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Assessing the Ecological Impact of Cassava Mill Effluent on Soil Microbiology and Physicochemistry

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Abstract

This study examined the effects of cassava mill effluent on soil microbiological and physicochemical properties to evaluate its environmental implications. Soil samples from three sites (A-C) were analyzed for microbial diversity and abundance, with a focus on impacted soil contaminated with cassava effluent. Microbial analysis revealed the predominance of Bacillus sp. and Pseudomonas sp. (28.6 %) among bacterial isolates, while Penicillium sp. (27.2 %) was the most common fungal isolate. Impacted soil exhibited higher bacterial counts (7.8×10^5 cfu/g), but fungal growth was suppressed. Physicochemical analysis indicated significant alterations in soil chemistry due to cassava mill effluent, with elevated levels of nitrogen (797 mg/l), potassium (459 mg/l), and phosphorus (432 mg/l) in impacted soil. However, the study also highlighted substantial heavy metal contamination, including copper and iron, posing toxicity risks. The findings suggest that cassava mill effluent enhances microbial activity but concurrently degrades soil quality. Antibiotic sensitivity testing revealed the resilience of certain isolates, such as Bacillus sp., with Ciprofloxacin exhibiting the highest inhibitory activity. The study concludes that while cassava mill effluent introduces beneficial nutrients for microbial growth, its acidity, heavy metal content, and potential to foster antibiotic-resistant microorganisms pose environmental and health risks. These findings emphasize the urgent need for effective effluent management practices to mitigate soil degradation and ecological imbalances.

Keywords: microbiological, physicochemical, effluent, environmental, contamination.

1. Introduction

Cassava (Manihot esculenta Crantz) is a widely cultivated root tuber crop in tropical regions worldwide. As a primary food source, cassava tubers are rich in carbohydrates (85.9 %) and low in protein (1.3 %), with notable amounts of cyanogenic glucoside (Nwabueze, 2007; Nwinanee, 2021). This crop is a staple food item, particularly for low-income earners in Africa and Asia (Desse, Taye, 2001; Edamisan, 2020).

Originating from South America, cassava was introduced to Africa in the 16th century and has since become a major food source in sub-Saharan Africa (Adewoye, 2005; Nwinanee, 2021). In West Africa and Nigeria, cassava is commonly consumed as garri, a fermented cassava product (Cheesbrough, 2005; Okoye, 2020). Nigeria is currently the world's largest producer of cassava, with significant growth in domestic and international demand (Desse, Taye, 2001; Edamisan, 2020).

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The cassava processing industry has expanded in Nigeria, with many small-scale processing units operating in residential areas. However, traditional garri production generates substantial amounts of wastewater, hydrocyanic acid, and organic waste, posing environmental and health risks (Akinfala, Tewe, 2004; Nwinanee, 2021). The indiscriminate discharge of this effluent contaminates agricultural lands, streams, and underground water, threatening the environment and human health (FAO, 2004; Okafor, 2008; Ehiadgonare et al., 2009; Nwinanee, 2021).

Studies have shown that the toxic concentrations of cassava mill effluent can prevent cereal seed germination and pose significant threats to humans and the environment due to its high cyanide content (Olorunfemi, 2008; Ezeogo et al., 2021). Therefore, investigating the environmental impact of cassava processing and implementing regulations for responsible waste management are crucial.

2. Materials and method

Study Area:

This research was conducted at the Department of Human Nutrition and Dietetics, Federal University of Health Sciences, Azare, Bauchi State. The study sample was collected from two distinct locations within Bauchi and Katagum Local Government Areas, situated in the North-Eastern region of Northern Nigeria.

Experimental details

Sample Collection and Preparation

Soil samples were aseptically collected from three cassava mill dump sites (A-C) in two locations within Bauchi State, Nigeria. Samples were collected at 5m and 50m distances from the dump site, with the latter serving as the control. Samples were transported in ice-cold containers to prevent microbial growth and analyzed accordingly.

Serial Dilution and Inoculation

Serial dilution of the samples was performed, and 0.1ml of the appropriate dilution was inoculated into nutrient agar and potato dextrose agar media in triplicate (Cheesbrough, 2005; Okoye, 2020). The inoculated plates were incubated at 37°C for 24h for bacterial enumeration and at room temperature for 5-7 days for fungal enumeration.

Characterization and Identification of Microbial Isolates

Discrete colonies were purified and identified based on cultural parameters, microscopic, and biochemical analysis. Biochemical tests, including Gram staining, motility test, oxidase test, catalase test, citrate test, indole test, methyl red test, and Voges Proskauer test, were conducted to further characterize the isolates (Fawole, Oso, 2004; Cheesbrough, 2005; Okoye, 2020).

Statistical Analysis

Simple ratios and percentages were used to determine relationships, while ANOVA was used to determine statistically significant differences between independent groups.

3. Results

A total of three soil samples were analyzed, with two samples from cassava mill effluent dumpsites and one from fertile, unimpacted farmland. Microbiological analysis revealed the presence of various bacterial and fungal species.

Bacterial Isolates

The identified bacterial isolates included Bacillus sp. (28.6 %), Pseudomonas sp. (28.6 %), Enterobacter sp. (7.1 %), Corynebacterium sp. (14.3 %), Proteus sp. (7.1 %), and Escherichia coli (14.2 %).

Fungal Isolates

The identified fungal isolates included Aspergillus sp. (18.2 %), Penicillium sp. (27.2%), Mucor sp. (9.1 %), Candida sp. (18.2 %), Saccharomyces sp. (18.2 %), and Rhizopus sp. (9.1 %).

Microbial Enumeration

The average microbial count of bacteria and fungi is presented in Figure 1. The total mean heterotrophic bacterial count ranged from $7.0\pm0.11 \times 10^5$ cfu/g for the control soil, while the effluent and polluted soil were $5.1\pm0.28 \times 10^5$ cfu/g and $7.8\pm0.19 \times 10^5$ cfu/g, respectively. The fungal mean ranged from $4.2\pm0.57 \times 10^5$ cfu/g for the control soil, while the effluent and polluted soil were $4.4\pm0.41 \times 10^5$ cfu/g and $3.9\pm0.42 \times 10^5$ cfu/g, respectively.

The impacted soil had the highest bacterial count of 7.8±0.19x10^5 cfu/g. Bacterial counts were higher than fungal counts in all samples.

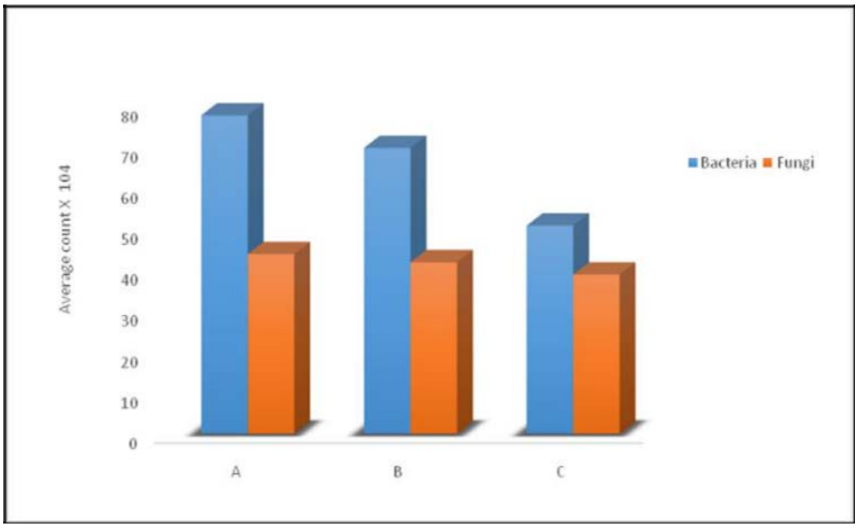

Fig. 1 Bacterial and fungal counts at the impacted soil, un-impacted soil and cassava effluent (A – Impacted soil, B – Un-impacted soil and C – Cassava effluent)

Physico-Chemical Analysis of Cassava Effluent and Soil Samples

The results of the physico-chemical analysis of cassava impacted soil, un-impacted soil, and cassava effluent samples are presented in Table 1. Duplicate data for each parameter were analyzed using One-Way ANOVA at a 0.05 (95 %) significance level.

pH and Nutrient Levels

The cassava effluent sample had an acidic pH of 3.13, while the un-impacted and impacted soil samples had slightly alkaline pH values of 7.87 and 7.21, respectively. The cassava effluent sample contained the highest concentration of nitrogen (797.00 mg/l), followed by the un-impacted soil (95.15 mg/l) and impacted soil (7.51 mg/l). Potassium levels were significantly higher in the cassava effluent sample compared to the impacted and un-impacted soil samples.

Metal Concentrations and Other Parameters

Magnesium levels were similar in the cassava effluent (657.75 mg/l) and impacted soil (660.85 mg/l) samples, but lower in the un-impacted soil sample (36.95 mg/l). Calcium, sodium, and electrical conductivity levels were higher in the effluent and un-impacted soil samples compared to the impacted soil sample. The biological oxygen demand (BOD5) was highest in the impacted soil sample (7.18), followed by the cassava effluent sample (5.73) and un-impacted soil sample (4.54). Dissolved solids were highest in the effluent sample (478.75 mg/l) and lowest in the impacted soil sample.

Heavy Metal Concentrations

The concentration of copper was two- to three-fold higher in the effluent sample compared to the un-impacted and impacted soil samples. Iron, cobalt, and ammonia concentrations were highest in the cassava effluent sample. Zinc concentrations were relatively constant across all three samples. Nitrate levels were highest in the cassava effluent sample (12.01 mg/l), while nitrite levels were highest in the un-impacted soil sample (88.05 mg/l).

Table 1. Physicochemical analysis of cassava effluent, impacted and un-impacted soils samples

Parameters	Un-impacted soil	Impacted soil	Cassava effluent
pH	7.87±0.01a	7.21±0.01b	3.13±0.01c
Nitrogen (mg/l)	95.15±0.10	7.51±0.01	797.00±1.41
Phosphorus (mg/l)	288.85±0.10	79.51±0.01	432.00±1.41
Potassium (mg/l)	66.65±0.10	56.25±0.10	56.25±0.10
Magnesium (mg/l)	36.95±0.10	660.85±0.10	657.75±0.21

Parameters	Un-impacted soil	Impacted soil	Cassava effluent
Calcium (mg/l)	34.15±0.10	6.71±0.01	64.30±1.41
Sodium (mg/l)	53.75±0.10	4.51±0.01	30.90±0.14
Conductivity(µs/cm)	26.35±0.10	8.99±0.01	46.20±0.01
BOD (28 °C)	4.54±0.01	7.18±0.01	5.73±0.01
TDS (mg/l)	57.12±0.01	4.51±0.01	478.75±0.01
Copper (mg/l)	8.40±0.01	14.73±0.01	29.34±0.01
Zinc (mg/l)	9.76±0.01	11.13±0.01	10.17±0.00
Nickel (mg/l)	0.66±0.00	0.68±0.00	1.75±0.00
Cobalt (mg/l)	BDLd	0.78±0.00	8.80±0.01
Iron (mg/l)	13.95±0.01	18.09±0.01	31.95±0.01
Ammonia (mg/l)	3.76±0.01	3.50±0.00	10.50±0.01
Nitrate (mg/l)	BDLd	1.10±0.01	12.01±0.01
Nitrite (mg/l)	88.05±0.01	0.61±0.01	6.10±0.01

Notes: a, b, c Represent Mean±SD readings that are significant ($p < 0.05$) across the rows for each of the parameters and samples while BDLd equals below detection level, respectively.

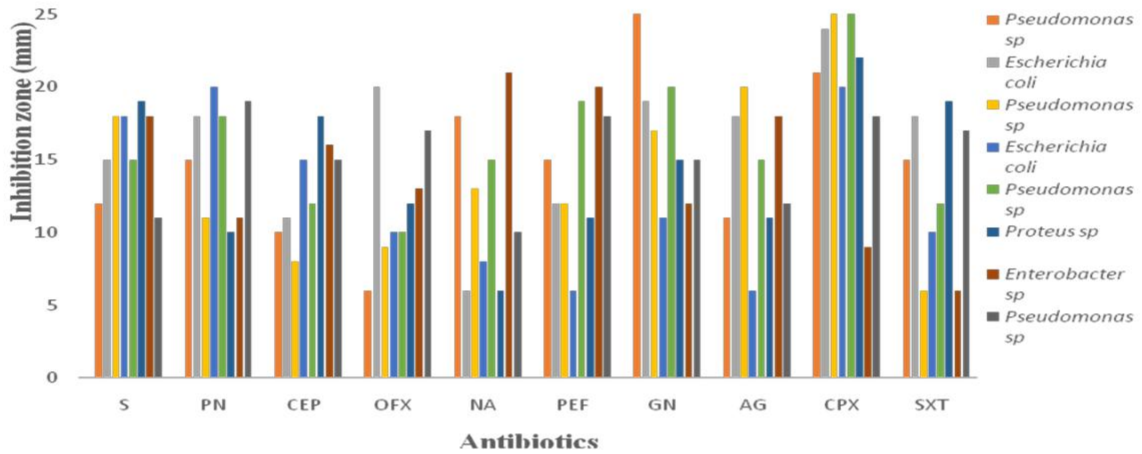

Fig. 2. The sensitivity of different gram negative organisms and antibiotics

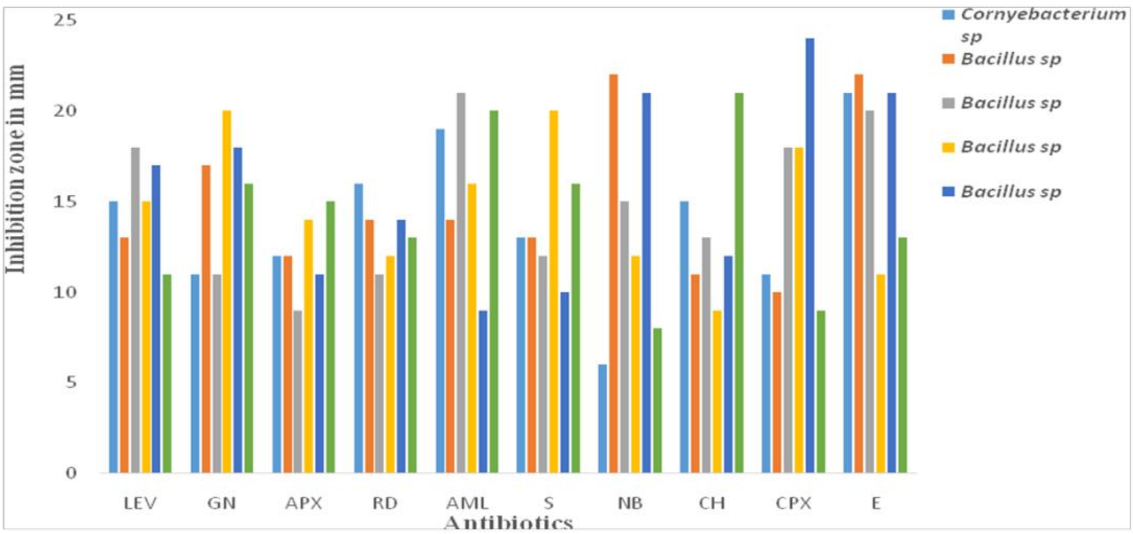

Fig. 3. The sensitivity of different gram positive organisms and antibiotics

Statistical Significance and Antibiotic Susceptibility Testing

Values marked with a, b and c represent significant mean ± standard deviation readings ($p < 0.05$) across rows for each parameter and sample. The symbol BDLd indicates values below the detection level.

Antibiotic Susceptibility Testing Results

Figures 2 and 3 present the results of the antibiotic susceptibility testing on Gram-negative and Gram-positive organisms isolated from different soil samples. The antibiotics used in this study were: Streptomycin; Ciprorex; Ampicillin; Tarivid; Nalidixic Acid; Perflacine; Gentamicin; Augmentin; Ciprofloxacin; Septrin; Levoflacin; Ampiclox; Rifampin; Amoxil; Norfloxacin and Erythromycin.

Pseudomonas sp., Proteus sp., and Escherichia coli isolated from all three sites were tested against these antibiotics.

Key Findings

1. Ciprofloxacin efficacy: Ciprofloxacin demonstrated consistent activity against the test isolates, with zones of inhibition of 25 mm and 24 mm against Pseudomonas and Escherichia coli, respectively.
2. Least effective antibiotics: Tarivid and Septrin showed the least zones of inhibition (6 mm) against Pseudomonas, E. coli, and Proteus species.
3. Pseudomonas sensitivity: Pseudomonas species isolated from impacted and effluent soil samples were most sensitive to the test antibiotics.
4. Gram-positive organism sensitivity: The highest zone of inhibition (24 mm) was observed for Bacillus sp. isolated from cassava effluent-impacted soil with Ciprofloxacin.

Antibiotic Efficacy Ranges

1. Levoflacin, Streptomycin, and Gentamicin: Showed zones of inhibition between 10 mm and 20 mm on all test isolates.
2. Remaining antibiotics: Had zones of inhibition between 6 mm and 24 mm.

Most Isolated Species

1. Bacillus sp.: Most frequently isolated from all three locations.
2. Corynebacterium and Enterobacter: Followed in terms of frequency of occurrence.

4. Discussion

Microbial Isolates and Counts

The study revealed the presence of various bacterial and fungal species in the soil samples. The bacterial isolates included Bacillus sp., Pseudomonas sp., Enterobacter sp., Corynebacterium sp., Proteus sp., and Escherichia coli. Eleven species of fungi were also isolated, including Aspergillus sp., Penicillium sp., Mucor sp., Candida sp., Saccharomyces sp., and Rhizopus sp. These findings are consistent with previous studies (Ehiagbonare et al., 2009; Igbinosa, Igiehon, 2015).

Microbial Counts and Comparison

The impacted soil had the highest microbial count of 7.8±0.19x105 cfu/g. Bacterial counts were higher than fungal counts in all samples. The total mean heterotrophic bacterial count ranged from 7.0±0.11x105 cfu/g to 7.8±0.19x105 cfu/g, while fungal counts ranged from 4.2±0.57x105 cfu/g to 3.9±0.42x105 cfu/g. These findings suggest that fungal counts were significantly lower than bacterial counts ($p < 0.05$), consistent with previous reports (Aiyegoro et al., 2007).

Effects of Cassava Mill Effluent on Soil Microorganisms

The study revealed that soil samples not affected by cassava mill effluent had a higher microbial population compared to those impacted by the effluent. This difference can be attributed to the harmful effects of cyanide acid content in cassava mill effluent on soil microorganisms. These findings support previous reports (Akubuenyi, 2022; Dike et al., 2022; Ukaegbu-Obi et al., 2018).

5. Conclusion and Recommendations

The study highlights the adverse environmental effects of cassava mill effluents on soil microorganisms and physicochemical properties. To mitigate these effects, it is recommended that:

1. Relocation of Cassava Mills: Cassava mills should be relocated to areas away from residential and agricultural areas.
2. Regulations and Enforcement: Governments should establish and enforce regulations for the disposal of cassava mill effluents.
3. Public Awareness and Education: Public awareness campaigns should be conducted to educate cassava farmers and processors about the dangers of cassava mill effluents and proper disposal methods.
4. Further Research: Additional research should be conducted on the effects of cassava mill effluents on surrounding water bodies and human health.

6. Acknowledgements

We extend our deepest and sincerest gratitude to the Tertiary Education Trust Fund (TETFUND) for their generous and invaluable financial support, which made this research possible. Their full sponsorship of this study is a testament to their commitment to advancing knowledge and education, and we are truly grateful for their trust in our work.

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**The Journal "European Reviews of Chemical Research" (2014–2024):
A Thematic Review of Published Research over the last 10 years**

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Abstract

This article is devoted to the ten-year (2014–2024) results of the publication activity of the journal "European Reviews of Chemical Research" and its authors. The materials for this work are the studies that were published in the journal in the period from 2014 to 2024. The research methodology is presented by both general scientific and special research methods. In the article, the authors provide a thematic analysis of the works that were published in the journal "European Reviews of Chemical Research". In conclusion, the authors note that over the ten-year period of the journal's existence, 76 articles on various areas of chemical science were published in it. Separately, the authors note the language ratio of publications, where 80 % of all articles were published in English and the remaining 20 % were published in Russian, which allows expanding the reach of the journal's readership.

Keywords: chemistry, chemical research, scientific journal, chemical journal, research review, intellectual capital, publication activity, work results.

1. Introduction

Nowadays the journal "European Reviews of Chemical Research" is an open chemical scientific publication, publishing various studies in the field of chemical science, as well as publishing studies on multidisciplinary issues.

The purpose of this work is to provide a thematic review of studies that were published in the journal "European Reviews of Chemical Research" in the period from 2014 to 2024.

This article is an attempt by the author to summarize the publication activity of the journal "European Reviews of Chemical Research" and its authors over the past 10 years (2014-2024).

2. Materials and methods

The materials for this study were studies that were published in the journal "European Reviews of Chemical Research" in the period from 2014 to 2024.

The research methodology is based on general scientific and special research methods. General scientific research methods are represented by: analysis, synthesis, induction, deduction. Special research methods are represented by: content analysis (to analyze the content of studies that were published in the journal), bibliographic method (in terms of selection, classification and ranking of studies that were published in the journal), and narrative method (to form the narrative line of this work).

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3. Discussion

The journal was founded in 2014 and is still being published. In 2014, 2 issues of the journal were published, then in 2015 and 2016, 4 issues of the journal were published each year. From 2017 to 2019, the journal was published twice a year. From 2020 to the present, the journal is published once a year (issue in December) (Figure 1).

The mission of the journal "European Reviews of Chemical Research" is to present the results of modern researches in the chemistry. The priority direction of development journal is coverage of related multidisciplinary problems, the use of new methods and approaches in the context of practical application.

The journal's objective is to familiarize the specialists and all interested readers with the contemporary achievements in chemical sciences, developing the inter-institutional and international scientific cooperation in the framework of the research problems, improving research ethics and publication activity of young researchers.

Fig. 1. Cover of the chemical scientific journal "European Reviews of Chemical Research".

Currently, review articles that are devoted to various aspects of the analysis of published studies (Amobonye et al., 2024; Çalik, Wiyarsi, 2021; Ültay, Çalik, 2012) or the publication activity of journals are quite common. This type of scientific work is not only purely review in nature for the formation of certain conclusions, but also helps new (often young) researchers to better navigate the area of scientific interests that interests them (Mamadaliev, 2023), enhancing their research potential (Shtuts i dr., 2023). Thus, this article is review and at the same time introductory in nature, and will be useful not only for potential authors of the journal and researchers, but also for a wide range of readers.

4. Results

For ease of understanding and ease of navigation through the work, all studies were distributed by year of publication and subject matter.

2014 year.

Defining the Conditions of 3D Printing Using Abs Plastic was presented in the paper of Marat I. Abdullin, Azamat A. Basyrov, Sergey N. Nikolaev, Yuliya A. Koksharova and Nikolay V. Koltaev (Abdullin et al., 2014). Antimicrobial Potential of Nicotinic Acid Derivatives Against Various

Pathogenic Microbes was presented in the paper of Mohammad Asif (Asif, 2014). Heterofunctional Condensation of α,ω – Bis (Aminodimethylsilil) Tetramethyl – Cyclodisilazanes With Dichloranhydride Tereftalic Acids and α,ω – Bis (β – Carboxyethyl) Dimethylsiloxanes was presented in the paper of Lili Janiashvili, Giuli Andronikashvili, Archil Varadashvili and Mzia Gagolishvili (Janiashvili et al., 2014). Repellency and Antifeedant of Ticks Through Ethno Plant Extracts and Ivermectin on Buffalo Calves was presented in the paper of Mehmood A. Kalwar, Hakim A. Sahito, Barkat A. Kalwar, Madan Lal and Shahnawaz Fazlani (Kalwar et al., 2014). Microbiological Synthesis of 2H- and 13C-Labeled Amino Acids and Proteins With Various Levels of Isotopic Enrichment was presented in the paper of Oleg Mosin, Ignat Ignatov, Dmitry Skladnev and Vitaly Shvets (Mosin et al., 2014b). Biosynthesis of 2H-labeled Photochrome Trans-membrane Protein Bacteriorhodopsin by Halobacterium Halobacterium Halobium was presented in the paper of Oleg Mosin and Ignat Ignatov (Mosin, Ignatov, 2014).

The Thermodynamic Estimation of Forming Possibility of Cu2–xSe thin Films and Investigation of the Composition and Morphology was presented in the paper of Ekaterina A. Fedorova, Larisa N. Maskaeva and Vyacheslav F. Markov (Fedorova et al., 2014). Computer Simulation as a way to Determine the Conditions of Purposeful Synthesis of AgxPb1 – xS Solid Solutions by Hydrochemical Deposition was presented in the paper of Aleksey Y. Kirsanov, Vyacheslav F. Markov and Larisa N. Maskaeva (Kirsanov et al., 2014). Biosynthetic incorporation of deuterium-labeled aromatic amino acids - [2,3,4,5,6-2H5]phenylalanine, [3,5-2H2]tyrosine and [2,4,5,6,7-2H5]tryptophan into the molecule of transmembrane protein bacteriorhodopsin from halobacterium Halobacterium halobium E was presented in the paper of Oleg Mosin, Ignat Ignatov, Dmitry Skladnev and Vitaly Shvets (Mosin et al., 2014). Microbiological Synthesis of 2H- and 13C-Labeled Amino Acids and Proteins With Various Levels of Isotopic Enrichment was presented in the paper of Oleg Mosin, Ignat Ignatov, Dmitry Skladnev and Vitaly Shvets (Mosin et al., 2014a).

2015 year.

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The Methods of non-equilibrium Spectrum (NES) and Differential non-equilibrium Spectrum (DNES) in Studying the Interaction of Carbonaceous Mineral Shungite and Aluminosilicate Mineral Zeolite with Water was presented in the paper of Ignat Ignatov and Oleg Mosin (Ignatov, Mosin, 2015). Studying the Biosynthesis of 2H-labeled purine Ribonucleoside Inosine by a Chemoheterotrophic Bacterium Bacillus subtilis B-3157 was presented in the paper of Oleg Mosin and Ignat Ignatov (Mosin, Ignatov, 2015). A Review Fundamentals in Colors, Dyes and Pigments Chemistry was presented in the paper of H.A. Shindy (Shindy, 2015).

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Hydrochemical Deposition of Cu2S Films Using Thiocarbamide was presented in the paper of Irina A. Glukhova, Larisa N. Maskaeva, Vyatcheslav F. Markov, Stanislav S. Tulenin and Anna I. Shemyakina (Glukhova et al., 2016). The Reactions of Condensation–Dehydration Occurring in Aqueous Alkaline Solutions at Pн = 9–11 and T = 65–95 0C in the Process of Modeling of Primary Hydrosphere was presented in the paper of Ignat Ignatov and Oleg Mosin (Ignatov, Mosin, 2016).

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2017 year.

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A quantum-chemical topological analysis of the formation of the C-O bond in the 32CA reaction involving the zwitterionic type was presented in the paper of Abdelilah Benallou, Zouhair Lakbaibi, Habib El Alaoui El Abdallaoui and Hocine Garmes (Benallou et al., 2018b). A theoretical explanation of the mechanism and regio-stereoselectivity of the cycloaddition between nitronylides and electron-deficient methacrylonitrile was presented in the paper of Abdelilah Benallou, Habib El Alaoui El Abdallaoui, Hocine Garmes (Benallou et al., 2018a).

A DFT study of the mechanism, regio- and stereoselectivity of the epoxidation reaction of methyl 2-((2R,4aR)-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalen-2-yl)acrylate using m-CPBA was presented in the paper of M. El Idrissi, A. El Haib, Y. Hakmaoui, M. Zoubir, M. El Ghozlani, S. Mouatarif, N. Ourhriss and R. El Ajlaoui (El Idrissi et al., 2018). A study of the impact of pest control agents used in closed production facilities on food quality using SPME in combination with GC-MS was presented in the paper of A. Lakhili, M. Fekhaoui, A. Bellaouchou, L. Tahri, A.E. Abidi and M. El idrissi (Lakhili et al., 2018). Synthesis and visible spectral studies of new pyrazolo/oxazolemerocyanine dyes were presented in the paper of H.A. Shindy, M.A. El-Maghraby, M.M. Goma and N.A. Harb (Shindy et al., 2018).

Purification of toxic gaseous HCN emissions by gas-phase O2 oxidation: quantum chemical modeling was presented in the paper of Abdelilah Benallou, Habib El Alaoui El Abdallaoui and Hocine Garmes (Benallou et al., 2018). Extraction and phytochemical analysis of Vernonia Amygdalina (Shuwaka) leaves were reviewed in the paper of I.A.A. Ibrahim, M. Chindo, M.M. Mohammad, A.A. Faisal, H. Musa (Ibrahim et al., 2018). Analysis Phytochemical and antimicrobial activity of Ficus Plat Platyphylla stem bark extract. Extraction and phytochemical analysis of Vernonia Amygdalina (Shuwaka) leaves was presented in the paper of I.A.A. Ibrahim, M. Chindo, M.M. Mohammad, A.A. Faisal and H. Musa (Ibrahim et al., 2018a). Regioselective and stereoselective synthesis of 2,5-dichloro-2,5,9,9-tetramethyl-decahydro-benzocycloheptene via stepwise addition reactions between α-himachalene and HCl: an experimental and theoretical study was presented in the paper of N. Ourhriss, A. Zeroual, C. A. Gadhi, A. Benharref, A. Abourriche, A. Bennamara and A.El Hajbi (Ourhriss et al., 2018). Novel polyheterocyclic cyanine dyes: synthesis, photosensitization and solvent/electron correlation transitions were reviewed in the paper of H.A. Shindy (Shindy, 2018).

2019 year.

Origin of life in hot mineral water of hydrothermal springs and ponds. Effect of hydrogen and hydrogen produced. Spectral, pH and ORP analyses were presented in the paper of Ignat Ignatov (Ignatov, 2019). The results of IR spectroscopy of CortiNon+ on the development of experimental Graffiti tumor in hamsters were presented in the work of Ignat Ignatov, Reneta Toshkova, Georgi Gluhchev and Elisaveta Tzvetkova (Ignatov et al., 2019). Physicochemical and microbiological characteristics of thermal healing springs of the Burgas region were presented in the paper of Nedyalka Valcheva (Valcheva, 2019).

A theoretical study of the regioselectivity of the reaction between diethyl (trichloromethyl-1)phosphonate and triethyl phosphite using the DFT method was presented in the paper of A. Barhoumi, M.El idrissi, A. Zeroual, S. Bakkas, A. El Hajbi and A. Tounsi (Barhoumi et al., 2019). Quantum mechanical descriptors of indazole-containing derivatives using the DFT method were considered in the paper of H. Essassaoui, M. El idrissi, R. Bouhdadi, M. Echajia, A. Zeroual, A. Tounsi and M. Mbarki (Essassaoui et al., 2019). The experimental and theoretical development of the phase diagram of the ternary system Ni(NO3)2-Al(NO3)3-H2O at 20°C was presented in the work of M. Jouaiti, B. Mekkaoui, A. Barroug, M. El idrissi, R. Lbibb, L. Lâallam, A. Jouaiti (Jouaiti et al., 2019). A review of the synthesis of various classes of polyheterocyclic cyanine dyes was presented in the paper of H.A. Shindy (Shindy, 2019).

2020 year.

A molecular docking study of primaquine-favipiravir-based compounds as potential inhibitors of the main COVID-19 protease was presented in the paper of Olawale F. Akinyele, Emmanuel G. Fakola, Oluwatoba E. Oyeneyin, Omolara O. Adeboye, Ayowole O. Ayeni, Justinah S. Amoko and Temitope A. Ajayeoba (Akinyele et al., 2020). The biochemical effect of aqueous extract of Abeere leaves (Hunteria umbellata) on liver enzymes of white rats was examined in the paper of Iliyasu A.A. Ibrahim and Labaran A. Magashi (Ibrahim, Magashi, 2020). Solvatochromic and halochromic evaluation of some biheterocyclic cyanine dyes was reviewed in the paper of H.A. Shindy, M.A. El-Maghraby and F.M. Eissa (Shindy et al., 2020).

2021 year.

A mini-review of the biological potential of pyrazole and triazole derivatives was presented in the work of Mohammad Asif (Asif, 2021). Isolation and identification of bacteria from used masks at Bauchi State University, Gadau was presented in the paper of Iliyasu A. A. Ibrahim, Hafizah Sani Sulaiman, Sulaiman Maikudi and Habibu Musa (Ibrahim et al., 2021).

2022 year.

Innovations in the preliminary preparation of biological samples in chemical-toxicological studies were studied in the work of Viktor N. Bekhterev, Svetlana N. Gavrilova, Eugenia G. Neskubina and Igor N. Shipanov (Bekhterev et al., 2022).

2024 year.

A review of methods for increasing the thermal conductivity of phase-change materials was presented in the paper of Ashraf AL-Nassar and Andrei N. Makeev (AL-Nassar, Makeev, 2024). Exotic multichannel and position-sensitive high-resolution methods for physicochemical archaeometry were reviewed in the paper of Oleg V. Gradov and Andrew A. Skrynnik (Gradov, Skrynnik, 2024).

In last time the journal has also published papers that examine the biographies of chemists who have been significant for the development of science (Mamadaliev, 2022; Taran, 2023; Cherkasova, 2023; Mamadaliev, 2024).

Languages of publications.

Data on the languages of articles published in the journal “European Reviews of Chemical Research” from 2014 to 2024 are presented in Table 1.

Table 1. Data on the language distribution of publications in the journal “European Reviews of Chemical Research” from 2014 to 2024

№	Language of publication	Number of articles	Percentage of all publications
1.	English	61	80 %
2.	Russian	15	20 %
3.	Total number of all articles: 76.		

Thus, having publications in two languages at its disposal, the journal “European Reviews of Chemical Research” solves the important task of expanding the base of potential readers. The English language is focused on presenting research results at the global level, and the Russian language is focused on presenting research results at the level of the post-Soviet space, since the dominance of the Russian language here is still great.

5. Conclusion

Thus, based on the conducted review study, it can be concluded that over the entire period of the journal's existence, 76 articles on a wide range of topics have been published. The language coverage of publications is also impressive. Most of all works were published in English (80 %), which helps in the international promotion of the research results presented in them. Also, some of the articles were published in Russian (20 %), which also increases the dissemination of knowledge in the post-Soviet space.

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Integrated Spectrozonal/Multispectral Lens-Less Optical Microscopy and Scanning Electron Microscopy as Novel Combined Chemometric Instruments for Food Science

Part I. The brief history of optical and electron microscopy in food industry

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Abstract

Food samples are heterogeneous and structured media that require investigation with spatial resolution or positional sensitivity for meaningful metrological data interpretation. When analyzing such materials at a specific scale characteristic to the sample's texture, any averaged information loses its significance without structural context. This leads to overlapping peaks in analytical signals, which could be effectively separated only by introducing position-specific resolution adequate to the size of structures carrying biochemical properties and descriptors responsible for these signals. The solution lies in quantitative analytical microscopy. These methods include various spectral ranges ensuring qualitatively different measurement approaches and characterization possibilities, all crucial for comprehensive understanding of target substances' structure and composition. This article discusses the integration of multiple diagnostic tools and provides analytical reasoning supporting this approach as well as highlighting current methodological and technical limitations preventing broader implementation. It serves primarily as a background resource for potential users from the food industry seeking consultation on adopting such advanced techniques, particularly when considering their applicability to particular research objects described herein or following general technical guidelines provided.

Keywords: chemometrics, food biochemistry, optical microscopy, NMR microscopy, SEM, ESEM, ASEM, CLEM, CryoSEM, multispectral optical microscopy, history of microscopy for food chemical analysis.

1. Введение

Являясь гетерогенной и структурированной средой, пищевой образец либо аналит, как правило, требует исследования свойств с пространственным разрешением или позиционной чувствительностью. На определенном масштабе рассмотрения, специфичном для текстуры образца, любые метрологические данные теряют смысл без привязки к структуре, поскольку аддитивная информация, усредненная по множеству структурно неидентичных/химически неэквивалентных зон (ROI) является источником множества интерференций аналитических сигналов, приводящих к наложению пиков, которые были бы эффективно разделены только лишь при внедрении позиционной привязки данных с разрешением, адекватным размерам структур – носителей тех или иных биохимических свойств и биофизических дескрипторов,

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то есть источников аналитического сигнала. Решение данной проблемы рационально может реализоваться только с применением методов количественной аналитической микроскопии, причем именно того пула методов количественной аналитической микроскопии, который, в комплексе, может быть источником максимального количества аналитических сигналов (из числа целевых). Множественность используемых методов аналитической микроскопии есть, в данном случае, прежде всего, множественность пределов разрешения, соответствующих, в соответствии с критериями Рэлея (аналогичное можно показать для критерия Аббе), длинам волн, а, следовательно – спектральным диапазонам возможной аналитической метрологии, идентификации и амплитудного оценивания концентрации образца (не абсолютного лишь, но и относительного – «рациометрического», с нормированием к диапазонам спектральной чувствительности и изменением принципов расчетов при переходе к качественно-отличным по физике диапазонам – например, от UV-Vis-анализа содержания аналита к инфракрасной области для реконструкции строения аналита, идентификации функциональных групп etc.). Соответственно, максимально полное покрытие спектральных диапазонов, обеспечивающих качественно разные методы измерений и характеризации структур образца, представляется, в случае позиционно-чувствительного измерения-картирования, пререквизитом получения полного комплекса данных о всех целевых измеряемых веществах с минимальным уровнем ROI-локализованной интерференции метрологических сигналов. Задачей настоящей статьи (в аспекте обеспечения вышеуказанных требований) видится, во-первых, комплексирование нескольких методов подобной диагностики пищевого материала (как proof of concept / proof of principles) на аппаратном уровне, а, во-вторых, реализация аналитического рассмотрения, необходимого для обоснования данного подхода.

2. Обсуждение и результаты

2.1. Методы оптической и МР-микроскопии в пищевой индустрии.

Применение методов микроскопии в пищевой индустрии в технологических процессах и контрольно-надзорных – включая уровень ОТК – квалиметрических и сертификационных целях, начавшись в XIX веке, получило грандиозное развитие в середине XX века, поскольку индустриальная революция и автоматизация в пищевой промышленности интродуцировала метрологические устройства непосредственно в линии переработки, а массовый, посменный характер производства сделал необходимостью «сверять часы» при переходах смен, партий, диверсифицированных источников поставок, дублирующих конвейерных линий и режимов работы отдельных агрегатов, что, несомненно, стало давать свой вклад в гетерогенность (или статистическую невоспроизводимость) качеств продукта – источника целевых аналитов. Это обстоятельство заставило индустриальный сектор прибегать к использованию на линиях и в пищевых лабораториях (не только на этапе становления производства, но и в регулярной его фазе) серьёзных микроскопических и микро-метрологических инструментов, включающих в себя не только обычную световую микроскопию, но и весьма экзотичные для периода 1960-х гг. инструменты электронной микроскопии. Ниже приводится аналитический обзор данных тенденций, разделенный по технико-методическому принципу.

Во-первых, конечно, нужно упомянуть хрестоматийные методы прямой микроскопии, перечисленные в перечне практического руководства 1995 г. (van Laarhoven, 1995), аннотированного в журнале «Trends in Food Science and Technology» в середине 1990-х гг. (Flint, van Laarhoven, 1995). Эта номенклатура до сих пор актуальна и, если говорить о реальной промышленности, к ней мало что возможно добавить; особенно, если речь идёт о квалиметрии в условиях технической вооруженности развивающихся стран. Начиная с 1960-х гг., световая микроскопия входит в широкий обиход за гранью анализа непосредственно биоматериала – в приложениях, связанных с анализом продуктов его переработки, практически повсеместно – включая пережившую атомную бомбардировку Японию (Nakanishi et al., 1967). В 1970-е гг. в обиход пищевых оптико-микроскопических исследований интенсивно внедряется кино- и теле- микроскопия (Bell et al., 1975). К 1980-м гг. оформляются возможности квалиметрии с использованием комбинированного анализа образцов с использованием оптической и электронной (как просвечивающей, так и сканирующей) микроскопии (Fretzdorff et al., 1982). Не ранее 1990-х гг., в силу необходимости достижения определенного технического уровня, для широкого внедрения становятся доступными компьютеризованная криомикроскопия, особо – в цейтраферных (time lapse) режимах, демонстрирующих динамику процессов заморозки и разморозки (дефростинга, в том числе – в СВЧ-дефростерах, криомикроскопия в которых была реализована группой Градова) и микроскопия в условиях термо- и крио-циклирования (также разрабатывавшаяся нашей группой в середине 2010-х, в приложении к потенциальным синтетическим носителям кода). В большинстве же случаев анализ замороженных пищевых продуктов производится с выемкой их из условий охлаждения – за редким исключением (Caillet et al., 2003), не *in situ* (по аналогии с операндо-спектроскопией можно было бы назвать анализ подобных процессов *in situ* – операндо-криомикроскопией). Исключение же составляют лишь методы криоэлектронной микроскопии молокопродуктов (Ong et al., 2011). Впрочем, если говорить *sensu lato*, и ранее применялись методы электронной микроскопии пищевых продуктов с охлаждаемым столом (Gordon, Barbut, 1990), не называвшиеся CryoEM/CryoSEM и аналогичными модными аббревиатурами. Менее высокоразрешающее и вместе с тем существенно более экзотичное направление – магниторезонансная микроскопия (ЭПР и ЯМР микроскопия) в исследованиях охлаждения продуктов питания, пищевой инженерии также применялась (Serša et al., 2011).

Из специальных видов микроскопии, используемых в пищевой индустрии, инженерии, можно назвать методы поляризационной микроскопии (Swatland, 1990), иммерсионной или лазерно-микродифрактометрической иммерсионной микроскопии (Varcoe, Jones, 1983), ИК, в частности ИК-Фурье- (FTIR) микроскопии (Suutarinen et al., 1998), микроспектроскопии и микроспектрометрии комбинационного рассеяния либо, почти тождественно, рамановской микроскопии (Yang et al., 2017). Если говорить о технических возможностях визуализации, то они отличны – от двумерного сверхразрешающего сканирования в методах сканирующей ближнепольной микроскопии (Zakharia, 2009) до трёхмерной репрезентации сканирующей конфокальной микроскопии (Velinov et al., 1990; Dürrenberger et al., 2001; van de Velde et al., 2002; Ko, Gunasekaran, 2007; Sheen, 2008; Adedeji et al., 2011; Moreno, Bouchon, 2013; Moreno, Bouchon, 2013). Про магниторезонансную микроскопию говорилось выше – она не представляет интереса для повседневного пользователя, в силу сложности бэкграунда, на котором основана, и интерпретации данных (Maeda et al., 2009).

Помимо обычной микроскопии в разных диапазонах, для анализа пищевых продуктов часто используют люминесцентную или флуоресцентную микроскопию, в том числе методы иммунофлуоресцентной микроскопии (Fritz, Greaser, 1991; Parkkonen et al., 1997; Corich et al., 2004; Heilig et al., 2009). Не является исключением и рентгеновский поддиапазон, в котором с использованием синхротронного излучения детектируются неорганические нутриенты и/или поллютанты (Ohi et al., 2016). Надо сказать, что рентгеновская микроскопия достаточно существенно прогрессировала последние годы и теперь возможно регистрировать не только рентгеновские дифрактограммы пищевых продуктов, сопоставляемые с электронными либо иными микрофотографиями (Kitada et al., 1986), но и полно-информативные рентгеновские микрофотографии данных изображений на синхротронном микроскопе (Seim et al., 2015). До этого ни один промышленно выпускавшийся рентгеновский микроскоп (в том числе МИР – разработка советских специалистов, допускавший лишь микронные точности/разрешения) не достигал таких возможностей. Единственным методом, конкурирующим с рентгеновской микроскопией современного уровня в полноатомном восстановлении структуры soft matter / частично упорядоченных сред является собственно атомно-силовая микроскопия, активно и постоянно совершенствуясь внедряющаяся в технологии анализа пищевой индустрии (Kirby et al., 1995; Morris et al., 2001; Yang et al., 2007; Cardona et al., 2008; Handojo et al., 2009; Sigua et al., 2010; Sigua et al., 2010; Neethirajan et al., 2012; Phinney et al., 2017). Однако предметами её использования в пищевой индустрии, как правило, являются микроструктуры неатомного масштаба – представляет практический интерес использование технологий атомно-силовой микроскопии для определения адгезии, гидрофильности-гидрофобности / контактного угла (Handojo et al., 2009; Sigua et al., 2010), реологических параметров пищевых продуктов, сред и их прекурсоров (Morris et al., 2001; Ikeda et al., 2013), что ранее делалось с использованием стандартных вискозиметрических (и реометрических) сред с электронно-микроскопической визуализацией и визуальным «вынесением вердикта» по продукту (Hoskin, Dimick, 1980).

Электронная микроскопия также находит известное применение в пищевой индустрии – но, как правило, это не является просвечивающей электронной микроскопией, которая, по известным данным, позволяет визуализировать, а не реконструировать, атомную структуру кристаллов. Более распространена сканирующая электронная микроскопия низкого уровня увеличения и просвечивающая микроскопия цитофизиологических масштабов увеличения. Активно используется электронная микроскопия в пищевой миологии, в ультраструктурном анализе результатов термической обработки на структуру говядины (Cheng, Parrish, 1976) и других источников мясного сырья (Cassens et al., 1963; Stromer, Goll, 1967; Schaller, Powrie, 1971; Schaller, Powrie, 1972; Yang et al., 1974; Ruddick, Richards, 1975; Cheng, Parrish, 1976; Jones et al., 1976; Hägerdal, Löfqvist, 1977; Otwell, Hamann, 1979), включая моллюсков и ракообразных. Сканирующая электронная микроскопия несколько позже вошла в практику пищевой индустрии (Alli, Baker, 1981; Rosenberg et al., 1985; Zilberboim et al., 1986), однако же, она также достаточно широко используется как в мясомолочной индустрии, так и в анализе и исследованиях вегетарианских или фруторианских продуктов (Tatsumi et al., 1991; Indrani et al., 2003; Careli et al., 2009; Scussel et al., 2014). Широкое использование нашел контроль с помощью сканирующих электронных микроскопов результата ультразвуковой сонификации продуктов или микроволновой обработки (Bermúdez, Barbosa-Cánovas, 2008; Wambura et al., 2010; Bermudez-Aguirre et al., 2012). Существуют программные подходы поиска особенностей микроструктуры образцов пищевых продуктов при сканирующей электронной микроскопии (Amboni et al., 1999). Если для простых образцов – типа жировых кристаллов – можно лишь охарактеризовать геометрию (Heertje, Leunis, 1997), то для пищевых образцов с развитой поверхностью есть возможность охарактеризовать около трёх десятков дескрипторов текстуры, морфологии поверхности (Hayta, Ertop, 2018). Это переводит электронную микроскопию как метод анализа в пищевой индустрии из раздела иллюстраций в раздел метрологического её обеспечения.

2.2. Электронная микроскопия в пищевой индустрии и её нюансы.

С 1970-х гг. идёт речь о внедрении в практику не только электронно-микроскопической визуализации, но и количественной электронной микроскопии сырья и пищевых продуктов (Flint, 1977), причём количественный анализ подразумевает не только морфометрию тех или иных структур, но и привязку электронно-микроскопических изображений к их конкретным условиям получения или конкретным условиям формирования/обработки образца, особо – термической, в силу специфики большинства кулинарных процедур для подготовки образца (Lin, Ito, 1986). Несомненно, гранулометрия и калибровочный сайзинг распределений неких частиц по размерам также интересует и современного пользователя (Tan, Balasubramanian, 2017), однако на этом мы не останавливаемся, так как этому посвящены сотни статей с 2000-го г.. Более интересны рентгеновские методы структурного и спектрального микроанализа – как волнодисперсионной, так и энергодисперсионной микроспектрометрии/спектроскопии вторично-эмиссионного рентгеновского излучения, а также рентгеновской дифрактометрии (James, 2005; Dharmaraj et al., 2014; Ivanov et al., 2017). Ранее была актуальна также и задача оценки и картирования микронеоднородностей рельефа, решавшаяся количественным YMD – Y-модулированным детектированием, ныне перешедшая в компетенцию атомно-силовой микроскопии (Yang et al., 2005). Нами ранее были реализованы процедуры картирования на YMD-подобных режимах не только для морфологии, но и для распределения элементов (как микрозондовый анализ) и заряда поверхности пищевых и биомиметических продуктов.

Надо сказать, что специальные методы электронной микроскопии являются средством физико-химического качественного и количественного определения (или оценки) структур, систем, фаз, микрообъектов пищевого назначения – вплоть до тонких soft matter – частично упорядоченных сред (например, продуктов инкапсуляции (Kaláb, Larocque, 1996), эмульсий (Borchert et al., 1967), белковых мицелл (Karlsson et al., 2007), белок-содержащих оболочек и мембран (Tung, Richards, 1972)). С помощью электронной микроскопии пищевых объектов и сред можно устанавливать: изменения продуктов и состояние дрожжевых культур хлебного производства при воздействии давления при обработке, хранении и изготовлении отличных видов изделий (Bang, Swanson, 2008); потерю воды при дегидратации, при эмульсификации (Hsu et al., 1980); причины возникновения жесткости мясных продуктов при окоченении тех или иных мышечных структур после смерти животного (Varriano-Marston et al., 1978) [но это зависит ещё и от техники пробоподготовки образцов, если речь не идет о SEM-микроскопии во влажной среде или же в камерах в специализированных газо-паровых атмосферах, ESEM – Environmental Scanning Electron Microscopy или, аналогично ей – ETEM (McDonough et al., 1993; James, 2009; James, 2009; James, Yang, 2011; James, Yang, 2011; Gradov, Gradova, 2016)] или же явления агрегации белков (Badii et al., 2004). Эффекты заморозки среды, затронутые в последней цитированной работе, можно воспроизводить in situ – непосредственно в SEM и TEM на охлаждающем столе (Kaláb, Larocque, 1996). Быстрая заморозка является достаточно весомой альтернативой как низковольтной (LVEM) либо сверхнизковольтной ESEM (James, Smith, 2009), так и классическим методам контрастирования и фиксации, деформирующим микроструктуру (или ультраструктуру – если речь о клеточных системах) пищевого образца (Lee, Morr, 1993). Однако для некоторых объектов может понадобиться вакуумный нагрев, а не вакуумное охлаждение, или атмосферный нагрев, а не «естественная» газовая атмосфера ESEM; пример – крахмал, изменения которого при данных типах нагрева в воде и маслах *in situ* могут быть проанализированы вакуумной микроскопией с нагреваемым столом (Ovalle et al., 2013), ибо обычные микроскопические методы наблюдения изменений крахмала здесь бессильны (Freke, 1971). Аналогичное утверждение может касаться фрагментов мяса, особо – говядины, которые логично анализировать не просто «электронной термомикроскопией», а коррелированной световой и электронной термомикроскопии (CLETM – Correlated Light and Electron ThermoMicroscopy), в частности – фазово-контрастной её модификацией, поскольку прецеденты успешного применения и сопоставления микрофото сканирующей электронной микроскопии и фазового контраста для фрагментов говядины и свинины имели место уже в 70-е (Hearne et al., 1978). Динамические измерения *in situ* в данном случае оправданы даже в столь экзотических случаях, так как данные о динамике химических или постбиологических процессов в пищевых аналитах могут быть источником для построения макрокинетических моделей со структурной интерпретацией (хотя это не будет «структурной макрокинетикой» в её стандартном значении). Нужно учитывать, однако, что «дух времени» в данном аспекте не всегда себя оправдывает (Yada et al., 1995). В отличие от световой микроскопии, которая, в частности, допускает многодневный мониторинг биодеградации пищевых продуктов или их прекурсоров (Yau, Murphy, 2001), в большинстве электронных микроскопов возможно – без ущерба инфраструктуре и образцу – проводить быстрые «операндо-микроскопические» измерения контрольного плана, но не многосуточный мониторинг.

3. Заключение

Поэтому нужна техника, которая бы позволила это реализовывать. На роль такой техники хорошо подходит безлинзовая многоцветная (мультиспектральная и гиперспектральная) микроскопия, о которой пойдёт речь в ближайших статьях данного цикла, в том числе безлинзовая микроскопия в «субкамерах» электронных микроскопов на нагреваемых столах и столах-термоциклерах (например, с элементами Пельтье).

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Корреляционная спектрозональная/мультиспектральная безлинзовая оптическая микроскопия и сканирующая электронная микроскопия как новый комбинированный инструментарий хемометрики для пищевой биохимии

Часть I. Краткая история применения оптической и электронной микроскопии в пищевой промышленности

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Аннотация. Пищевые образцы представляют собой гетерогенные и структурированные среды, требующие исследования с пространственным разрешением или позиционной чувствительностью для осмысленной метрологической интерпретации данных. При анализе таких материалов в масштабе, характерном для текстуры образца, любая усредненная информация теряет свою значимость без структурного контекста. Это приводит к перекрывающимся пикам в аналитических сигналах, которые можно эффективно разделить только путем введения позиционно-специфического разрешения, адекватного размеру структур, несущих биохимические свойства и дескрипторы, ответственные за эти сигналы. Решение заключается в количественных методах аналитической микроскопии. Эти методы включают различные спектральные диапазоны, обеспечивающие качественно различные подходы к измерениям и возможности

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характеризации, что имеет решающее значение для всестороннего понимания структуры и состава целевых веществ. В данной статье обсуждается интеграция множества диагностических инструментов, а также освещаются существующие методологические и технические ограничения, препятствующие более широкому внедрению. Она служит в первую очередь справочным ресурсом для потенциальных пользователей из пищевой промышленности, обращающихся за консультацией по внедрению таких передовых методов, особенно при рассмотрении их применимости к конкретным исследовательским объектам, описанным в данной статье.

Ключевые слова: хемометрика, пищевая биохимия, оптическая микроскопия, ЯМР-микроскопия, СЭМ, ESEM, ASEM, CLEM, КриоСЭМ, мультиспектральная оптическая микроскопия, история микроскопии для химического анализа пищевых продуктов.