemaking under EU Regulation 2019/934 [13, 14].

Bioprotective yeasts function through an elegant ecological principle: when inoculated directly into fresh grape must at the pre-fermentative stage (immediately after crushing and pressing), they rapidly colonize the available microbial niche before indigenous spoilage organisms can establish [9,15]. Operating at cool pre-fermentative temperatures (10–16°C), these non-*Saccharomyces* yeasts consume fermentable sugars and nutrients competitively, produce inhibitory metabolites, trigger oxygen-dependent stress responses that suppress acetic acid bacteria and lactic acid bacteria, and establish cell-contact inhibition mechanisms [16]. Critically, they achieve this protective action *without* initiating alcoholic fermentation—a role reserved for subsequently inoculated *Saccharomyces cerevisiae* strains, which take over once ethanol concentration exceeds the ethanol tolerance threshold (~5% v/v) of the bioprotectors [17,18].

1.4 State of International Scientific Evidence

Recent peer-reviewed literature demonstrates quantifiable success across diverse wine regions and varieties. Field trials conducted in France (INRAE, Université de Bordeaux), Italy (Università di Modena e Reggio Emilia), Spain, Australia, and California consistently show SO₂ reduction of 30–50% depending on grape variety, regional climate, harvest sanitary status, and inoculum viability and confirm applicability across both white and red wine production, with minimal impact on fermentation kinetics and often with measurable improvements in polysaccharide and glycerol content—desirable sensory markers of wine complexity and mouthfeel [19–22]. The mechanistic basis underlying bioprotection has also been substantially elucidated. *Metschnikowia pulcherrima* produces pulcherrimin, a red iron-chelating pigment with documented broad-spectrum antimicrobial activity against acetic acid bacteria, lactic acid bacteria, and spoilage yeasts [23,24]. *Torulaspora delbrueckii* accumulates extracellular polysaccharides while consuming fermentable sugars under semi-anaerobic conditions, thus reducing osmotic stress on subsequent *Saccharomyces* fermentation [25, 26]. Both species excrete secondary metabolites—organic acids (lactic, acetic), phenolic compounds, and bioactive peptides—that create a biochemically hostile microenvironment for spoilage microbiota. Recent transcriptomic and proteomic studies have revealed that

direct cell-cell contact between bioprotective yeasts and competing organisms may play an underappreciated role in suppression, independent of metabolite production alone [27].

This comprehensive article synthesizes current scientific evidence on bioprotective yeasts in winemaking, integrates quantitative efficacy data from international field trials, presents mechanistic understanding grounded in recent molecular studies, and provides original analytical validation from 2025 white wine vinification in Codru, Moldova [28]. Specific objectives are to: (1) comprehensively document bioprotective mechanisms with full scientific citation; (2) quantify SO₂ reduction outcomes across diverse wine varieties and regions; (3) present chemical safety profiles and regulatory compliance validation; (4) demonstrate practical applicability within Moldavian viticulture conditions; (5) discuss sustainability and market positioning implications; and (6) identify future research directions for optimization.

2. Materials and Methods

2.1 Materials

Grapes. Two dry still white wines were produced during the 2025 vintage in the Codru wine region (46°N, 28°E), Republic of Moldova. Codru is an Indication of Geographic Production (IGP) zone, representing approximately 30% of Moldova's vineyard area and specializing in cool-climate white wine production [29]. For this study, two cultivars with distinct phenological profiles were selected:

- **Fetească Regală** (*Vitis vinifera*) : An endemic Moldavian variety cultivated for over 200 years. It is characterized by high vigor and excellent adaptation to the Codru region, maintaining a stable balance between titratable acidity and phenolic maturity even under high harvest temperatures. It was chosen for its natural resistance to oxidation and its role as a cornerstone of Moldavian wine identity [28].
- **Glera** (*Vitis vinifera*) : An international cultivar originating from northeastern Italy [28]. It was strategically selected due to its high sensitivity to enzymatic and microbial oxidation. This allowed for testing the capacity of bioprotection to preserve delicate primary aromas (floral, stone fruit, and herbaceous notes) in the absence of high sulfur dioxide doses at the crushing stage.

Both varieties were harvested in September 2025 from commercially managed vineyards (minimum 5 years old). Harvesting coincided with optimal maturity, determined by Brix measurements (Glera: 190 g/L; Fetească Regală: 180 g/L), pH stability, and phenolic ripeness assessment.

Bioprotective yeasts. For bioprotection, the commercial preparation Zymaflore Égide TDMP (Laffort Groupe, Bordeaux, France) was used, consisting of a certified mixture of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima*, intended for environmental colonization, with no fermentation activity detected (no assimilation of sugars or nitrogen, no differences in turbidity levels at the end of the settling process); for restricting the growth of indigenous flora, in accordance with the current EU regulation n° 2019/934.

2.2 Pre-Fermentation Must Preparation

Processing protocol:

Step 1: Destemming and Crushing (30.09.2025, ambient temperature 16-18°C): Grapes were gently destemmed (vibrating destemmer, low-intensity setting) and crushed using pneumatic press (1.5–2 bar, Vaslin Bucher Inertia, stainless steel) to achieve optimal juice extraction (yield 65–70%) while minimizing tannin extraction from seeds and skin fragments [11].

Step 2: Static Settling (30.09.2025): Must was transferred to temperature-controlled vessels and settled statically at 13°C for 6 hours, permitting heavy particles (skin fragments, pulp debris) to precipitate to vessel bottom [11].

2.3 Bioprotective Yeast Inoculation Protocol

Immediately after static settling ($t_0 = 19:00$ PM, 30.09.2025), clarified must for each variety was divided into Bioprotected lots (2 tanks per variety (100 hL Glera) and (100 hL Feteasca Regala)).

Bioprotection Protocol: Commercial preparation: Zymaflore Égide TDMP (Laffort Groupe, Bordeaux, France), consisting of a certified blend of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima* strains, supplied as active dry yeast (ADY) at certified viability $\geq 2 \times 10^{10}$ CFU/g, non-GMO, EU Regulation 2019/934-compliant [14].

Rehydration: Zymaflore Égide TDMP was rehydrated according to manufacturer specifications: 1000 g (total for 100 hL / 100 hL musts) dissolved in 10x volume (10000–20000 mL) of 45°C sterile distilled water for 20 minutes, maintaining constant gentle stirring. Rehydrated suspension was immediately dispersed over must surface using circulation pump (low-speed setting, 2–3 minutes) to ensure uniform inoculum distribution [14].

Inoculation target: Standard commercial dose of 5 g/hL (equivalent to $\sim 1 \times 10^7$ CFU/mL after complete inoculation and mixing) [10, 14].

Critical conditions maintained during pre-fermentation (0–48 hours):

- Must internal temperature: 14–16°C (monitored and maintained using temperature-controlled tanks with immersion heaters/coolers)
- Facility ambient temperature: 16–18°C (typical cellar conditions during harvest period)
- No SO₂ addition during initial 24–48 hours (the defining characteristic of bioprotection strategy—relying on biological suppression rather than chemical preservation).

Organoleptic assessment (qualitative):

Trained sensory panel (5 WSET certified judges) evaluated must samples for presence of off-flavors: vinegary character (acetobacter spoilage), moldy/musty notes (fungal contamination), oxidized/sherry-like character (oxidation), or other irregularities. Assessment conducted in standardized conditions (20–22°C, ISO standardized white light, tasting glasses rinsed with distilled water and dried).

2.4 *Saccharomyces cerevisiae* Inoculation and Alcoholic Fermentation

Inoculation with *Saccharomyces cerevisiae* was performed for 48 hours (01.10.2025, 19:00).

All bioprotected lots were inoculated with selected *Saccharomyces cerevisiae* strain:

- Dose: 20 g/hL (standard commercial dose), rehydrated 35°C for 20 minutes as per manufacturer specifications [5]
- Inoculation: Rehydrated yeast suspension dispersed uniformly over must surface and gently mixed.

Fermentation conditions:

- Temperature: 16–18°C (monitored fermentation temperature maintained using temperature-controlled cellar environment and tank cooling/heating systems)

Vessel type: Stainless steel fermentation tanks (100 hL capacity), fitted with airlock valves to permit CO₂ release while preventing air re-entry

- Monitoring: daily measurement of specific gravity (density hydrometer, to nearest 0.0001), temperature (immersion thermometer), and organoleptic assessment of fermentation vigor (aroma intensity, CO₂ release rate)
- Fermentation endpoint: duration 8–10 days at 16–18°C.

2.5 Post-Fermentation Processing

Upon completion of alcoholic fermentation (determined by specific gravity plateau <0.998 and absence of CO₂ evolution, typically 8–10 days post-inoculation), wines underwent post-fermentation stabilization. A 14–17-day holding period at 14–16°C permitted enzyme-mediated polysaccharide sedimentation, tannin precipitation, and yeast autolysis before sample collection (28.10.2025). This post-fermentation maturation period, standard in Moldavian practice, allows completion of secondary metabolic reactions while maintaining reductive conditions that preserve wine freshness [4].

1. Racking: Wine transferred to clean stainless steel tanks, leaving yeast sediment (lees) at vessel bottom
2. pH Adjustment and Wine Stabilization: Final pH was measured potentiometrically using a calibrated pH meter (calibration buffers pH 4.0 and 7.0, uncertainty ± 0.05 units). Results: Fetească Regală pH 3.2 ± 0.1 ; Glera pH 3.1 ± 0.1 . Final pH values fell within optimal ranges for white wine stability (3.0–3.3) [1], and no pH adjustment was required.
3. Cold stabilization: Wine held at -4°C for 7 days to precipitate excess tartrates.
4. Filtration: 0.45 – 0.65 μm pore size filter used to achieve commercial clarity.
5. Bottling: December 2025 (Feteasca Regală).

2.6 Analytical Determinations: Accredited Laboratory Methods

All analytical measurements of wines were performed according to the methods approved by the Technical Regulation "Analysis methods in the field of wine production" [30] within the laboratory for testing wine and alcoholic products, an ISO 17025 accredited unit, specialized in wine analysis. The following validated analysis methods were applied [31]:

1. Ethanol content: PS-08-NIR-DE ed.1 (near-infrared spectroscopy), uncertainty $\pm 0.10\%$ vol
2. Sugars (glucose + fructose): OIV-MA-AS311-10 (enzymatic method, glucose oxidase/hexokinase), uncertainty ± 0.2 g/L
3. Acidity:
 - Titratable acidity: OIV-MA-AS-313-01 (potentiometric titration with 0.1 M NaOH), uncertainty ± 0.2 g/L
 - Volatile acidity: Gas chromatography with flame ionization detection, uncertainty ± 0.08 g/ dm³ as acetic acid
4. SO₂: OIV-MA-AS323-04B (Ripper iodometric titration), uncertainty ± 9 mg/ dm³ (total SO₂), ± 5 mg/L (free SO₂)
5. Heavy metals (ICP-AES inductively coupled plasma-atomic emission spectrometry, uncertainty ± 0.01 – 0.1 mg/L depending on element):
 - Lead (Pb): <0.2 mg/L regulatory limit
 - Cadmium (Cd): <0.01 mg/L regulatory limit
 - Copper (Cu): <1.0 mg/L regulatory limit
 - Zinc (Zn): <5.0 mg/L regulatory limit
 - Iron (Fe): <14.0 mg/L regulatory limit
6. Sodium: Flame atomic absorption spectrometry (FAAS), uncertainty ± 1 mg/L, regulatory limit 80 mg/L

2.7. Data treatment

All experiments were performed in triplicate, and the mean and standard deviation were calculated. Results were considered statistically significant if $p < 0.05$ (95% IC, $k=2$).

3. Experimental Results

3.1. The must quality indices are presented in table 1.

Table 1

Physicochemical parameters of Fetească Regală and Glera musts at harvest

Parameter	Feteasca Regala	Glera
Total acidity (g/L)	7.5±0,2	4.3±0,2
Total SO ₂ (mg/L)	12±2	12±2
Free SO ₂ (mg/L)	4±1	4±1

Low SO₂ reflects minimal chemical intervention, testing bioprotection under moderate microbial pressure.

The post-fermentation analytical results of the bioprotected wines (28.10.2025) are presented in table 2.

Table 2

Post-fermentation analytical results of the bioprotected wines

Parameter	Feteasca Regală	Glera
Total acidity (g/L)	7.7±0,2	5.3±0,2
Total SO ₂ (mg/L)	44±4	67±6
Free SO ₂ (mg/L)	20±4	13±4
Ethanol (% vol)	10.90±0,10	10.40±0,10

The post-fermentation results indicate a balanced and controlled fermentation process for both wine variants. Fetească Regală exhibited higher total acidity (7.7 g/L) and slightly higher ethanol content (10.9% vol), contributing to a fresher and more structured profile. In contrast, the Glera wine showed lower acidity (5.3 g/L) and a marginally lower alcohol level (10.4% vol), suggesting a softer and rounder sensory expression.

In both wines, free and total SO₂ levels remained conservative (13–20/44–67 mg/L), representing less than 35% of the maximum limit allowed by EU regulations, confirming the effectiveness of the bioprotective approach in limiting sulfur dioxide usage.

3.2. Wine analytical profile

The physicochemical and safety parameters of the for Feteasca Regala and Glera wines are detailed in Table 3.

Table 3

Parameters of Feteasca Regala and Glera wines, in comparison with the regulatory limits established by the EU/OIV.

Parameters	Reference Value	Feteasca Regala	Glera
Ethanol (% vol)	min 8.5	10.58 ± 0.10	10.91 ± 0.10
Sugars (g/L)	max 9.0	1.6 ± 0.3	3.3 ± 0.3
Titrateable acidity (g/L)	min 3.5	6.4 ± 0.2	6.0 ± 0.2
ph	min 3.0- 3.3	3.2 ± 0.1	3.1 ± 0.1
Volatile acidity (g/L)	max 1.08	0.43 ± 0.08	0.44 ± 0.08
Total SO ₂ (mg/ L)	max 200.0	99 ± 9	75 ± 9
Free SO ₂ (mg/ L)		56 ± 5	18 ± 5

Continuation Table 3

Na (mg/L)		23 ± 4	16 ± 3
Pb (mg/L)	max 0.2	<0.012	$<0,012$
Cd (mg/L)	max 0.01	<0.002	$<0,002$
Cu (mg/L)	max 1.0	<0.10	<0.10
Zn (mg/L)	max 5.0	$0.35 \pm 0,04$	$0.27 \pm 0,03$
Fe (mg/L)	max 14.0	$0.3 \pm 0,1$	$0.3 \pm 0,1$

For Fetească Regală, the data reflect the finished product following the bottling process, at which stage stabilization is complete, ensuring the preservation of the variety's specific aromatic profile. Conversely, the results for Glera were recorded prior to bottling. As this batch is intended as a base wine for sparkling wine production, maintaining it in bulk is essential to preserve freshness and ensure low free SO₂ levels (18 mg/L), preventing yeast inhibition during secondary fermentation. Both varieties strictly comply with EU Regulation 2019/934. Notably, the heavy metal content (Pb, Cd, Cu, Zn) and iron levels are significantly below the maximum legal limits, confirming that the bioprotection treatment does not compromise the toxicological safety of the wine. The volatile acidity (0,43–0,44 g/L) further demonstrates the success of *Metschnikowia pulcherrima* in suppressing acetic bacteria throughout the process.

4. Discussion

The results in Tables 1 and 2 confirm the success of the bioprotection strategy. Despite minimal initial sulfites (12 mg/L), non-*Saccharomyces* yeasts ensured stability without massive SO₂ additions. For Fetească Regală, the high total acidity (7.7 g/L) indicates effective protection against spoilage bacteria. For Glera, the chemical profile meets modern demands for reduced intervention while preserving the freshness required for sparkling wines.

The implementation of a bioprotective strategy using non-*Saccharomyces* yeasts (*Metschnikowia pulcherrima*/*Torulaspora*) led to a significant reduction in sulfur dioxide requirements (Figure 1):

- Feteasca Regala: Total SO₂ was recorded at 99 ± 9 mg/L, representing a 37–41% reduction compared to conventional Moldavian practices (~140–160 mg/L). The molecular SO₂ (~1.8 mg/L) ensures robust antimicrobial protection for the bottled product.
- Glera: Total SO₂ reached 75 ± 9 mg/L, a 32–42% reduction from the 110–130 mg/L baseline. The free SO₂ (18 ± 9 mg/L) is intentionally kept lower than in the Feteasca Regala to prevent yeast inhibition during the upcoming secondary fermentation.

Combined, these values represent only 38–50% of the EU maximum limit (200 mg/L), aligning with international benchmarks for low-sulfite vinification in regions like Bordeaux and Italy [32, 33].

Volatile acidity analysis is an important measure of wine quality and is commonly used as an indicator of wine spoilage. Volatile acidity served as the primary indicator for the success of bioprotection. The efficacy of the implemented bioprotective strategy is most prominently reflected in the volatile acidity levels recorded for both experimental variants (Feteasca Regala: 0.43 ± 0.08 g/L; Glera: 0.44 ± 0.08 g/L) (Figure 2).

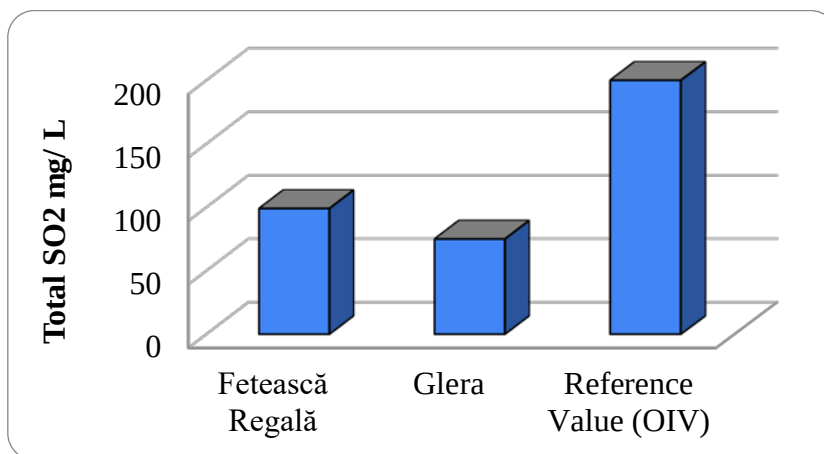


Figure 1. Comparative analysis of Total SO₂ levels (mg/ dm³) in experimental wines versus EU/OIV maximum limits, demonstrating a 32–42% reduction through bioprotection.

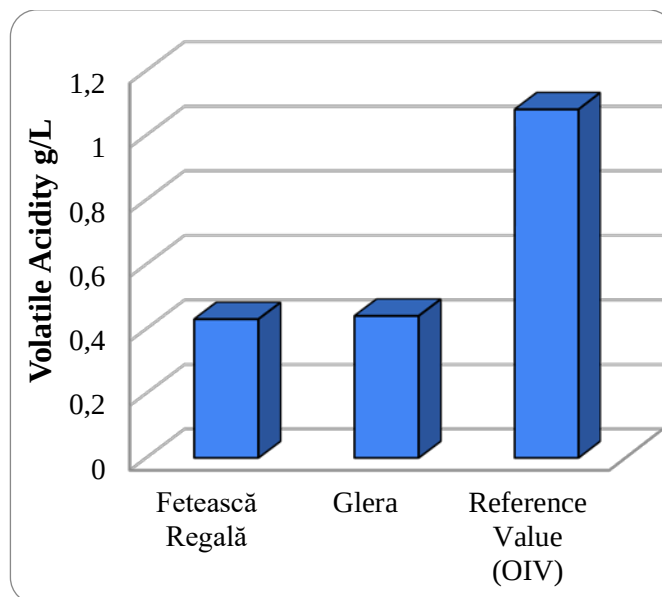


Figure 2. Volatile acidity levels (g/L acetic acid) of Feteasca Regala and Glera wines relative to the EU quality threshold.

Despite the deliberate reduction of SO₂ dosages during the crushing stage, these values remained significantly below the maximum EU quality threshold of 1.08 g/L, representing only about 40% of the legal limit. In grape must, the presence of acetic acid bacteria (AAB) can produce ethyl acetate and acetic acid, the main components of the volatile acidity of wine. Three genera are mainly associated with must and wine spoilage: *Acetobacter*, *Gluconobacter* and *Gluconacetobacter*. *Gluconobacter oxydans* is the main species in the must, although, in the case of grapes with gray rot, *Acetobacter aceti* can also be found at high population levels. It is possible that the bioprotection composed of two non-*Saccharomyces* species (*Torulaspora delbrueckii* and *Metschnikowia pulcherrima*) exerts antimicrobial activity by occupying the niche, thus reducing the relative abundance of AAB, *Hanseniaspora uvarum* and filamentous fungi during the prefermentation stages.

This marked suppression of AAB is a direct consequence of the rapid colonization of the must by the non-*Saccharomyces* consortium of *Metschnikowia pulcherrima* and *Torulaspora*

delbrueckii. Within the critical first 24 hours post-inoculation, these bioprotective yeasts established ecological dominance through several key mechanisms:

- Nutrient competition: Exhausting available resources required by spoilage microflora.
- Oxygen depletion: Reducing the oxygen tension necessary for AAB proliferation.
- Biological antagonism: Neutralizing potential contaminants before the onset of alcoholic fermentation.

The success of this biological intervention is further highlighted when compared to typical control scenarios. Under similar low-sulfite conditions without bioprotection, volatile acidity levels frequently escalate to 0.8–1.0 g/L. Consequently, the results obtained in this study (all below 0.45 g/L) validate that bioprotection is a robust alternative for maintaining microbiological stability while reducing chemical inputs.

Heavy Metals and Sodium Content. The mineral profile of both experimental wines confirms the high purity of the processing environment and the safety of the bioprotective vinification approach [34]. The results are fully compliant with the rigorous requirements of international markets.

- **Heavy Metals:** Lead (Pb), Cadmium (Cd), and Copper (Cu) concentrations remained below the limits of quantification in both wines. Specifically, Pb (<0.012 mg/L) represents less than 6% of the legal limit (0.2 mg/L), while Cd (<0.002 mg/L) and Cu (<0.10 mg/L) represent less than 20% and 10% of their respective thresholds. Zinc (Zn) and Iron (Fe) levels were also negligible: Zn was recorded at 0.35 ± 0.04 mg/L for Fetească Regală and 0.27 ± 0.03 mg/L for Glera (only 5–7% of the limit), while Fe remained constant at 0.3 ± 0.1 mg/dm³ for both (approximately 2% of the allowed threshold).
- **Sodium (Na):** The sodium content was recorded at 23 ± 4 mg/L for Fetească Regală and 16 ± 3 mg/L for Glera. These values represent only 20–29% of the 80 mg/L OIV reference limit, confirming the absence of excessive salts and the high quality of the process water and cellar hygiene.

This exceptionally clean mineral profile ensures unrestricted access to premium international markets (EU, USA, Japan) and qualifies the wines for "low-sulfite" or organic certification labeling, adding significant commercial value [35].

Acid Composition and Stability. The acidity balance supports both freshness and microbial stability. Fetească Regală exhibited a titratable acidity of 6.4 ± 0.2 g/L, while Glera followed with 6.0 ± 0.2 g/L. High tartaric acid levels (3.4–3.5 g/L) provide natural antimicrobial buffering. Lactic acid remained <0.2 g/L in both variants, confirming that malolactic fermentation was effectively controlled or not yet initiated at the time of sampling. The retention of malic acid (~1.9 g/L) is particularly vital for the Glera base wine, providing the crispness and acidity required for high-quality sparkling wine production [36].

The scientific understanding of bioprotection, developed over past five years through mechanistic studies [10–12], translates directly into the field trial outcomes observed. The multiple non-redundant bioprotective mechanisms (pulcherrimin-mediated iron chelation, polysaccharide production, volatile metabolite accumulation, nutrient competition, oxygen depletion, cell-contact inhibition) functioned synergistically in practice to suppress acetic acid bacteria. This mechanistic redundancy has profound practical implication: bioprotection is robust across variable vineyard and oenological conditions [15]. A single mechanism failing

(e.g., iron saturation in naturally iron-rich must) does not eliminate overall protective efficacy, as complementary mechanisms continue to suppress spoilage. There is experimental evidence that strains of *Metschnikowia pulcherrima* can inhibit the growth of *Brettanomyces bruxellensis* and *Hanseniaspora uvarum*, although the specific mechanisms that determine this inhibition remain unclear [37]. Furthermore, the combination of *M. pulcherrima* (dominant iron-chelating activity) and *T. delbrueckii* (dominant polysaccharide production, oxygen scavenging) in commercial formulations like Zymaflore Égide TDMP provides synergistic protection likely exceeding efficacy of either species alone [10,14].

The results obtained in the 2025 in the region Codru trial demonstrate that bioprotective yeast application is an effective and robust strategy for significantly reducing sulfur dioxide (SO₂) usage while maintaining microbiological stability, analytical compliance, and market readiness of white wines. The achieved SO₂ reduction of 38–50% aligns closely with previously reported European field trial outcomes, including Bordeaux Sauvignon Blanc (40%; INRAE, 2023) [11], Italian Prosecco (35%; University of Modena, 2022) [10], and Spanish Albariño (45%; 2023 trials) [16]. This strong concordance with literature benchmarks validates the working hypothesis that bioprotection can reliably replace a substantial proportion of conventional SO₂ inputs under controlled pre-fermentative conditions.

The Codru region's climatic and technological parameters—cool pre-fermentative temperatures (13–16°C), must pH values between 3.2 and 3.5, and moderate initial microbial pressure—proved optimal for rapid bioprotective yeast colonization. Within 24 hours, bioprotective species such as *Torulaspora* and *Metschnikowia* established dominance, effectively preventing the proliferation of spoilage organisms prior to *Saccharomyces cerevisiae* inoculation. These findings corroborate previous observations that bioprotection is particularly effective in cool-climate viticultural zones [37].

Grape must protection also includes protection against oxidation. The species *M. pulcherrima* is known as a major oxygen consumer. The high consumption of this resource could make O₂ rapidly unavailable in grape must and therefore prevent oxygen from entering the redox pathways, preventing color darkening and the production of undesirable aromas. In addition, *M. pulcherrima* is known to secrete pulcherriminic acid, which, once in the medium, chelates Fe³⁺. This ion is also involved in redox mechanisms through the Fenton reaction. The depletion of iron in the medium by pulcherriminic acid could also contribute to a lesser extent to protecting the matrix against oxidation [38].

Mechanistically, the observed SO₂ reduction is consistent with well-established bioprotection pathways reported in the literature. Ecological niche exclusion plays a central role, as bioprotective yeasts rapidly colonize the must at low temperatures where *Saccharomyces* and acetic acid bacteria remain metabolically limited. Additionally, the antimicrobial activity of *Metschnikowia pulcherrima*—mediated through pulcherrimin-mediated iron sequestration—limits the growth of iron-dependent spoilage microorganisms [39]. This mechanism is supported by the low volatile acidity values observed (0.43–0.44 g/L), significantly below spoilage thresholds and consistent with acetic acid bacteria suppression. Oxygen consumption by bioprotective yeasts further contributes to early establishment of quasi-anaerobic conditions, restricting aerobic spoilage metabolism prior to active alcoholic fermentation [40]. Sequential inoculation of *Saccharomyces cerevisiae* 24–48 hours after bioprotective yeast addition proved critical to fermentation success. By the time *Saccharomyces* was introduced, a stabilizing pH and ethanol gradient had already formed, inhibiting wild yeast development and allowing fermentation to proceed without microbial

competition or fermentation arrest [41]. This controlled succession aligns closely with previously described optimized bioprotection protocols.

4.6 Limitations and Research Needs:

This field trial, while validating the efficacy of bioprotective yeasts in Moldavian conditions, operates within several important constraints that should be acknowledged:

1. *Absence of concurrent control group*: A parallel fermentation lot receiving standard SO₂ dosing (140–160 mg/L, representative of traditional Moldavian practice) was not conducted alongside bioprotected wines. This limits statistical isolation of bioprotection effects from other process variables (temperature management, equipment cleanliness, vintage quality). Future studies must include simultaneous control fermentations for robust comparison.
 2. *Single-vintage data*: Results represent the 2025 vintage under specific Codru climate conditions (September harvest, 16–18°C ambient, moderate microbial pressure on grapes). Bioprotection efficacy may vary significantly under different vintage conditions (extreme heat, heavy rain, high botrytis pressure, early harvest, late harvest). Multi-vintage validation (2026, 2027, 2028 minimum) across diverse climate scenarios is essential before recommending widespread producer adoption.
 3. *White wine production only*: Bioprotection was validated exclusively in white wine vinification. Applicability to red wines—which require longer maceration (5–14 days), higher fermentation temperatures (18–25°C), different yeast strains, and extended contact with tannin-rich skins—remains completely unexplored. Dedicated red-wine field trials (Feteasca Neagra, Rara Neagra, Merlot) are required.
 4. *Absence of microbial population dynamics*: The trial did not quantify temporal population dynamics of bioprotective yeasts, *Saccharomyces cerevisiae*, spoilage bacteria, or wild yeasts through Colony-Forming Unit (CFU) enumeration or molecular methods (qPCR, DGGE). Such microbial kinetics data would validate the theoretical mechanistic basis and confirm colonization timing and suppression efficacy at the cellular level.
 5. *Limited sensory evaluation*: Organoleptic assessment was qualitative (absence/presence of off-flavors only). Quantitative sensory analysis (trained panel, descriptive analysis, GC-olfactometry for volatile profiles, consumer preference testing) was not performed. Comparative sensory quality versus control wines remains unknown.
 6. *Post-bottling stability data incomplete*: Wines were analyzed 14–17 days post-fermentation (approximately 3 months vintage age at manuscript submission). Long-term aging data (18–36 months post-bottling) is critical to assess color stability, oxidative resistance, SO₂ preservation efficacy during bottle storage, and microbiological safety.
- These limitations are acknowledged and should guide future research prioritization.

4.7 Future Research Directions:

Immediate priority (2026–2027):

1. Multi-vintage field trial (2026, 2027 vintages) across Moldavian regions (Codru, Ștefan Vodă, Valul lui Traian) to assess robustness under variable precipitation and harvest conditions.
2. Parallel control fermentations with standard SO₂ dosing (140–160 mg/L) to enable statistical comparison and isolate bioprotection contribution.
3. Quantitative sensory analysis (descriptive analysis, triangle test) and consumer preference testing versus control wines in blind conditions.

Medium-term priority (2027–2028):

4. Application to red wine varieties (Feteasca Neagră, Rara Neagră, Merlot) with optimized bioprotection protocols for higher fermentation temperatures (18–25°C) and longer maceration (5–14 days).
5. Microbial population dynamics (CFU enumeration via serial dilution on selective media, qPCR targeting *Metschnikowia*, *Torulaspora*, and acetic acid bacteria) to elucidate temporal colonization patterns.
6. Extended post-bottling monitoring (6, 12, 18, 36 months) assessing color stability, SO₂ preservation efficacy, and microbiological shelf-life.

Long-term priorities (2028+):

7. Molecular mechanisms of cell-contact-mediated inhibition (co-culture transcriptomics, proteomic analysis of bioprotective metabolites).
8. Integration with malolactic fermentation timing optimization (co-inoculation protocols with *Oenococcus oeni* strains).
9. Regional certification framework development (low-SO₂ Moldavian wine standard for IGP designation).

5. Conclusions

The research conducted in the Codru wine region during the 2025 vintage confirms that bioprotection using non-*Saccharomyces* yeasts (*Metschnikowia pulcherrima* and *Torulaspora delbrueckii*) is a highly effective and robust alternative to conventional sulfur dioxide (SO₂) usage in white winemaking. The key findings of this study are:

Substantial SO₂ reduction: The implementation of a bioprotective strategy achieved a 32–42% reduction in total sulfur dioxide levels compared to traditional Moldavian practices. The final concentrations (99 mg/L for Fetească Regală and 75 mg/L for Glera) represent only 38–50% of the maximum legal limit established by EU Regulation 2019/934.

Effective Microbial Control: Volatile acidity levels (0.43–0.44 g/L) remained at approximately 40% of the EU quality threshold, demonstrating the successful suppression of acetic acid bacteria and other spoilage microorganisms during the critical pre-fermentative stage.

Safety and Regulatory Compliance: Analytical data from ISO 17025-accredited testing confirm that both wines fully comply with OIV standards and international market requirements. The mineral profile—including heavy metals and sodium—remained significantly below safety limits.

Preservation of Varietal Integrity: The bioprotective approach supported a controlled fermentation process that maintained the natural acidity and aromatic freshness of both the endemic Fetească Regală and the international Glera cultivars, proving its suitability for both still and sparkling base wine production.

This strategy enables Moldavian wineries to position their products in the premium 'low-sulfite' segment, meeting the rigorous standards of demanding international markets such as the EU and Japan.

In conclusion, bioprotective yeast application provides a scientifically validated pathway for Moldavian producers to meet the growing global demand for "low-sulfite" and natural wines. This strategy aligns environmental sustainability with high-quality enological standards, ensuring competitive positioning in premium international markets.

Research Data Availability Statement: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request. Raw analytical data and laboratory certificates are stored at LTPA CNSAPSA, Chisinau, and may be accessed for research purposes upon formal application.

Acknowledgments: *The authors would like to thank the Institutional Project, subprogram 020405 “Optimizing food processing technologies in the context of the circular bioeconomy and climate change”, Bio-OpTehPAS, being implemented at the Technical University of Moldova and the Laboratory for Testing Alcoholic Products (LTPA CNSAPSA) for analytical support.*

Contribution of authors

Nicolai Cupcea: Conceptualization, experimental design, sampling, data curation, literature review, writing—original draft, writing—review & editing.

Rodica Sturza: Supervision, validation, methodology, writing—review & editing, project administration.

Conflicts of Interest: The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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Citation: Cupcea, N.; Sturza, R. The use of bioprotective yeasts to reduce sulfite consumption in winemaking. *Journal of Engineering Science*. 2025, XXXII (4), pp. 120-136. [https://doi.org/10.52326/jes.utm.2025.32\(4\).09](https://doi.org/10.52326/jes.utm.2025.32(4).09).

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Submission of manuscripts:

jes@meridian.utm.md

[https://doi.org/10.52326/jes.utm.2025.32\(4\).10](https://doi.org/10.52326/jes.utm.2025.32(4).10)
UDC 663.25:634.8:004.8:004.738.5



SMART TECHNOLOGIES AND ARTIFICIAL INTELLIGENCE IN VITICULTURE

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Received: 11. 15. 2025

Accepted: 12. 22. 2025

Abstract. Every technological innovation is a step forward in the development of human society. Almost all areas of life have been affected by the digital industry, including winemaking. The digitization process is widely implemented in order to obtain high-quality products, increase crop yields, verify counterfeit wine products, and automate the work process. Any digital technology can be used for the benefit of mankind, and the use of drones in winemaking is already accepted and recognized. The International Organization of Vine and Wine (OIV) has launched a new digital monitoring center with the aim of identifying key trends in digitalization and how new technologies can be applied in the vine and wine sector. The initiative aims to provide updates on digital/technological trends in the vine and wine sector. This paper analyzes various digital technologies used throughout the value chain: vineyard, winery, and distribution. Artificial intelligence, robotics, satellite imaging, the Internet of Things (IoT), and blockchain are some of the technologies included. For each of these, a definition has been included, as well as their application in the wine sector and their future prospects. Digital transformation offers the wine sector an opportunity to gain efficiency, transparency, productivity, open up to new business models/value propositions, and improve sustainability.

Keywords: *digitization in winemaking, modern wineries, digital technologies, viticulture, robotics, blockchain.*

Rezumat. Fiecare inovație tehnologică este un pas înainte în dezvoltarea societății umane. Aproape toate domeniile vieții au fost afectate de industria digitală, printre care și domeniul vinificației. Procesul de digitalizare este implementat pe larg în cadrul obținerii unor produse de înaltă calitate, majorării volumului de recoltă, verificarea produselor vitivinicole contrafăcute și automatizarea procesului de muncă. Orice tehnologie digitală poate fi folosită în folosul omului, iar utilizarea dronelor în domeniul vinificației este deja acceptată și recunoscută. Organizația Internațională a Viei și Vinului (OIV) a lansat un nou centru de monitorizare digitală cu scopul de a identifica principalele tendințe în digitalizare și modul în care noile tehnologii pot fi aplicate în sectorul viei și vinului. Se propune inițiativa de a oferi actualizări privind tendințele digitale/ tehnologice din sectorul viei și vinului. În lucrarea dată s-au analizat diferite tehnologii digitale utilizate pe parcursul etapelor lanțului de valoare: vie, cramă și distribuție. Inteligența artificială, robotica, imagistica prin satelit, Internet of Things (IoT) și blockchain sunt câteva dintre tehnologiile incluse. Pentru fiecare

dintre acestea a fost inclusă o definiție, precum și aplicația lor în sectorul vinicol și perspectivele lor viitoare. Transformarea digitală oferă oportunitate sectorului vinicol de a câștiga eficiență, transparență, productivitate, de a se deschide către noi modele de afaceri/propuneri de valoare și de a îmbunătăți durabilitatea.

Cuvinte cheie: *digitalizare în vinificație, crame moderne, tehnologii digitale, viticultură, robotica, blockchain.*

1. Introduction

The wine industry is undergoing a profound transformation due to the adoption of digital technologies and process automation, which are necessary for producing high-quality wines while reducing operational costs and energy consumption. In many wine-producing countries, conceptual projects have been launched to address the challenges of an increasingly complex and fast-paced global market. The Italian project VINO 4.0 is dedicated to integrating the latest technologies in the wine industry with new models of organization and promotion of the wine business. Advanced technologies, such as infrared spectroscopy integrated with artificial intelligence (AI), offer extensive possibilities for analysis and optimization. Spectroscopy detects and analyzes the chemical parameters of grape varieties, and AI processes this data by comparing it with existing databases to identify deviations or opportunities for improvement. Based on historical and predictive analyses, AI enables an assessment of grape quality over several years, providing winegrowers with strategic information on evolutionary trends and harvest quality.

Technologies such as the Internet of Things (IoT), Laser Imaging Detection and Ranging (LIDAR), satellite imaging, and geographic information systems (GIS) contribute significantly to the digitization of agricultural processes in viticulture. IoT integrates high-precision sensors for continuous monitoring of essential parameters such as soil moisture, air humidity and temperature, and wind speed. Complex, integrated analysis of these parameters, correlated with decision-making models based on effective practices tested over many years, allows for the optimization of irrigation, reduction of water consumption, and identification of the nutrients needed by the vines.

The intelligent recommendations of the digitized system are adapted to variable weather conditions and other impact factors. LIDAR scanning technology is a remote sensing method used to assess the characteristics of a specific area in detail, such as studying the terrain, vegetation, and obstacles, and recording this information.

This method is already being used in viticulture, including to prevent accidents in vineyards, namely through the use of a detailed 3D map of the terrain and satellite images are used to map and analyze vineyards, providing detailed data on terrain topography, crop conditions, and areas with high productivity potential. In wineries, sensors monitor critical fermentation and storage parameters, contributing to precise adjustments and ensuring consistent quality of the final product [1-3].

The main objective of digitization in the wine industry is the complete integration of data and processes to create an agile, sustainable production system capable of consistently delivering high-quality wines, regardless of fluctuations in various important factors. According to the report by International Organization of Vine and Wine (OIV) experts [4], digitization in the wine sector focuses on the following key components: efficiency, productivity, transparency, proposing new values and business models, and sustainability.

In order to effectively achieve this goal in the field of production and storage, the following basic trends of the digital revolution are evident: IoT/sensorisation, AI, robotics, satellite imaging/ GIS, LIDAR systems, blockchain, e-label, e-certificate, smart storing.

The economic, social, and environmental benefits of implementing these components can only be relevant if all stages of product realization—raw material production, processing, distribution, and marketing—are integrated into the digitized system in order to optimize the organization and management of the wine-producing entity [5]. Certain digital technologies and techniques are present at all stages of production and distribution, while others are specific to certain stages (Table 1) [6].

Table 1

Digital Transformation of Production and Distribution Processes [5,6]

Production	Transformation	Distribution	Consumption
Field sensors, technology and equipment. Drones. Satellite imagery. Decision support systems (DSS).	Quality control sensors. Big Data Analytics. Dematerialization systems. Integrated traceability.	Logistics optimization systems. E-commerce platforms. Blockchain in food delivery.	Smart labels. Sensors for waste reduction. Food sets. Big data analytics.

In fact, DSS must be present at all stages of the production-distribution chain as an indisputable tool for analyzing data and recommending executive decisions, free from human error.

2. Smart Technologies in Vineyards

During the grape production stage, real-time data acquisition provides winegrowers with an overview of the phytosanitary status of the plantation, temperature and water conditions, light radiation, vegetative development, nutrient sufficiency, chlorophyll content, wind direction, and wind speed. The data provided (input) feeds various predictive models and, as a result, is reflected in relevant conclusions about the risk of the occurrence and development of various pathogenic diseases (downy mildew and powdery mildew), the activity phases of pests, and the course of biochemical processes that impact grape quality [7]. In this context, the oenologist also has a priority of information about the potential quality of the grapes, enabling them to organize the winemaking process appropriately.

Precision viticulture (PV) is closely linked to smart technologies and depends on their level of development and implementation. Smart technologies can already become a reliable aid to winegrowers in establishing vineyards by providing comprehensive training at all essential stages: mapping with geodetic positioning (satellite, aerial, and drone imagery), LIDAR, physical and chemical soil analysis, assessment of water reserves, nutrients, etc.

Planting a vineyard cannot be successful unless all the impact factors that will determine the quality of the grapes are taken into account: soil quality, exposure, slope, wind rose, composition, granulometry, water retention capacity, organic matter, erosion risk, etc. Data on these essential parameters can be obtained using remote monitoring techniques (satellites, aircraft, drones), assisted by intelligent equipment and sensors. The dedicated software can also be fed with the primary information collected without the involvement of operators, thus excluding possible errors caused by human factors. In conclusion, reports are obtained on the feasibility of planting, as well as recommendations on the suitability of the land for the intended varieties or the most suitable varieties for planting. If in-depth soil studies of the land already exist, they can be digitized and made available for consultation

on web-based platforms using geographic information system, such as the one developed by the Agricultural Institute of the Edmund Mach Foundation (S. Michele all'Adige, Trentino, Italy) [8]. Formation on water requirements for irrigation can also be consulted, providing sufficient data for calculations according to the Irri4web mathematical model based on soil characteristics [8,9]. Other useful information is also available, processed in QGIS software—shading, exposure, slope, hours of sunlight, potential global radiation.

All forms of real-time agricultural imaging provide information about plant vigor, development trends, and possible nutrient deficiencies. Images taken from lower altitudes (airplanes and drones) (Figure 1) are more suitable for this topic, as the sectors between rows can be individualized to exclude them from the estimation of vine vigor [10,11].



Figure 1. Low-Altitude Drone-Based Agricultural Imaging System for High-Resolution Monitoring of Vine Vigor, Growth Dynamics, and Nutrient Stress Indicators [11].

Projects such as Chianti and Bolgheri [12], launched in this context in Tuscany, Italy, in vineyards of 15 and 3 ha respectively, using drones equipped with multispectral and thermal cameras, have demonstrated their effectiveness and the importance of information for tactical decisions appropriate to precision viticulture. Thus, thermal and multispectral images, processed using various specialized programs (PIX4D, NDVI-Normalized Difference Vegetation Index, and CWSI, Crop Water Stress Index), enabled the creation of 3D maps of temperatures, four classes of water insurance, and vegetative vigor (Figure 2).

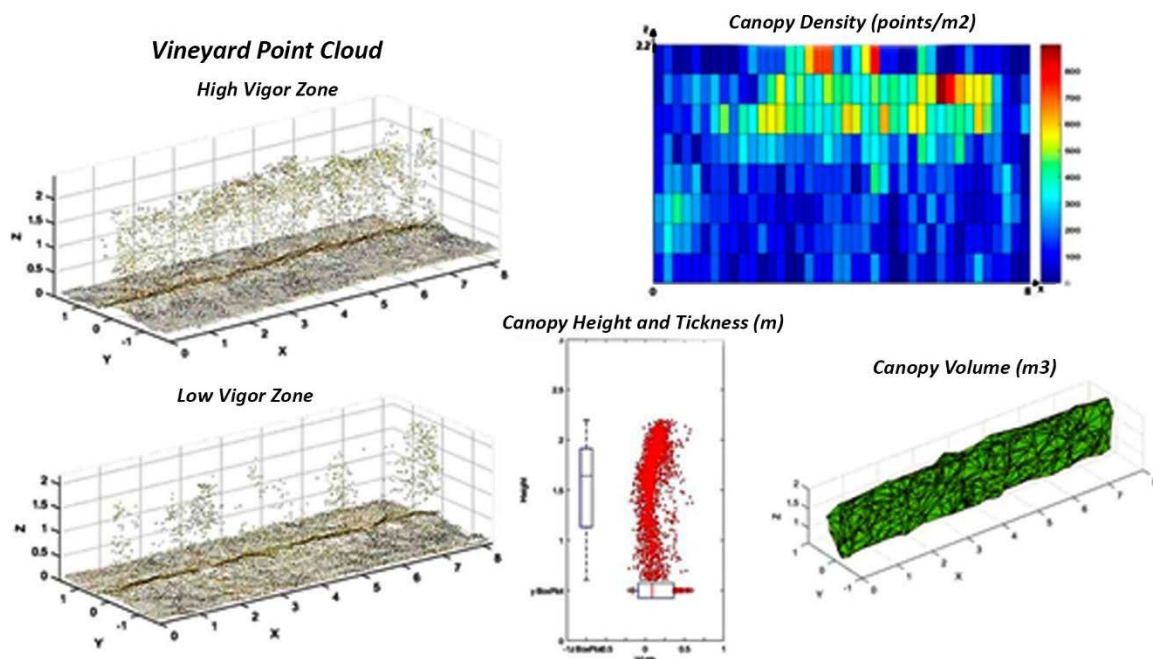


Figure 2. Vine vigor map, created in 3D based on multispectral images captured using drones [12].

The image resolution ranged from 1.76 to 8.6 cm/pixel, which ensured accurate details. Based on these results, schemes were developed for differentiated irrigation and staggered harvesting according to grape quality.

3. Precision Viticulture and Robotics

Determining the geographical coordinates of plants showing symptoms of certain diseases or nutritional deficiencies can be integrated into the decision-making system by programming the executive machine for local processing and fertilization according to the specific situation, without the need to treat the entire vineyard excessively. This results in significant savings in pesticides and fertilizers and ensures increased sustainability, with particular care for environmental organisms, pollinating insects, flora diversity, and soil quality [13]. Artificial intelligence is responsible for managing each stage of vineyard work, including harvesting, given that automated systems have already been developed for the operational sorting of grapes by quality during mechanized harvesting [14,15]. The labor shortage is forcing builders and IT specialists to develop robots to efficiently replace humans, even in the most complex operations. Similar technologies are being progressively implemented in large companies in the field. For example, Bouchaine Vineyards (California, USA) has secured its production throughout the wine-making chain with IoT sensors from Cisco, not only to optimize the quality of grapes and wines, but also to avoid wasting any resources and ensure care for the environment [15]. To emphasize sustainability, together with researchers from Oregon State University's Wine Research Institute, they are studying and developing smart technologies to combat pests without harming pollinating insects. To do this, they use robotic devices powered by solar energy to generate vibrations of a certain frequency and intensity to capture and destroy harmful insects (Figure 3). The frequency of the vibrations coincides with that used by insects to meet and mate.



Figure 3. The Pied Piper robot (prototype) for selective pest control in vineyards [15].

Robots are also welcome when it comes to performing complicated and responsible tasks to replace professional operators, such as cutting and shaping the crown of grapevines. In New Zealand, the Maara Tech project has been launched with the involvement of researchers from the Universities of Auckland and Ontago, focusing on virtual pruning and correct crown formation. Virtual reality headsets are also being designed to teach the

principles of dry and green pruning. Continuous training of the curriculum takes place with the help of Artificial Intelligence. Initially, the rows are scanned using a robot [17].

Although fully automated cutting has not yet been achieved, numerous robots, including those already in industrial production, are available for other maintenance tasks—chipping removed wood material, loosening soil, mowing grass between rows and between plants, spraying pesticides and fertilizers, and the trend toward replacing scarce labor with robots is steadily increasing. The automation of vineyard work can also be achieved by towing various equipment with autonomous tractors equipped with sophisticated positioning and monitoring systems, with multiple video cameras, radars, and LiDARs. Increasingly, these are powered by electric motors rather than combustion engines. AI ensures instantaneous response to deviations from the planned route - 0.1 s [17]. Communication platforms already allow remote control of multiple executive units. For example, six tractors can be controlled simultaneously via a smartphone in Android or iOS environments.

4. The era of Drones in Viticulture

The global market for agricultural drones (Unmanned Aerial Vehicles – UAVs) is experiencing rapid growth, reaching an estimated value of USD 1.92 billion in 2024 and projected to expand to approximately USD 10.46 billion by 2034 [18]. According to additional sources, the European agricultural drone market alone was valued at around USD 5.8 billion in 2024, with expectations of growth to USD 7.62 billion in 2025 and a long-term forecast indicating an increase to approximately USD 55.72 billion by 2033 [19]. A substantial share of this market is attributed to drones designed for crop health management, particularly those employing aerial spraying technologies, commonly referred to as unmanned aerial spray systems [20].

A major challenge is the use of drones for phytosanitary treatments and vineyard fertilization (Figure 4). Industrial technologies are advancing rapidly, and agricultural drones are now being proposed, specialized for various operations, assisted by complex software, cameras, GPS localization, and impressive technical features.

The well-known Chinese company DJI (global leader) recently launched its top-of-the-range Agras T100 model, which has a 75-liter pesticide tank and a 100-liter fertilizer tank, with a takeoff weight of 150 kg and lifting capacity of 85 kg, a 360° horizontal and 180° vertical viewing system consisting of five cameras, LiDAR, radar, and AI. These features allow the Agras T100 to be used even at night, and to increase the efficiency of treatments, the droplet size can be adjusted by adjusting the spray nozzles accordingly [21].

While EU legislation is slowly evolving, with many reservations in drone regulations, including for security reasons, in some countries - the US, China, Japan [22], drones are increasingly being used for these important tasks, generating a number of advantages [23,24]:

- ✓ the ability to intervene when traditional techniques cannot be applied, at any phenological stage, regardless of soil moisture, without compacting it;
- ✓ savings in pesticide consumption of up to 30%, with the prospect of reducing the amount of pesticides needed by 5-10 times;
- ✓ reduced water consumption (more than 10 times);
- ✓ operational efficiency—about 5 times faster than traditional methods [23];
- ✓ possibility of localized treatments, with mapping and exact GPS positioning.

To overcome excessive regulatory constraints, facilitate the adoption of drones in agricultural practices, and support the integration of emerging technologies, several

countries, including Poland and Germany, have been compelled to approve official derogations from existing European Union regulations [25].

At the same time, the problems that need to be solved are also highlighted, including uncertainties regarding the effectiveness of drone-based treatment, possible drift of sprayed preparations from target objects and damage to the ecological system, including pollinating insects, potential phytotoxic effects caused by active ingredient concentrations that are much higher than in traditional treatments, etc.

Work safety for operators is no less important. As tests carried out in Lombardy (Italy) have shown, when treating vineyards with drones in weather conditions that meet the requirements, the operator (pilot) is not at risk of contamination except when filling the tank with the necessary preparations [26].



Figure 4. Drone-Based Phytosanitary Treatment System for Precision Protection of Vineyards [26].

Another limitation is the development of an appropriate legal framework, which in the EU requires a complex approach, as well as the certification of pilots in accordance with European Regulations 2019/945 and 2019/947.

A pragmatic, rational approach to the use of drones in plant protection is demonstrated by Switzerland, a country that has developed concise, precise, and intuitive scenarios for the application of drones (Figure 5) [27]. Drones can be used at a maximum wind speed of 3 m/s, with a maximum flight speed of 7 m/s. The maximum flight altitude is 30 m above ground level, with spraying possible up to 6 m above ground level. The distance to the buffer zone (10 m) and its width are specified. In this way, the Swiss have omitted excessive bureaucracy from the permissive documents, which hinders the application of drones in agriculture.

In recent years, a severe and incurable disease capable of compromising entire vineyards has been identified, namely grapevine yellows, commonly referred to as *Flavescence dorée* [28,29]. The disease is caused by a phytoplasma—a unicellular pathogenic organism—transmitted by the insect vector *Scaphoideus titanus* (grapevine leafhopper).

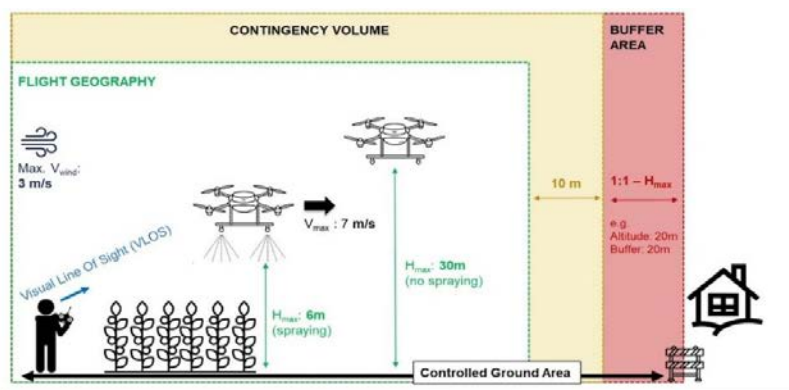


Figure 5. Example of a scenario sheet for the use of drones for phytosanitary treatments in Switzerland [27].

The manifestation of the disease in vineyards is well defined and exhibits distinct symptomatology depending on grape variety, particularly between white and red cultivars. Based on a comprehensive set of physiological and phenotypic parameters, the disease can be reliably identified, allowing for an accurate assessment of infection severity and the subsequent development of vineyard sanitation and containment measures. A critical step in the monitoring of phytoplasmosis is the determination of infection rates, which is essential for establishing effective control strategies. However, this task becomes particularly challenging in large-scale vineyards, especially considering the highly active but temporally limited phenophases during which vector insects exhibit peak activity. To facilitate the detection of disease symptoms, identify outbreak hotspots, and monitor disease spread over extensive areas within short timeframes, drone-based technologies equipped with multispectral and hyperspectral imaging systems have been developed. A key contribution to the advancement of these technologies was made by researchers at NASA, whose findings were subsequently commercialized by the Canadian–French–American consortium SkySquirrel Technologies. With proven field experience in vineyards across France, Switzerland, and Romania, SkySquirrel Technologies demonstrated the operational effectiveness of this approach in 2017 through the pilot project FlaveDor. The project was conducted in the vineyards of Sălcuța LLC (Căușeni district), with full involvement of the National Office of Vine and Wine and support from international partners, including USAID and the Government of Sweden [30]. The drone-based system achieved a detection accuracy of 95%, surveying 20 ha of vineyards in just 24 min. In addition to Flavescence dorée, the integrated sensor suite successfully identified another highly destructive disease - Black Wood (Bois noir). Following the demonstrated high efficiency of this technology, the role of specialized drones in monitoring vineyard health is expected to expand significantly in the near future, becoming an essential component of precision viticulture and sustainable plant protection strategies.

5. Evaluating Grape Ripeness

During the grape ripening stage, it is crucial to estimate the optimal harvest period, using not only sugar and acid content as reference parameters, but also polyphenol concentration and their extractability from the berries. To this end, several laboratory methods have been developed, but they require special equipment and are time-consuming and laborious. To overcome these drawbacks, mobile methods have been developed to instantly assess phenolic ripeness [31,32], based on the principle of global radiation absorption in a specific spectral range (Figure 6).



Figure 6. Hand-device Spectron (a) and Multiplex (b) for grape quality proximal quality [32].

Phytosanitary status assessment and grape maturation monitoring can be fully automated through robotic and drone-based systems (Figure 7) [33], incorporating advanced technologies for data acquisition, transmission, integration, and intelligent interpretation.



Figure 7. Robotic monitoring of phytosanitary status and grape ripeness [33].

Assessing the phenolic maturity of red grapes is an essential criterion for obtaining quality wines. To quantify this parameter, equipment based on the interaction of waves with grapes is proposed. These can be waves in the infrared range or the fluorescence effect (Pellenc Spectron and Multiplex Terrain portable devices) [32].

Prof. E. Celotti et al. from the University of Udine (Italy) [34] developed an instrumental photometric method for easy, instant assessment of ripeness directly in the vineyard, comparing the results with those obtained by traditional methods in laboratory conditions, but also with the color intensities of vineyard plots, obtained by satellites using cameras with different spectral filters (Figure 8) [34,35].

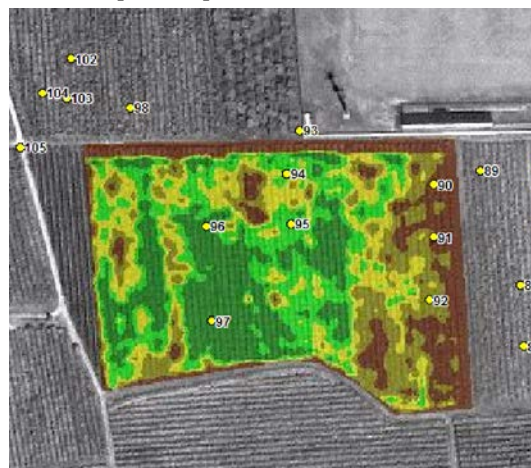


Figure 8. Satellite image of a vineyard and elaboration of spectral information into vigor indices [34].

For vineyards, a satisfactory correlation was established between the abstract values obtained in the vineyard and satellite spectral analysis. For vineyards in the Valpolicella area, a high correlation was determined between vine vigor and anthocyanin content ($R^2=0.8997$), while the correlation between vigor and carbohydrate content ($^{\circ}\text{Brix}$) is much lower ($R^2=0.3244$) (Figure 9). The evolution of drone technology can make this procedure very efficient for plantations of all sizes and for the most diverse grape varieties.

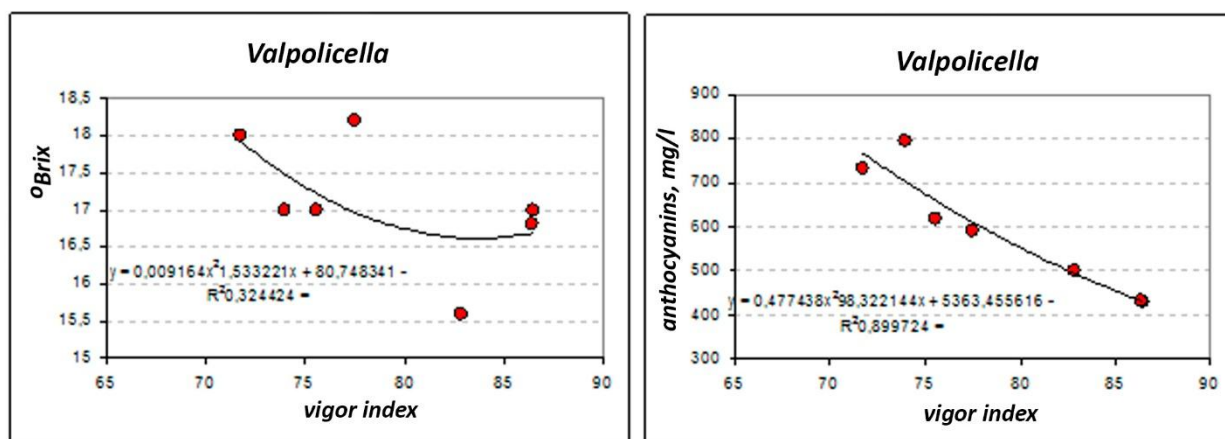


Figure 9. Correlations between vine vigor, determined using satellite imagery, and various grape parameters [35].

Regenerative agriculture, based on accurate data, improves soil quality and vineyard productivity, helping to mitigate the impact of climate change. In this context, digitization not only reduces the risks associated with harvesting and production, but also allows processes to be customized to meet market demands and consumer preferences. Through automation, smart monitoring, and advanced analytics, the wine industry is building a modern future focused on excellence and sustainability [36,37].

From exotic to widespread implementation, with the full involvement of wine industry specialists, through their training and involvement in the adjustment and development of smart technologies—this is their natural path in the face of multiple challenges—climatic, economic, ecological, and social. Familiarization with the latest smart technologies and their use in successful economies is no longer an option but a necessity, as confirmed by numerous dedicated events, such as the robotics and digitization festival in viticulture in Italy, organized on the platform of the University of Pisa with the participation of numerous national and international producers, researchers, and consumers of such products [38,39].

6. Conclusions

The implementation of smart technologies in viticulture represents a fundamental change in the mechanization, automation, and optimization of wine grape production, bringing tangible benefits at both the operational and strategic levels.

Although the adoption of smart technologies is currently at an early stage, they promise undeniable valuable potential.

The main task of developing smart technologies in the wine sector, associated with artificial intelligence, is to help wine growers throughout the entire production chain to:

- ✓ identify areas suitable for growing grape varieties;
- ✓ monitor the vegetative state of plantations, climatic conditions, and the plants' water and fertilizer needs;

- ✓ optimize irrigation and fertilization of vineyards;
- ✓ detect pests and diseases of vines and optimize phytosanitary protection;
- ✓ simplify and automate quality control of grapes to optimize their harvesting.

Acknowledgment: The research was funded by Institutional Project 020405, „Optimizing food processing technologies in the context of the circular bioeconomy and climate change, Bio-OpTehPAS”, running at Technical University of Moldova.

Conflicts of Interest: The authors declare no conflict of interest.

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Citation: Scutaru, I.; Sclifos, A. SMART technologies and artificial intelligence in viticulture. *Journal of Engineering Science*. 2025, XXXII (4), pp. 137-149. [https://doi.org/10.52326/jes.utm.2025.32\(4\).10](https://doi.org/10.52326/jes.utm.2025.32(4).10).

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Submission of manuscripts:

jes@meridian.utm.md

[https://doi.org/10.52326/jes.utm.2025.32\(4\).11](https://doi.org/10.52326/jes.utm.2025.32(4).11)

UDC 663.222:543.544(478)



APPLICATION OF GAS CHROMATOGRAPHY-ION MOBILITY SPECTROMETRY(GC-IMS) TO CHARACTERIZE VOLATILE SIGNATURES OF FETEASCĂ NEAGRĂ WINES FROM THE IGP ȘTEFAN VODĂ REGION

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Received: 11. 15. 2025

Accepted: 12. 22. 2025

Abstract. Wine aroma is a key determinant of quality and typicity, shaped by complex mixtures of volatile compounds. Traditional methods such as GC-MS provide detailed chemical information but are often time consuming and less suited for rapid terroir studies. This study hypothesized that gas chromatography-ion mobility spectrometry (GC-IMS) can differentiate wines from neighboring vineyards within the same protected geographical indication (PGI). The objective was to evaluate its applicability for profiling volatile compounds in wines from three vineyards of the PGI Ștefan Vodă region in Moldova. Microvinification was carried out with Fetească Neagră grapes harvested at technological maturity in 2022, and the resulting wines were analyzed by GC-IMS. Three dimensional spectra, two dimensional projections, differential mapping, and fingerprint plots revealed vineyard specific volatile profiles. Wine from Purcari was characterized by higher levels of esters, Cimișlia by carbonyls and terpenes, and Căușeni by higher alcohols and acetate esters. These results confirm that GC-IMS can capture subtle vineyard level differences and provide unique chemical fingerprints.

Keywords: *Fetească Neagră, wine aroma, volatile compounds, Ștefan Vodă, gas chromatography-ion mobility spectrometry.*

Rezumat. Aroma vinului este un determinant esențial al calității și tipicității, fiind modelată de amestecuri complexe de compuși volatili. Metodele tradiționale, precum GC-MS, oferă informații chimice detaliate, dar sunt adesea consumatoare de timp și mai puțin adecvate pentru studii rapide asupra terroirului. Acest studiu a emis ipoteza că cromatografia gazoasă cu spectrometrie de mobilitate ionică (GC-IMS) poate diferenția vinurile provenite din vii învecinate, aflate în cadrul aceleiași regiuni cu indicație geografică protejată (IGP). Obiectivul a fost evaluarea aplicabilității acestei metode pentru profilarea compușilor volatili din vinurile a trei vii din regiunea cu IGP Ștefan Vodă din Republica Moldova. Microvinificarea a fost realizată cu struguri Fetească Neagră recoltați la maturitate tehnologică în 2022, iar

vinurile obținute au fost analizate prin GC-IMS. Spectrele tridimensionale, proiecțiile bidimensionale, hărțile diferențiale și diagramele de tip „fingerprint” au evidențiat profiluri volatile specifice fiecărei plantații de viță de vie. Vinul din Purcari s-a caracterizat prin niveluri mai ridicate de esteri, cel din Cimișlia prin compuși carbonilici și terpene, iar cel din Căușeni prin concentrații mai mari de alcooli superiori și de esteri ai acidului acetic. Aceste rezultate confirmă faptul că GC-IMS poate detecta diferențe subtile la nivel de vie și poate furniza amprente chimice unice.

Cuvinte-Cheie: *Fetească Neagră, aroma vinului, compuși volatili, Ștefan Vodă, cromatografia gazoasă cu spectrometrie de mobilitate ionică.*

1. Introduction

Wine's aroma is shaped by a complex mixture of volatile compounds produced from grape material, metabolism during fermentation, and subsequent aging [1,2]. Thousands of volatile compounds have been identified in wine, which can be divided into alcohols, esters, aldehydes, ketones, terpenes, sulfur compounds, and methoxypyrazines [3]. Although many of the volatile compounds are present in low concentrations in wine, with very low odor thresholds, they still contribute to the wine's aroma.

Advances in analytical chemistry over the past few decades have enabled the study of volatile compounds in wine to become more extensive. Gas chromatography-mass spectrometry (GC-MS) has long been considered the gold standard for qualitative and quantitative analysis [4,5]. More comprehensive methods, such as comprehensive two-dimensional gas chromatography (GC×GC-MS) and proton transfer reaction mass spectrometry (PTR-MS), have further improved resolution and sensitivity [6,7]. However, these methods are generally time-consuming (complex sample preparation, analysis, and data processing). In recent years, gas chromatography-ion mobility spectrometry (GC-IMS) has been considered as an alternative for the analysis of volatile compounds in wine [8]. GC-IMS combines gas chromatographic separation with ion mobility-based detection to provide rapid, highly sensitive measurements and generate two- or three-dimensional “fingerprints” that allow visualization of complex volatile mixtures [9-11].

Previous studies have demonstrated the utility of GC-IMS in food authenticity and quality control, including applications in olive oil, honey, ham, beer, and dairy products [12-14]. In recent years, GC-IMS has been introduced into oenological research and has been shown to be effective in differentiating wines based on grape variety, vintage, and fermentation conditions [15-17]. However, the application of this technique in terroir related studies remains rare, especially at the vineyard level within a single geographical indication.

There is still debate about whether GC-IMS can replace traditional GC-MS for detailed chemical characterization [18]. Some researchers argue that GC-IMS has limitations in terms of compound identification and quantification and is best considered a complementary tool [19]. While others emphasize its advantages in terms of speed, sensitivity to trace volatile substances (e.g., methoxypyrazines, volatile sulfur compounds), and pattern recognition capabilities, which are closely related to authenticity of origin studies [20]. It is hypothesized that GC-IMS would be able to differentiate between wines from neighboring vineyards under the same PGI, providing a unique chemical fingerprint associated with the specific terroir of the vineyard.

The aim of this study was therefore to evaluate the applicability of GC-IMS for profiling volatile compounds in wines from three vineyards located in the IGP Ștefan Vodă region of

Moldova, with a focus on assessing its value as a rapid and informative tool for vineyard level discrimination.

2. Materials and Methods

This research was conducted in three vineyards located within the IGP Ștefan Vodă region: Purcari, Cimișlia, Căușeni. The Fetească Neagră grape harvest in the 2022 vintage, sampling showed a sugar content of 255 g/L. For microvinification, 3 kg of grapes per trial were destemmed and crushed, and 100 mg/dm³ potassium metabisulfite (PMS) was added. After must clarification, 80 mg/dm³ of commercial *Saccharomyces cerevisiae* yeast was inoculated. During alcoholic fermentation, pigeage and remontage were performed twice daily, and the must density was continuously monitored. When the density decreased to 1010 - 1020 g/dm³, 25 mg/dm³ of lactic acid bacteria were added to initiate malolactic fermentation. Upon completion of malolactic fermentation, the wines were racked, treated with 50 mg/dm³ PMS, and bottled in 750 mL glass bottles.

The analysis of volatile organic compounds was performed using a GC-IMS instrument FlavourSpec® (G.A.S., Germany), equipped with an automatic CTC-PAL 3 injection system. An aliquot of 1 g of wine was placed in a 20 mL headspace vial, incubated at 60 °C for 20 min, and then injected, with each sample analyzed in triplicate. The headspace injection conditions were as follows: incubation temperature 60 °C, incubation time 20 min, injection volume 100 µL, splitless mode, incubation agitation speed 500 rpm, and injector needle temperature 85°C. GC conditions included a column temperature of 60 °C and high-purity nitrogen as the carrier gas (≥99.999%). The initial flow rate was 2.0 mL/min (held for 2 min), increased linearly to 10.0 mL/min within 8 min, then to 100.0 mL/min within 10 min, and held for 20 min, giving a total run time of 40 min; the drift gas flow was 75.0 mL/min, and the injector temperature was 80°C. IMS conditions included a tritium ionization source, a drift tube length of 53 mm, an electric field strength of 500 V/cm, a drift tube temperature of 45°C, high purity nitrogen (≥99.999%) as the drift gas, and positive ion mode. Data processing was carried out using VOCal software, with compound identification based on retention time and drift time matched against the built in NIST 2020 GC retention index and IMS drift time libraries. The Reporter and Gallery Plot modules were used to generate three dimensional spectra, two dimensional spectra, various maps, and fingerprint plots.

All samples were measured in triplicate, and the VOC content results are expressed as mean values ± standard deviation. Statistical analysis was first performed in Excel for raw data organization, followed by subsequent processing in R package (version 4.4.3). R package is a programming language and statistical computing environment widely used for data analysis. One-way analysis of variance (ANOVA) was used to evaluate differences among vineyards, and Tukey's post hoc test was applied for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

3. Results

Figure 1 shows a three-dimensional GC-IMS spectrum, with drift time on the X-axis, retention time on the Y-axis, and signal intensity on the Z-axis. It is immediately apparent that the main diagonal line (representing the reaction ion peak and its associated cluster) is consistent across all spectra. At the same time, subtle differences in the distribution and intensity of secondary peaks were evident. In particular, the front region of the spectra (corresponding to lower retention times) contained multiple minor clusters with variable intensities across samples, suggesting vineyard specific variations in light volatiles, such as

alcohols and esters. Although the overall complexity appeared comparable, these local differences justified further visualization through two dimensional projections, as illustrated in Figure 2.

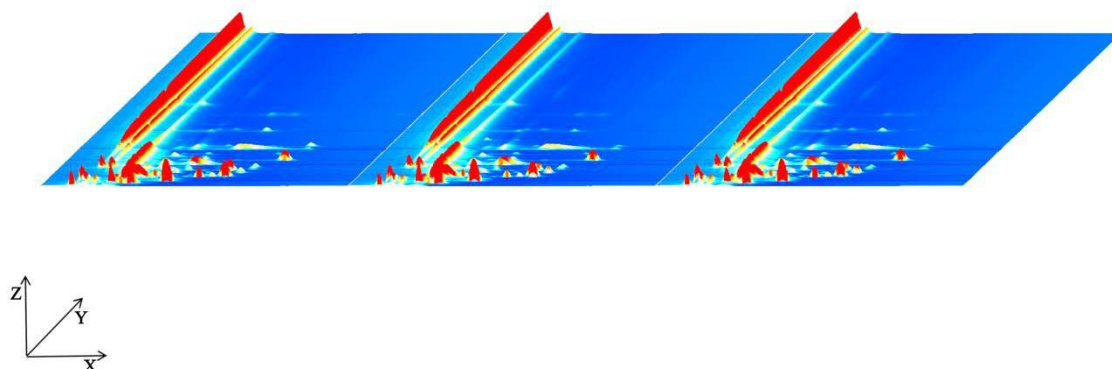


Figure 1. Three-dimensional GC-IMS spectra of wines from three vineyards in the IGP Ștefan Vodă region.

The two-dimensional GC-IMS spectra (Figure 2) provides a clearer picture of the distribution of volatile compounds than the three-dimensional plots. While the overall spectral structure of the three samples is similar, there are differences in the peak intensities, particularly in the region with lower drift times, which are typically associated with light compounds such as alcohols and esters.

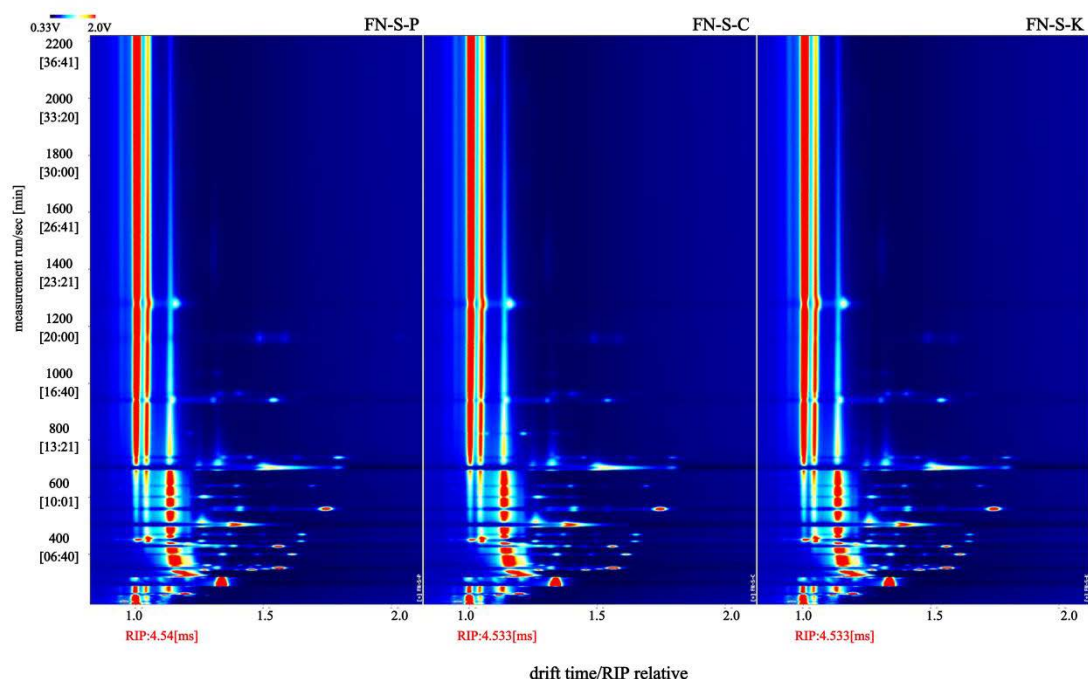


Figure 2. Two-dimensional GC-IMS spectra of wines from three vineyards in the IGP Ștefan Vodă region. (FN-S-P: Purcari, FN-S-C: Cimișlia, FN-S-K: Căușeni).

To further highlight these variations, differential mapping was performed using the spectrum of FN-S-P (Purcari) as the reference (Figure 3). In the differential plots, the red vertical line at drift time 1.0 corresponds to the normalized reaction ion peak (RIP), with the vertical axis representing gas chromatographic retention time(s) and the horizontal axis representing relative drift time (normalized, a.u.). Each point adjacent to the RIP peak

corresponds to a volatile organic compound, and the color gradient from blue to red indicates signal intensity, with deeper colors denoting higher peak responses. In comparative analysis, a white background indicates no difference between the target and reference compounds, while red represents higher concentrations of the compound and blue represents lower concentrations. The results showed that compared to the Purcari sample, Cimișlia and Căușeni showed weaker signals in the intermediate drift time region, which may be related to aldehydes and esters. Overall, the combination of two-dimensional visualization and differential mapping demonstrated that GC-IMS is a powerful tool for capturing and emphasizing subtle vineyard specific variations in wine aroma profiles.

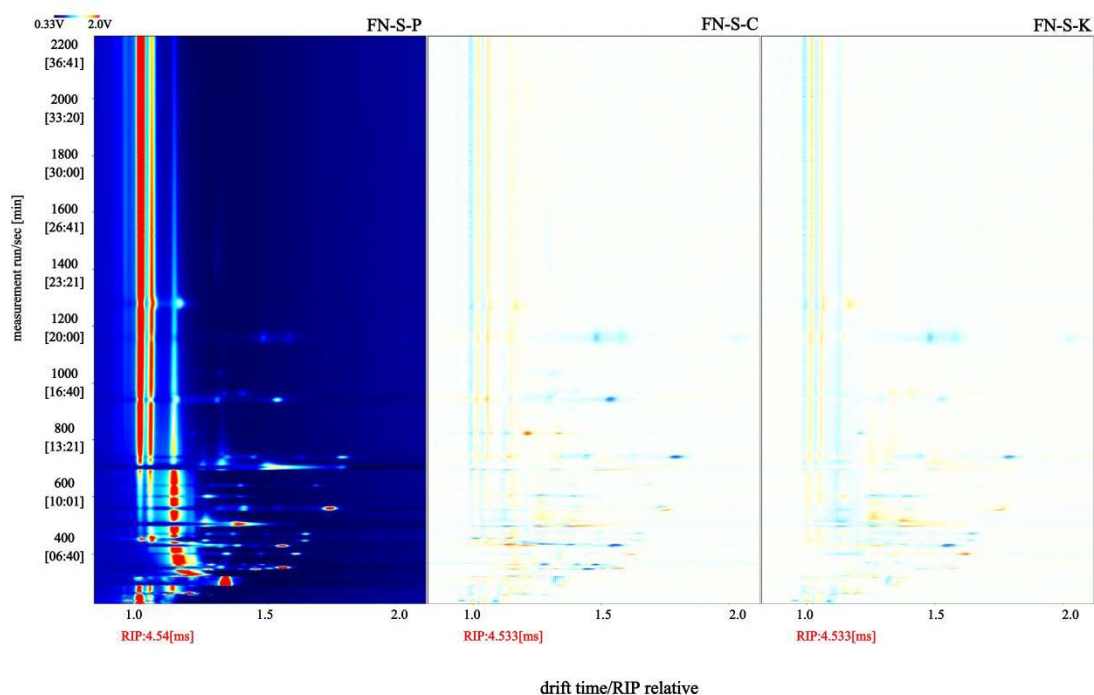


Figure 3. Difference maps of GC-IMS spectra (from left to right: Purcari, Cimișlia, Căușeni).

Volatile compounds were identified by matching their RI and Dt values with the NIST (2020) and VOCal IMS libraries, as summarized in Table 1. A total of 44 peaks were detected across the three samples, corresponding to 33 volatile compounds (where M denotes monomer and D denotes dimer).

Table 1

Details of Volatile Compounds				
Peak#	RI*	Rt* (s)	Dt* (RIPrel.)	Compound
1	1720.5	2016.961	1.09249	γ -Butyrolactone
2	1720.3	2016.456	1.20485	Acetophenone
3	1509.6	1278.873	1.15744	Acetic acid
4	1462.8	1155.942	1.49214	Ethyl octanoate-M
5	1462.2	1154.366	2.02767	Ethyl octanoate-D
6	1410.6	1032.574	1.32225	(E)-2-Hexenol
7	1410.2	1031.615	1.24315	Z-3-Hexenol
8	1367.9	941.534	1.14236	Ethyl lactate-M
9	1367.9	941.534	1.53785	Ethyl lactate-D
10	1379	964.533	1.33118	1-Hexanol-M
11	1377.7	961.658	1.64119	1-Hexanol-D

Continuation Table 1

12	1306	823.661	1.21126	Terpinolene
13	1305.4	822.573	1.40112	Octanal
14	1255.5	742.174	1.34641	Ethyl hexanoate-M
15	1255.5	742.174	1.8005	Ethyl hexanoate-D
16	1230.8	705.804	1.24902	3-Methyl-1-butanol-M
17	1230.4	705.166	1.48865	3-Methyl-1-butanol-D
18	1182.5	631.148	0.94264	1-Penten-3-ol
19	1168.6	601.796	1.26543	1-Butanol-M
20	1169.2	603.072	1.38799	1-Butanol-D
21	1146.1	557.13	1.31358	Isoamyl acetate-M
22	1146.7	558.406	1.75236	Isoamyl acetate-D
23	1116.9	504.169	1.25559	2-Methyl-1-propanol-M
24	1117.3	504.807	1.36829	2-Methyl-1-propanol-D
25	1095.6	469.075	1.27091	Ethyl 3-methylbutanoate-M
26	1095.6	469.075	1.66154	Ethyl 3-methylbutanoate-D
27	1079.2	447.38	1.25449	Ethyl 2-methylbutanoate-M
28	1079.2	447.38	1.65278	Ethyl 2-methylbutanoate-D
29	1065.1	429.514	1.24793	1-Propanol
30	1064.1	428.237	1.56415	Ethyl butanoate
31	1040.5	400.162	1.33875	2-Methylpropyl acetate-M
32	1041.1	400.8	1.61339	2-Methylpropyl acetate-D
33	1012.3	368.896	1.35625	2-Pentanone
34	1006.3	362.515	1.41315	Pentanal
35	1005.7	361.877	1.48209	Propyl acetate
36	997	352.944	1.56525	Ethyl isobutyrate
37	989	346.563	1.45801	Ethyl propanoate
38	957.6	323.592	1.12319	Ethanol
39	918	296.792	1.34094	Ethyl acetate
40	861.5	262.336	1.1965	Methyl acetate
41	852.5	257.231	1.11225	Acetone
42	832.7	246.384	1.06301	Propanal
43	810.9	234.898	0.95796	Dimethyl sulfide
44	1305.7	823.211	1.07067	3-Hydroxy-2-butanone

*RI - retention index; Rt - retention time; Dt - drift time; RIPrel - normalized relative to the RIP (reaction ion peak).

After merging the monomer and dimer signals, GC-IMS detected a total of 33 volatile compounds, including 9 alcohols, 12 esters, 4 aldehydes, 3 ketones, 1 acid, 1 sulfur compound, 1 terpenoid, and 2 others. Among them, 3-hydroxy-2-butanone and γ -butyrolactone were classified as "Others". The former, although possessing the dual characteristics of a hydroxyketone, contributes to wine aroma mainly through its involvement in the metabolic conversion of diacetyl, rather than behaving as a typical alcohol or ketone. The latter belongs to the lactone family, whereas the esters identified in this study were predominantly straight- and branched-chain acetate esters and ethyl esters of fatty acids. It is noteworthy that γ -butyrolactone has previously been reported as a natural constituent of wines and other beverages.

In Figure 4, each row represents all the selected signal peaks detected in each sample, while each column corresponds to the same volatile compound across different samples.

Continuation Table 2

Octanal	33.63	0.01	92.71	0.04	29.88	0.01
Pentanal	176.73	0.07	85.37	0.03	172.79	0.07
Ethyl propanoate	1658.27	0.67	1024.43	0.41	1551.60	0.63
Propanal	146.51	0.06	161.03	0.06	187.57	0.08
Ethyl octanoate	1497.40	0.61	759.96	0.30	732.84	0.30
Ethyl lactate	4005.18	1.62	3329.80	1.33	4005.56	1.62
Ethyl hexanoate	1730.79	0.70	932.32	0.37	1101.09	0.44
Isoamyl acetate	8000.17	3.24	8078.06	3.24	8494.70	3.43
Ethyl 3-methylbutanoate	1456.43	0.59	1298.07	0.52	1123.80	0.45
Ethyl 2-methylbutanoate	1468.79	0.59	1456.44	0.58	1321.14	0.53
Ethyl butanoate	2676.76	1.08	1608.67	0.64	1709.32	0.69
2-Methylpropyl acetate	2058.90	0.83	2278.15	0.91	2726.21	1.10
Propyl acetate	247.95	0.10	77.85	0.03	224.19	0.09
Ethyl isobutyrate	3684.68	1.49	3916.21	1.57	3755.78	1.52
Ethyl acetate	18839.30	7.63	18099.56	7.25	18560.51	7.49
Methyl acetate	2620.18	1.06	2810.98	1.13	2985.51	1.20
Acetophenone	349.79	0.14	415.67	0.17	371.04	0.15
2-Pentanone	89.89	0.04	98.94	0.04	60.81	0.02
Acetone	1699.29	0.69	1818.96	0.73	1508.18	0.61
Dimethyl sulfide	1041.23	0.42	773.49	0.31	762.15	0.31
Terpinolene	263.33	0.11	787.63	0.32	157.19	0.06
γ -Butyrolactone	443.88	0.18	513.54	0.21	492.11	0.20
3-Hydroxy-2-butanone	60.15	0.02	151.35	0.06	50.97	0.02

Table 2 shows the peak volumes (intensities) of volatile compounds in Fetească Neagră wines from three vineyards of Ștefan Vodă IGP.

Alcohols are among the most important compounds in wine. The results showed that ethanol content was highest (58.68% to 60.32%), significantly exceeding that of the other VOCs. Esters are among the most important volatile aroma compounds in wine. Within the acetate ester group, ethyl acetate, isoamyl acetate, ethyl lactate, ethyl isobutyrate, ethyl butanoate, and methyl acetate were detected. Within the acetate ester group, ethyl acetate, the most common volatile ester, generally contributes fruity aromas at moderate concentrations. Its content from 7.25% to 7.63%. Isoamyl acetate was one of the predominant esters detected, imparting intense banana and pear aromas. Its content from 3.24% to 3.43%. Within the ethyl ester group of fatty acids, ethyl lactate is an important compound detected in this study, contributing roundness and slightly creamy notes to wine, content from 1.33% to 1.62%. Ethyl hexanoate and ethyl octanoate, typical fatty acid ethyl esters that impart fruity and floral aromas.

Ketones are generally identified at low concentrations in wine. The content of acetone went from 0.61% to 0.69%. The content of 2-pentanone from 0.02% to 0.04%.

Dimethyl sulfide (DMS) was the only sulfur-containing compound detected in this study, with the highest content in 0.42% and the lowest in 0.31%. DMS, as a volatile sulfur compound, is further influenced by matrix effects: for instance, when added alone to deodorized wine, it is typically associated with off odors, but when combined with fruity aroma compounds, DMS can enhance overall complexity, imparting sweet fruity notes and green olive nuances to the wine.

Terpinolene, a monoterpene, generally imparts citrus, resinous, herbal, and subtle floral aromas to wine, and in grape varieties, it is primarily synthesized via the mevalonate pathway. However, terpenes are usually found in low concentrations in red wines due to the lower precursor content in red grape varieties, as well as the prolonged skin contact and higher phenolic levels during fermentation, which may suppress their expression. In this study, terpinolene ranged from 0.06% to 0.32%, suggesting that the Fetească Neagră variety may have relatively high terpene precursor levels or that this variety can accumulate higher amounts of free-form terpenes during ripening, which are then released during fermentation.

4. Discussion

Although all three vineyards lie in the Ștefan Vodă region, each site has unique attributes (e.g., slight differences in soil minerals, exposure, elevation, or viticultural practices) that can influence grape metabolism and fermentation outcomes, and the differences in subtle volatiles can be traced back to this terroir driven factors. This interpretation is consistent with the concept of terroir, which holds that even small scale environmental and viticultural differences manifest in the wine's chemical profile [21].

Many recent studies support our findings. A study carried out in Ningxia, China, showed that wines from sub regions with different climatic conditions exhibited different aroma profiles, and the temperature and soil nutrients were the main contributors [22]. Similarly, Goulioti et al. [23] found significant differences in the volatile composition and sensory profile of Xinomavro wines from different Greek Protected Designations of Origin (PDOs). Jin et al. [24] compared the volatile compound fingerprints of the variety Beibinghong from six vineyards and found that each region could be distinguished chemically and sensorially. These examples highlight that a wine's location can lead to measurable differences in its aroma compounds.

While the outcomes are encouraging, several limitations of this study should be acknowledged. First, we examined only a single vintage year, which means the observed differences could partially include vintage specific effects. Year to year climatic variability can influence grape composition; thus, our ability to generalize the findings across vintages is limited. Future studies should be carried out in multiple vintages to confirm that the vineyard differentiation holds under different growing seasons. Second, GC-IMS provides limited compound identification. This method is better at pattern recognition (fingerprinting volatiles), but its ability to conclusively identify each peak is constrained by the available spectral library and overlapping signals. Especially, some detected peaks remain unknown, whereas complementary methods like GC-MS would be supplemented to fully characterize the compounds. This limitation means we can pinpoint that “something” differs between vineyards (as shown in the differential maps), but not always name the specific chemical without additional analyses. Third, we did not conduct any sensory analysis in this study. As a result, it is unclear how the chemical differences observed translate to human perceived aroma or flavor differences.

Building on these findings, several avenues for future research are recommended. Expanding the scope to multiple vintages and additional vineyard sites within (and beyond) the Ștefan Vodă IGP will help validate GC-IMS as a robust tool for terroir differentiation. Including more years would clarify the consistency of vineyard specific volatile markers versus vintage induced variability. Similarly, experimental subjects should be expanded to different grape varieties. Another important future direction is to combine gas

chromatography-ion mobility spectrometry analysis with sensory evaluation and advanced data analysis techniques. By combining chemical fingerprinting with sensory descriptive analysis or consumer preference testing, researchers can determine which chemical differences are perceptible and crucial for the quality or typicality of wine. In parallel, applying chemometric tools and machine learning can greatly enhance the classification and predictive power of the data. For example, recent studies have shown that combining GC-IMS volatile data with machine learning algorithms (such as artificial neural networks) allowed >95% accuracy in predicting wine quality grades or classifying wines by type [25].

5. Conclusions

This study demonstrates that GC-IMS can effectively differentiate wines from three vineyards in the IGP Ștefan Vodă region of Moldova and reveal vineyard specific volatile fingerprints. The overall volatile composition of the samples was similar, significant differences were observed: FN-S-P wines were enriched in esters (such as ethyl octanoate, ethyl hexanoate, and ethyl acetate) and alcohols (such as 1-butanol and 1-penten-3-ol); FN-S-C wines were characterized by higher levels of carbonyl compounds and terpenes (such as γ -butyrolactone, acetophenone, and terpinolene); whereas FN-S-K wines showed elevated concentrations of higher alcohols and acetate esters (such as 3-methyl-1-butanol, isoamyl acetate, and ethyl lactate). These results confirm that GC-IMS can figure out the vineyard level differences in volatile composition. Although the study was limited to a single vintage, the findings highlight the potential of GC-IMS as a rapid, sensitive, and visual tool for vineyard level discrimination. Future research should include multiple vintages, more varieties, incorporate sensory evaluation, and apply chemometric and machine learning approaches to improve classification reliability.

Acknowledgments: The author (Fei Wang) is supported by China Scholarship Council.

Conflicts of Interest: The authors declare no conflict of interest.

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Citation: Wang, F.; Yao, M.; Arpentin, Gh. Application of gas chromatography-ion mobility spectrometry (GC-IMS) to characterize volatile signatures of Fetească Neagră wines from the IGP Ștefan Vodă region. *Journal of Engineering Science*. 2025, XXXII (4), pp. 150-160. [https://doi.org/10.52326/jes.utm.2025.32\(4\).11](https://doi.org/10.52326/jes.utm.2025.32(4).11).

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