NATIONAL ACADEMY OF SCIENCES OF UKRAINE
Palladin Institute of Biochemistry

BIOTECHNOLOGIA ACTA

Vol. 16, No 1, 2023

BIMONTHLY

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According to the resolution of the Presidium of the National Academy of Sciences of Ukraine from 27.05.2009 №1-05 / 2 as amended on 25.04.2013 number 463 Biotechnologia Acta has been included in High Attestation Certification Commission list of Ukraine for publishing dissertations on specialties "Biology" and "Technology".

Certificate of registration of print media КВ series №19650-9450ПР on 01.30.2013

Literary editor — H. **Shevchenko**; Computer-aided makeup — **O. Melezhyk**
Authorized for printing 2023/02/28, Format — 210×297. Paper 115 g/m^2. Gaqrn. SchoolBookC. Print — digital. Sheets 10.8. An edition of 100 copies. Order 6.6. Make-up page is done in Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine. Print — **O. Moskalenko** FOP

NATIONAL ACADEMY OF SCIENCES OF UKRAINE
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BIOTECHNOLOGIA ACTA

Scientific journal

Bimonthly

Vol. 16, No 1, 2023

REVIEWS

EXPERIMENTAL ARTICLES

НАЦІОНАЛЬНА АКАДЕМІЯ НАУК УКРАЇНИ
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BIOTECHNOLOGIA ACTA

Науковий журнал

Том 16, № 1, 2022

ОГЛЯДИ

ЕКСПЕРИМЕНТАЛЬНІ СТАТТІ

UDC 577.1 https://doi.org/10.15407/biotech16.01.005

COMBINED NANOCHEMOTHERAPY USING DOXORUBICIN AND CURCUMIN AS AN EXAMPLE

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Received 2022/12/02
Revised 2022/11/09
Accepted 2023/02/28

The *aim* of the work was to review literature data on combined nanochemotherapy using the example of two drugs doxorubicin and curcumin. Special attention was paid to the use of substances with synergistic properties in one nanoparticle, capable to penetrate into living cell.

The method of combined chemotherapy of nanopreparations improves processing efficiency. The technique of using nanocontainers with synergistic drugs in combination with ligands reduces the side effects of chemotherapy drugs.

Results. Literature data indicate that the use of nanopreparations contributes the rapid creation and use of synergistic combinations that were purposefully delivered to target cells, reducing dosage due to precise targeting. A promising direction of nanomedicine is the creation of multifunctional nanomaterials based on several active drugs having synergistic properties, with the simultaneous use of their enhancers and the strategy of active targeting. These structures enabled targeted and controlled penetration of medicinal compounds into the localization of pathological processes, reducing drugs toxicity for normal cells.

Conclusions. Combined chemotherapy using polymers and nanoparticles with ligands, in which synergistic drugs are included, ensures to reduce side effects and doses of chemotherapy drugs, and helps to overcome multiple drug resistance as well.

Key words: combined nanochemotherapy; doxorubicin; curcumin; synergism; active targeting.

Combined chemotherapy

The current advanced strategy in chemotherapy is aimed at combination therapy, which uses targeted delivery systems with a large number of drugs [1–4]. Therapy with one drug is limited due to the heterogeneity of cancer cells [5], therefore, today, combined chemotherapy has become a standard treatment scheme for cancer patients [2, 5] and is expedient and promising [1, 3, 4].

Combined therapy is becoming an important strategy for a better long-term prognosis with reduced side effects [6, 7]. The combination of chemotherapy drugs allows oncologists to use drugs in lower doses, reducing cytotoxicity [5] and increasing the effectiveness of treatment [1]. Most often, a combination of two agents is used, which leads to an improvement in therapeutic effectiveness and a reduction in the dosage of each agent and the achievement of several goals [1, 5].

Today, combined chemotherapy with a synergistic effect is a promising treatment scheme for cancer patients [5, 8, 9]. The combination of drugs that have different properties, such as solubility, usually requires the use of multiple carriers or solvents, limiting the probability of their simultaneous delivery to target cells. [5].

Consider a combination therapy using the example of two drugs with different properties, water-soluble doxorubicin (DOX) and hydrophobic curcumin (CUR). It is known that the combination of DOX and CUR

contributes to the treatment of several types of cancer [3, 5, 10–16].

Joint delivery of biomolecules in combination therapy is widely used in clinical diagnosis and treatment [17]. The combination of anticancer agents with chemosensitizers targets different signaling pathways in cancer cells, reduces side effects, and helps overcome multidrug resistance (MDR) [6].

In practice, combined chemotherapy increases the effectiveness of drugs and leads to improved survival compared to monotherapy [1]. A growing number of facts testify, that simultaneous treatment with chemotherapy and chemopreventive agents with antioxidant activity can increase the effectiveness of chemotherapy drugs [18]. It is recognized that the right combinations of drugs improve target selectivity and suppress the development of MDR [9].

Modern drug carriers based on nanotechnology are aimed at enhancing the effectiveness of the best properties of drugs [19]. Application of the recipe of formulations based on nanotechnology is aimed at reducing the dose due to more accurate targeting of drugs, which additionally increases the safety of drugs by minimizing off-target toxicity [19].

Nanoparticle-based drug delivery systems offer opportunities for the development of highly effective targeted therapeutics with improved half-life, bioavailability, biodistribution, and are indispensable for maintaining synergistic drug ratios in combination therapy [1].

For joint simultaneous delivery of chemotherapeutic drugs with different physicochemical properties, multifunctional carriers are used [5]. They can target several cancer hallmarks, which improves the efficacy of cancer treatment [20], complementing and extending the effects on tumors [21, 22].

Nanomaterials enhance the immunostimulating effect of chemopreparations due to synergistic action [2] and maintain an optimized synergistic ratio of drugs in one carrier until intracellular uptake [1].

Significant efficiency is ensured by joint simultaneous delivery of drugs by nanoparticles (NPs) into one cell [1, 23, 24], which leads to the use of chemotherapy drugs in smaller doses, reducing systemic toxicity and increasing therapeutic efficacy [9].

Synergism in combined nanopreparations

In combined chemotherapy, synergistic mechanisms are used, in which the synergistic [2, 8, 14] effect of two (or more) agents is directed at different pathways of the disease [1]. Combination chemotherapy is theoretically advantageous due to the synergistic effect of drugs with simultaneous suppression of MDR. Nanoparticle-assisted chemotherapy delivery, with multiple loaded chemotherapeutic agents, is a promising approach for the effective treatment of various cancers. NP with a combination of drugs simultaneously enhance therapeutic effects and reduce side effects [25]. NPs as means of drug delivery have promising possibilities in targeted and combination therapy [1]. For example, NPs with a gold surface with a structure stabilized by glutathione have the potential to carry various drugs. They are promising drug delivery systems to overcome MDR resistance, which is the main cause of ineffective chemotherapy [26].

The association of herbal products and anticancer drugs may become a new and highly effective therapeutic strategy for better cancer management and treatment. The identification of synergistic combinations of both synthetic and phytochemical substances is clinically important because they can be quickly transferred from the laboratory to practical use [27]. Future experiments should focus on enhancing the delivery of phytochemicals using their various synergistic combinations and studying the effects of *in vitro* and *in vivo* models [28]. It is necessary to investigate the mechanisms of synergistic action of several compounds with the aim of elucidating unique molecular mechanisms, that contribute to increasing the effectiveness of these phytochemicals when they are used in combination [29]. For example, indole-3 carbinol and resveratrol have both similar and unique molecular targeting profiles and together show synergistic antiproliferative effects [30]. The results indicate that gallic acid in combination with CUR synergistically increased the apoptosis of MDA-MB-231 breast cancer cells, reducing the amount of glutathione, increasing reactive oxygen species (ROS), carry out mitochondrial dysfunction [31]. The phytocompound ellagic acid showed chemopreventive and antitumor properties [27]. One of the candidates for effective phytochemicals in their combined use is curcumin, which is a powerful chemosensitizer and can cause an additive or synergistic effect with chemotherapeutic drugs against various cancer cell lines [32]. A study demonstrated that the potential antitumor activity of such phytochemicals as curcumin and ellagic

acid increases synergistically when they are combined, leading to apoptotic death of cancer cells [27].

The use of a combination of doxorubicin and curcumin is facilitated by a synergistic effect, thanks to which it is possible to eliminate the harmful effects of a greater concentration of doxorubicin [11, 33]. For example, improved co-delivery of DOX and CUR using nanocarriers showed synergistic antitumor efficacy against liver tumor [6].

Polymer therapy

Polymer therapy covers a variety of complex multicomponent high molecular systems with the presence of a rationally designed covalent bond between a water-soluble polymer carrier and bioactive molecules [34–36]. A DOX-curcumin composite nanoparticle was developed in which doxorubicin was covalently grafted to the carboxylic acid residue of the NVA622 polymer. It overcomes DOX chemoresistance and reduces DOX-induced multiple myeloma, acute leukemia, prostate cancer, ovarian cancer, and cardiotoxicity [37]. In order to overcome resistance to DOX, a composite nanoparticle DOX-curcumin was synthesized from covalently bound doxorubicin to the surface of the nanoparticle and curcumin, which demonstrated a complete absence of cardiac toxicity [38]. Curcumin improved the safety profile of DOX by reducing DOX-induced intracellular oxidative stress as indicated by total glutathione levels and glutathione peroxidase activity in heart tissue [38].

The well-known method of PEGylation (PEG) is the creation of bioconjugates using poly (ethylene glycol) (PEG) with proteins, peptides, oligonucleotides, drugs and NPs for nanomedicine, with the aim of extending the circulation time of drugs in the blood and increasing the effectiveness of drugs [1, 39]. However, treating patients with PEGylation drugs can lead to the formation of anti-PEG antibodies. Therefore, the development of alternative polymers to replace PEG is needed [40]. In other cases, it is necessary to have polymers that capture, solubilize and control the release of the drug without resorting to chemical conjugation [36], such as Pluronic® micelles [34, 41, 42], poly(lactic acid) (PLG), poly(lactic co-glycolic acid) (PLGA) [43], chitin [44, 45], chitosan [34, 35, 42]. For example, a multifunctional co-delivery system that co-encapsulates hydrophobic chemotherapeutic drugs (curcumin) and hydrophilic therapeutic drugs (Survivin shRNA genes) in polymer nanoparticles (PLGA polymer and conjugated triblock polymer) is a promising strategy for clinical application in cancer therapy [46]. Co-delivery of curcumin and Survivin shRNA increases tumor penetration and promotes synergistic inhibition (suppression) of SKOV-3 and Hela cells. The synergistic antitumor effect includes inhibition of tumor cell proliferation, induction of cell apoptosis, and activation of caspase-3 pathways [46]. For some drugs, it is desirable to have a high degree of homogeneity, which gives predictable conformations in solution and increased ability to load drugs [36].

Biodegradable polymers with a high molecular weight include: polypeptides, dextrins, polysialic acid, polyacetals [36], PLG, PLGA [43], chitosan [34, 35], PEG micelles [47], alginate [48], albumin [49–53], pluronic micelles (Pluronic®) [34, 41, 42, 49]. Recently, polyglutamic acid (PGA), which has a high potential among synthetic polypeptides, has been used in nanomedicine [36]. The use of platforms based on high molecular weight polymers is important for the treatment of diseases with chronic administration, such as neurological disorders or tissue regeneration [36].

PEG micelles have shown some ability to deliver both DOX and CUR to DOX-resistant A549/Adr cells and synergistically reverse their DOX resistance [54]. *In vivo* studies have confirmed that micelles have the ability to increase DOX or CUR plasma concentrations and prolong their respective circulation in the bloodstream [54]. PEG-stabilized Dox NPs also exhibit long blood circulation time, good biocompatibility and stability, fast release in acidic environment, and high accumulation in tumors. Compared with free Dox, such Dox*NPs dramatically increase the antitumor therapeutic efficiency in inhibiting the growth of tumor cells [55]. They can be combined with other anticancer drugs for the purpose of synergistic chemotherapy in the treatment of MDR cancer [55].

The use of nanoplatforms to transport Dox reduces its side effects during breast cancer treatment. In many cases, polymers increase the cytotoxicity of the drug and reduce the amount of drug needed to achieve a cytotoxic effect [42]. DOX and the p53 gene were loaded into the cyclodextrin polymer cavity to form a combined nanoparticle with greater drug efficacy against MCF-7 cancer cells. The synergistic effect of the drug and the gene is achieved by reducing the dose of the drug due to the high efficiency of

the combined nanoparticle and suppression of the MDR mechanism in cancer cells [24]. A bilayer phospholipid liposome coated with polyelectrolyte (poly(4-sodium styrenesulfonate) (PSS)) encapsulated with CUR and DOX demonstrated enhanced solubility of CUR, excellent biocompatibility, and commendable cytotoxic potential of combined chemopreparations [23].

Chitin

A pH-sensitive chitin-poly(caprolactone)-Dox nanogel composite system was developed against non-small cell lung cancer. Cellular internalization of nanogel systems was confirmed by fluorescence microscopy. Chitin-poly(caprolactone)-Dox particles showed dose-dependent cytotoxicity to A549 cancer cells. The results of *in vitro* analysis confirmed the compatibility of NPs with blood [44]. Chitin and Cur do not dissolve in water, but the gel NPs developed on their basis form a very good and stable dispersion in water with the size of spherical particles in the range of 70–80 nm [45]. Histopathological studies of pig skin samples treated with NP* Chitin* Cur showed loosening of the stratum corneum of the epidermis, through which the NPs penetrated without visible signs of inflammation. These results suggest that the designed gel NPs can be proposed for the treatment of skin melanoma [45].

Chitosan

Polymeric nanoparticles are an ideal delivery system. Modification of the surface of NPs, a biocompatible polymer of chitosan, with various macromolecules has a huge potential to improve the bioavailability and circulation time of the native drug in the blood [39]. Chitosan is used both independently and as a coating of NPs [34]. For example, PLGA NPs, grafted with chitosan and loaded with Cur, were used for lung delivery for sustained drug release using a controlled polymer architecture [56], and other PLGA NPs loaded with curcumin and surface-modified chitosan were used to improve the therapeutic efficacy of curcumin [39]. PLGA nanoparticles coated with chitosan (CS) and loaded with harmala alkaloid-rich fraction (HARF) are a promising antibacterial drug against Staphylococcus aureus and Escherichia coli for wound healing [57]. The three components of the developed nanopreparation (PLGA, chitosan, and HARF) have synergistic antibacterial and wound-healing properties for the treatment of infected wounds and were found to be biocompatible during testing on human skin fibroblasts [57].

Several studies have shown that the stability of Pluronic® micelles can be significantly improved after being coated with a chitosan layer [58, 59]. The use of chitosan provides numerous advantages due to its biodegradability, biocompatibility, ease of technical application, versatility and low toxicity. Chitosan has no immunogenicity, is not carcinogenic, and also has antibacterial properties [42].

DOX micelles based on Pluronic®

The process of self-assembly is an interesting tool [36]. In recent decades, Pluronic® [49, 60] has been developed to prepare micelles that can be modified [34, 41, 42]. It is known that micelles formed by pluronic triblock copolymers are a promising class of drug delivery agents [49].

Pluronic® micelles have attracted attention due to their low toxicity. Their molecules degrade in the biological environment [61], and due to the hydrophobicity of the core and steric factors, they have the ability to encapsulate hydrophobic agents [49, 42]. Pluronics L61, P85, P105, PF127 [34, 49, 62, 63], or P85 [42] and F68 [41, 49, 62] are most often used in micelles.

Pluronic® micelles cause a decrease in mitochondrial membrane potential and thus deplete ATP in tumor cells [34, 63]. Pluronic® 85 (P85) has been shown to inhibit P-glycoprotein (P-gp) activity and induce intracellular ATP depletion in MDR cells. Its action causes a lack of energy necessary for the work of transport proteins such as P-gp and for other protective mechanisms. The use of P85 polymer prevents the development of MDR in cells exposed to Dox [42].

The interaction of the drugs gemcitabine, cytarabine and hydroxyurea released from micellar media Pluronic® F68 and F127 with serum albumin, which was chosen as a model protein, was analyzed [49]. The conformational changes of BSA during the interaction between drugs in the presence of pluronic polymers were studied. All drugs showed improved distribution in F127 micelles and drug protein binding was shown to be enhanced when co-delivered with pluronic micelles. The results indicate that BSA retains its conformation when interacting with F127 or F68 micelles carrying gemcitabine, cytarabine, and hydroxyurea and that they do not have any negative effect on BSA protein stability [49].

NPs made using nanodiamonds (ND) are useful components for research in nanomedicine due to their relatively small size and chemical inertness. They have the possibility of flexible surface modification, which in general makes them positive elements for various biological applications [41, 64, 65]. *In vitro* studies have shown that ND-Dox + Pluronic® F 68 conjugates have slow and sustained drug release characteristics and tremendous cytotoxic potential against the MCF-7 breast cancer cell line. Pluronic®-coated ND conjugates are a promising and effective nanoplatform for anticancer drug delivery [41].

A mixed micelle with Pluronic® L61 and F127 polymers was used as a Dox delivery system in the preparation SP1049C, Supratek Pharma, Canada [62]. Double micelles are easy to fabricate, have high loading capacity, and co-deliver hydrophilic and hydrophobic components. In order to eliminate MDR, a double micelle of Pluronic® P105-Dox conjugate and paclitaxel conjugate with Pluronic® F127 was successfully developed, which had an antitumor synergistic effect against MCF-7/ADR cancer cells [34, 63].

Curcumin and doxorubicin

DOX is one of the important chemotherapeutic anticancer agents [3], which is widely used [11] but has limited therapeutic efficacy for cancer treatment [3]. DOX is a non-selective cytotoxic drug and has many side effects [11]. Clinical use of DOX is often associated with severe side effects, namely hepatotoxicity [66, 67], nephrotoxicity [10, 35, 47, 48, 66–68].] and dose-dependent cardiotoxicity [28, 54, 55, 66, 67]. Chemotherapy also causes damage to normal tissues of the bone marrow, gastrointestinal tract, neurons and auditory tissues, etc. [67]. DOX chemotherapy has been reported to induce inflammation, which is associated with DOX disruption of the intestinal flora, leading to the release and accumulation of endotoxins. They lead to systemic inflammation and damage to several organs [28]. Chemotherapy-induced cardiotoxicity includes oxidative stress, mitochondrial damage, altered calcium flux, activation of proapoptotic signaling cascades [67], and inflammation [10]. It is believed that the most debilitating consequences for organ tissues, especially the heart, occur as a result of ROS induction and a high cumulative dose of DOX [69].

Curcumin is an antioxidant, an anti-inflammatory agent [70], an inhibitor of amyloid fibrillation, a powerful anti-carcinogenic and even anti-metastatic agent. The potential of curcumin is recognized and its cytotoxic properties against many cancer cell lines are appreciated [71]. CUR helps in the treatment of oxidative and inflammatory conditions, metabolic syndrome, arthritis, anxiety and hyperlipidemia [72], and is involved in the inhibition of various growth pathways and pro-invasive signaling pathways [73]. Growing evidence suggests that CUR can prevent carcinogenesis [74], sensitize cancer cells to chemotherapy, and protect normal cells from chemotherapy-induced damage [67, 69]. CUR is known to have a preventive effect against chemotherapy-induced toxicity: cardiotoxicity [75], gastrointestinal toxicity, hepatotoxicity, nephrotoxicity, neurotoxicity, ototoxicity, and genotoxicity [67]. DOX-induced renal toxicity is closely related to oxidative stress, inflammation, apoptosis, and oxidative DNA damage [70]. Curcumin has protective efficacy against DOX-induced nephrotoxicity due to attenuation of oxidative stress, inflammation, and oxidative DNA damage. [70].

The ability of CUR to absorb free radicals is used to reduce toxicity during DOX chemotherapy [69]. Low-dose curcumin reduces the effective dose of doxorubicin and reduces its systemic toxicity [76]. It is able to reduce the toxicity of DOX on the heart and liver [13, 67], kidneys, brain, and reproductive organs, affecting the balance between the autophagy and apoptosis systems, reducing damage to energy production in the mitochondria of these organs [69]. The use of NPs significantly increases the bioavailability of CUR and its therapeutic effects [77, 78], which reduce DOX-mediated cardiotoxicity [10].

It has been shown in scientific works that binary preparations of DOX and CUR exhibit stronger antitumor activity *in vitro* and *in vivo* than DOX or CUR at the same concentrations [3, 5, 11, 15, 16]. Curcumin pretreatment of drug-resistant cancer cells restored their sensitivity to doxorubicin [76]. A synergistic effect between DOX and CUR in a nanosystem *in vitro* and *in vivo* has been shown, which enhances DOX-induced apoptosis in the endogenous mitochondrial pathway and may involve other apoptotic mechanisms [11].

The study of the improved effect of nanopreparations is aimed at various aspects of the properties of NPs, their interaction with target cells and the organism as a whole. CUR prevents oxidative stress, inflammation, DNA fragmentation and apoptosis, but co-administration of CUR and DOX enhances

the induction of apoptosis in cancer cells [10], and the apoptosis rates in cancer cells become significantly greater than CUR and DOX alone [3].

The efficacy of a pair of DOX and CUR drugs against various cancers has been demonstrated, namely:

1) against invasive B-cell lymphoma both *in vivo* and *in vitro* [11], where CUR negatively affects the metastasis of cancer cells, contributing to an increase in the effectiveness of DOX [10];

2) as a promising method for the treatment of liver cancer [13], when polymeric NPs or a specially designed micelle, which had an average diameter of approximately 110 nm, were used for joint delivery of DOX and CUR to hepatoma cells [15]. Encapsulation of the pro-apoptotic drug Dox and the anti-angiogenic agent Cur in pH-sensitive NPs provides a strategy for the effective treatment of human hepatocellular carcinoma in a synergistic manner [22].

3) the DOX — CUR pair significantly reduces the viability of formed tumor spheroids, migration and invasion in gastric adenocarcinoma (AGS) model cells [3].

4) polymeric micelles, for joint delivery of DOX–CUR, to improve antitumor efficacy in breast carcinoma [5]. Studying the molecular processes that stimulate the therapeutic effect of anticancer drugs, it was shown that the simultaneous administration of the drug from albumin NPs loaded with CUR-DOX led to greater intracellular accumulation of DOX and the destruction of MCF-7 breast cancer cells [12].

5) micellar joint delivery of DOX and CUR synergistically enhances the antitumor effect *in vivo* on spontaneous lung metastases formed by a 4T1 breast tumor [5].

6) showed the combinatorial effect of CUR and silibinin in sensitization of high-risk neuroblastoma cells to the chemotherapeutic drug DOX both *in vitro* and *in vivo* [73].

7) demonstrated the effectiveness of using CUR in combination with DOX to improve survival and improve the quality of life of patients with neuroblastoma [73].

The anticancer use of curcumin remains limited due to its low solubility in water. To increase the insufficient bioavailability of CUR, the inclusion of curcumin in the middle of the emulsome core is used, which allows it to reach its effective concentration inside the cell *in vitro* [79].

Due to the low bioavailability of CUR [80], the following nanomaterials were used for joint entry of DOX and CUR into the body: inorganic nanostructures, polymeric NPs, liposomes, micelles, nanogels [10], polydopamine [2], and albumin [14]. Small cross-linked cyclodextrin nanoparticles can function as a promising carrier for curcumin and protect curcumin against photodegradation [71].

The loading of several different drugs on one carrier allows the simultaneous delivery of these medicinal compounds, which leads to a synergistic effect and a general enhancement of anticancer activity [3, 5, 10, 16, 81], increasing the effectiveness of DOX chemotherapy [12, 73]. NPs are able to maintain an optimized synergistic ratio of drugs in one carrier until the moment of intracellular absorption by the target cancer cell [1]. Binary drugs show stronger antitumor activity than the delivery of a separate drug at the same concentrations and makes it possible to use such a pair with the addition of other antitumor drugs [11].

Co-conjugated DOX and CUR to zwitterionic polymer micelles have synergistically enhanced efficacy and the strongest cytotoxicity against drug-resistant MCF-7/Adr tumor cells [16]. Encapsulation of DOX in long-circulating liposomes demonstrated the antitumor efficacy of DOX, which could be significantly enhanced after its co-encapsulation with curcumin (CUR) compared to liposomal DOX [82].

Confocal laser scanning microscopy results indicated that curcumin- and doxorubicin-encapsulated albumin nanoparticles had synergistic cytotoxicity to B16-F10 cells and gradually released the drug over 24 h without a burst effect [83]. Co-loaded micelles of DOX and CUR should be monodisperse with small particle size, with high encapsulation efficiency and delayed release, enhanced uptake by tumor cells [5]. The joint encapsulation of DOX and CUR in micelles, which is carried out using a simple self-assembly procedure, even in the absence of organic solvents and surfactants [5, 14], is economically feasible.

To achieve combined therapy, biodegradable micelles (poly(ethylene glycol)-poly(3-caprolactone) (mPEG-PCL)) are used as a system for the joint delivery of hydrophilic DOX and hydrophobic CUR. Co-encapsulation of DOX and CUR in mPEG-PCL micelles was performed using a self-assembly procedure [5]. NPs synergistically enhance cytotoxic activity and apoptotic effects on breast tumors, both *in vitro* and *in vivo*, which is primarily due to increased cellular uptake of DOX and CUR [5].

Simultaneous administration of DOX and CUR using DOX/CUR-NPs on HepG2 cells *in vitro* and *in vivo* showed a synergistic effect of DOX/CUR-NP compared to DOX-NP and free DOX on the inhibition of liver cancer cells [6]. Also, the synergistic effect of simultaneous delivery of DOX and CUR was enhanced using a pH-sensitive nanocarrier. Such a delivery system helps realize a promising combination strategy for cancer treatment [22].

The selection of the best nanocarriers is based on the improvement of interaction with target cells, the accuracy and speed of penetration into the cells to the required organelles, the improvement of stability and the slower release of drugs over time. The developed polymer high molecular weight nanomaterial mPEG-b-P (Glu-co-Phe) loaded with DOX and CUR has high anti-lymphoma effect and low toxicity. These NPs increase the ability of drugs to penetrate the cell in a targeted manner, increasing their delivery to the cell nucleus [11].

Many studies have used chemosensitizers to increase the sensitivity of tumor cells to chemotherapeutic drugs. CUR is a good sensitizer that can regulate MDR protein expression and inhibit cancer cell proliferation [84]. CUR is able to inhibit ATP-binding cassette drug transporters (ABC), increasing the effectiveness of DOX chemotherapy [10, 69]. Once in the cytosol, CUR blocks the transport of DOX from cells by inhibiting the expression of P-gp [12]. The results proved that various [12, 16, 69, 84] micelles are promising means for co-delivery of drugs to fight against MDR [16]. If there is a decrease in the level of P-gp proteins, this indicates that the multidrug acts inversely to MDR [13].

Despite some improvements in the drug delivery system, the placement of combined chemotherapy drugs in a hybrid nanostructure remains a problem [23]. Optimizing the delivery system targeting the tumor microenvironment can be of great clinical importance [12]. The use of nanocarrier-based combination therapy requires considerable effort to study and confirm the benefits of synergistic effects [9] and fluorescence microscopy is often involved in the work [85]. Nanomedicine pays special attention to molecular imaging [4, 5, 8, 12, 23, 71, 83–91], and fluorescent probes [89] are widely used for diagnosis and treatment [55]. The intracellular distribution of nanoparticles and chemopreparations directly in different parts of the cell is visualized using images of confocal fluorescence laser scanning microscopy [12, 23]. The use of fluorescent dyes accelerates the study of drug transport processes by nanoparticles [12, 23, 87], the degree of synergistic cytotoxicity of chemopreparations is determined [30, 91]. Both fluorescence microscopy [88] and flow cytometry [30, 88, 92] are used to monitor apoptosis. Real-time fluorescence microscopic observation of cells [90] is a valuable tool for the creation of antitumor nanodrugs [71]. Thanks to fluorescence, the processes occurring with nanopreparations *in vivo* and *in vitro* [4, 5, 90] are visualized, their effectiveness is investigated [30, 83], and the movement, localization, and retention of nanopreparations are studied [90].

Multifunctional nanocarriers for pair of DOX and CUR delivery

Modern works are aimed at reducing the dose of cytotoxic drugs necessary for chemotherapeutic activity [86]. Selective delivery of DOX to tumors through the use of nanoscale carriers represents an attractive approach to address limitations in cancer therapy [42, 47]. MDR greatly inhibits the antitumor effect of DOX and leads to chemotherapy failure [34]. Therapeutic efficacy during chemotherapeutic treatment of breast cancer is significantly complicated by the emergence of MDR, severe cellular toxicity, and poor targeting of chemotherapeutic drugs [21].

Drug efflux and anti-apoptotic processes are the two most common mechanisms in cancer cells leading to MDR [84]. The decrease in their sensitivity to DOX is explained by the loss of drug accumulation in cells, the reduction of DNA damage and the attenuation of apoptosis [69]. Studies show that P-glycoprotein (P-gp) is involved in DOX resistance [10], and resistance to chemotherapy mainly develops through the activity of transporters that reduce the amount of DOX in the cell [69]. It has been shown that joint administration of synergistic drugs DOX and CUR reverses MDR [21].

The method of combining two or more therapeutic agents has great potential [55], for example, the joint use of CUR and DOX in the form of micelles significantly promotes not only their intracellular absorption, but also leads to greater efficiency in suppressing MDR [10].

Multifunctional nanocarriers for the DOX–CUR pair are constantly being optimized. On the way to creating promising and effective carriers for nanobiotechnology, it is necessary to use biodegradable micelles [5] as a co-delivery system for loading hydrophilic DOX

and hydrophobic CUR in order to achieve improved combined chemotherapy [5, 12, 15] and greater selective targeting and suppression of chemoresistance [12].

A polymer micelle was created using an amphiphilic copolymer linked with polyethylene glycol and d-tocopheryl PEG1000 succinate. These micelles were used to deliver DOX and CUR to reduce MDR in A549/Adr lung cancer cells, which enhanced the therapeutic efficacy of DOX [54].

Multifunctional micelles are an active strategy for delivery to cancer cells and attenuation of MDR. DOX is loaded into micelles by physical encapsulation or chemical bonding. The construction of «smart» polymer micelles sensitive to pH with the help of a hydrazone bond, which is cleaved in an acidic environment, was used for the joint delivery of DOX and the P-gp inhibitor Disulfiram [34].

There are certain limitations when using NPs, namely physiological barriers in tumor tissues and unwanted interactions with normal tissues. The use of multifunctional nanosystems requires that they have the smallest dimensions for greater efficiency [93], such as, for example, the use of gold NPs [26, 93], nanodiamonds [41, 64, 65], micelles [47].

Further research in nanomedicine is aimed at treating various types of cancer and creating improved carriers for drug combinations. The ability of CUR to suppress tumor growth is used in new treatment regimens and drug delivery systems to improve the effectiveness of chemotherapy [69]. Studying the movement of DOX, its localization and retention is necessary to further understand the mechanisms of toxicity and resistance to DOX, in order to establish a better treatment protocol in clinical settings [69].

Much remains to be learned in the emerging field of nanomedicine [1], and further studies are needed to study the effect of combination cancer treatment using *in vivo* models and the use of specific ligands. Such combined nanochemotherapy improves antitumor efficacy [84].

Ligands and combined nanochemotherapy

Progress in nanomedicine is due to the development of new nanocarriers and drug delivery technologies, and the search for the ideal nanocarrier continues [1]. There are some difficulties in achieving the optimal combination of physicochemical parameters for tumor targeting and drug release control, which hinders the use of nanomedicines in practical therapy [94]. Combined delivery system based on nanocarriers with ligands is superior to the conventional drug delivery system due to the ability to actively target specific cells/tissues, which makes it possible to reduce systemic distribution and unwanted side effects [9].

Research efforts are focused on the development of functionalized nanoparticles to deliver therapeutic agents to specific molecular targets overexpressed in cancer cells [94]. These include folic acid receptors, which are overexpressed on the surface of many types of tumors [95, 96]. Folic acid is widely used for diagnostic and therapeutic studies as a ligand, for imaging and therapy of cancer that expresses the folate receptor [95]. Folic acid conjugates can be used to target imaging molecules and therapeutic compounds directly to cancer tissues [21, 34, 35, 95–97]. For enhanced chemotherapy against drug resistance and cancer diagnosis, a drug with Dox and NPs of micellar PEG with incorporated folic acid with high payload has been developed. It has uniform spherical particles with a diameter of approximately 20 nm [47].

Folate-chitosan copolymer micelles were used for co-delivery of Dox and pyrrolidine dithiocarbamate [34]. Magnetic NPs with Dox loaded into the matrix were coated with folate-grafted chitosan. It was found that magnetic guidance of NPs with such a design enhances the local release of drugs and significantly reduces tumor growth [35]. Folate targets the folate receptor in cell walls [35] and inhibits MDR and thus increasing Dox in cancer cells [34].

To increase the selectivity of tumor targeting, folic acid-modified nanoparticles (DOX®CUR)-FA-NPs were developed based on star polyester [21]. *In vivo* results demonstrated that such NPs not only had significant MCF-7/ADR tumor targeting and antitumor efficacy, but also caused less pathological damage to normal tissues [21].

Lack of access to the brain is a major obstacle to the development of drugs for the central nervous system. A nanovector hybrid derived from grapefruit and polyethylenimine (PEI) coated with folic acid was developed for effective intranasal delivery of miR17 microRNA nanovector into folate receptor-positive GL-26 brain tumor [97].

Targeted multidrug delivery systems have become an advanced strategy for cancer treatment. They are used as ligands: antibodies, hormones, small peptides, tumor-specific ligands (P. Tuftsin) that were linked

to drug carriers [4]. The natural macrophage-stimulating peptide Tuftsin (P. Tuftsin) grafted to a liposome with co-encapsulated Cur and Dox was used as a ligand. This form of liposome has an enhanced synergistic therapeutic effect of the peptide tumor-specific ligand and the dual drug Cur and Dox [4].

Based on the conjugate of Cur with hyaluronic acid (HA), HA–Cur/Dox nanoparticles with dimensions of approximately 180 nm with excellent encapsulation efficiency and serum stability were generated. In this combination, Cur reversed MDR in tumor cells *in vitro* by inhibiting P-gp expression and activity, as well as inducing apoptosis through the mitochondrial pathway. The effect of targeted delivery of NPs of chemotherapeutic agents occurs thanks to CD44 receptors [84].

The conclusions of the work are that the combination of chemotherapeutic drugs allows oncologists to use drugs in smaller doses, reduce cytotoxicity and increase the effectiveness of treatment. The combination of antitumor agents with chemosensitizers targets different signaling pathways in cancer cells. During the chemotherapeutic treatment of cancer, strong cellular toxicity develops due to the poor targeting of therapeutic agents and the emergence of MDR, which significantly complicates the therapeutic effectiveness of chemotherapeutic drugs. The right drug combinations improve target selectivity and inhibit the development of MDR.

The anticancer drug doxorubicin (DOX) has limited chemotherapeutic efficacy for cancer treatment, due to poor selectivity and severe side effects. During DOX chemotherapy, dose-dependent cardiotoxicity, hepatotoxicity, nephrotoxicity develops, oxidative stress, inflammation, apoptosis, and other disorders occur. The appearance of MDR leads to the failure of chemotherapy.

Combined therapy is used to increase the effectiveness of chemotherapy. Simultaneous treatment with chemotherapeutic and chemopreventive agents with antioxidant effect reduces the non-selective cytotoxicity of drugs. Recently, special attention has been paid to herbal preparations with antioxidant effect in combination with anticancer drugs, which synergistically enhance the effect of the main drug. A number of synergistically effective drug combinations have already been selected.

Herbal preparations have not only an antioxidant effect, but also exhibit antitumor properties. Phytochemicals have both similar and unique molecular targeting profiles, which enables the selection of the desired effective synergistic drug combinations against the respective cancer cells. Among promising herbal preparations, curcumin (CUR) stands out. It is an antioxidant, anti-inflammatory agent, anti-carcinogenic and anti-metastatic agent. CUR can cause additive or synergistic effects with chemotherapeutic drugs against various cancer cell lines. CUR protects normal cells from damage caused by DOX chemotherapy, helping to increase the effectiveness of DOX.

The combination of DOX and CUR drugs is considered as a general scheme to increase the effectiveness of chemotherapy. The combination of DOX and CUR has a synergistic effect and is able to influence the balance between the autophagy and apoptosis systems, reduce mitochondrial damage caused by DOX chemotherapy. Binary preparations of DOX and CUR show stronger antitumor activity *in vitro* and *in vivo* than DOX or CUR at the same concentrations and represent a novel and highly effective therapeutic strategy for better cancer treatment.

Chemotherapeutic drug delivery using nanoparticles is a promising approach for the effective treatment of various cancers. The use of NPs simultaneously enhances the therapeutic effects and reduces the side effects of anticancer drugs. Such an effective cancer treatment strategy is based on the joint delivery of several drugs by nanocarriers into one cell, which complement and extend the effect on tumors. The search and selection of the best nanocarriers is based on improved interaction with target cells, greater accuracy and enhanced penetration into the cells to the required organelles, improved stability of drugs and slower release of the combination of drugs over time.

In the scientific literature, it is reported that increasing the solubility and bioavailability of CUR is achieved by coincapsulating it in NPs and water-soluble polymers, which increases its therapeutic effects. Incorporating curcumin into albumin, Pluronic®, or other polymers allows it to reach its effective concentration inside the cell *in vitro*. For example, enhanced co-delivery of DOX and CUR using nanocarriers has shown synergistic antitumor efficacy against liver, breast, and lung tumors. NPs are able to maintain an optimized synergistic ratio of drugs in one carrier until intracellular absorption by the target cancer cell.

Biodegradable micelles are used as promising and effective carriers for

nanobiotechnology, which jointly deliver hydrophilic and hydrophobic drugs loaded into them. Such drugs have the ability to increase the concentration of DOX and CUR in the blood plasma, as well as to prolong the time of existence of the multidrug in the blood circulation. Biodegradable polymers often used in nanomedicine include micelles of PEG, Pluronic®, chitosan, PLG, PLGA, and albumin. For most NPs, the encapsulation of DOX and CUR is carried out using the self-assembly procedure, which is the most economically feasible. The peculiarity of using chitosan is related to its biodegradability, biocompatibility, ease of technical application, versatility and low toxicity. The stability of the Pluronic® micelle is significantly improved after coating it with a chitosan layer.

MDR and anti-apoptotic processes are two of the most common mechanisms leading to chemotherapy resistance in various cancers. Resistance to chemotherapy mainly develops due to the activity of transporters that reduce the amount of DOX in the cell. Studies demonstrate that P-gp is involved in DOX resistance. Joint administration of synergistic drugs DOX and CUR reverses MDR. This is because, once in the cytosol, CUR is able to block the transport of DOX from cells by inhibiting the expression of P-gp. It was shown that the combination with DOX and CUR changes MDR in tumor cells by inhibiting the activity of P-gp, as well as the induction of apoptosis through the mitochondrial pathway. The method of combining two or more therapeutic agents, which are included in NPs, or micelles, has great potential due to synergistic effects in overcoming MDR. In addition, the use of some polymers significantly improves the results in suppressing MDR. The use of biodegradable polymers is becoming the norm to achieve improved combination chemotherapy and greater selective targeting to suppress MDR. Loading into NPs with synergistic systems of additional chemotherapeutic agents can increase the antitumor effectiveness of the combination. The creation of such multifunctional NPs is an active strategy to mitigate MDR. Multifunctional nanocarriers for the DOX - CUR couple are constantly optimized in order to increase the cytotoxicity of the drug and reduce the amount of the drug needed to achieve a cytotoxic effect. Polymers that inhibit MDR are used to increase targeted drug delivery.

A combination drug delivery system based on multifunctional ligand-nanocarriers is superior to the conventional drug delivery system due to the ability to actively target cancer cells, which reduces unwanted side effects. For example, NPs modified with folic acid as a ligand selectively target tumors that express the folate receptor. Such NPs have increased antitumor efficiency, strongly inhibit MDR and cause less pathological damage to normal tissues.

There are some difficulties in achieving the optimal combination of drugs in nanoparticles, accuracy of tumor targeting and controllability of drug release. Such problems prevent the use of developed nanomedicines in practical therapy. This is due to the fact that during the use of nanomedicines there are certain limitations associated with physiological barriers in tumor tissues and non-selective interaction of drugs with normal tissues.

It is necessary to investigate and identify the mechanisms of synergistic action of several compounds with the aim of elucidating the molecular mechanisms that contribute to increasing the effectiveness of phytochemicals when they are used in combination for the treatment of oncological diseases. Future experiments should focus on enhancing the delivery of phytochemicals and creating different combinations of them that act synergistically, using *in vitro* and *in vivo* models. Creation of combined nanopreparations based on them, with optimized proportions of drugs, enhanced with ligands for the purpose of selective targeting of cancer cells, will become a standard scheme of treatment for cancer patients.

Despite some improvements in the drug delivery system, the placement of combination chemotherapeutics in the hybrid nanostructure remains a challenge, but the optimization of the delivery system targeting the tumor microenvironment may have great clinical significance. Advances in nanomedicine are driven by the development of new and improved carriers for drug combinations and drug delivery technologies. Research efforts are focused on the development of functionalized nanoparticles for the targeted delivery of therapeutic agents to specific molecular targets overexpressed in various cancer cells. The use of optical research methods helps to a great extent in the development of multifunctional combined nanopreparations with a synergistic effect. Such nanomaterials support an optimized synergistic ratio of drugs in one carrier until the moment of intracellular absorption. The use of nanocarrier-based combination therapy requires significant efforts to study the molecular

processes that drive the therapeutic effects of synergistic anticancer drugs. The search for the ideal nanocarrier and combination of natural and synthetic synergistic drugs continues. The development of NPs with a combination of synergistic drugs with known phytochemicals is clinically important because they can be rapidly transferred from the laboratory to clinical practice.

Financing of the work was within the basic subject of the department Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine.

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КОМБІНОВАНА НАНОХІМІОТЕРАПІЯ НА ПРИКЛАДІ ДОКСОРУБІЦИНУ ТА КУРКУМІНУ

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Мета. Огляд даних літератури щодо комбінованої нанохіміотерапії на прикладі двох препаратів —доксорубіцину (Dox) та куркуміну (Cur). Особливу увагу приділено використанню речовин із синергічними властивостями в одній наночастинці (НЧ), здатних проникати в живу клітину.

Метод комбінованої хіміотерапії нанопрепаратами дозволяє підвищити ефективність лікування. Техніка використання наноконтейнерів із синергічними препаратами в поєднанні із лігандами зменшує побічні ефекти хіміотерапевтичних препаратів.

Результати. Дані літератури свідчать про те, що застосування нанопрепаратів сприяє швидкому створенню та використанню синергітичних комбінацій, які цілеспрямовано доставляються до клітин-мішеней, зменшуючи дозування за рахунок точного націлювання. Перспективним напрямком наномедицини є створення багатофункціональних наноматеріалів на основі кількох активних препаратів із синергічними властивостями, з одночасним використанням їх підсилювачів та стратегією активного націлювання. Ці структури дозволяють цілеспрямовано та контрольовано проникати лікарським сполукам у місця локалізації патологічних процесів, знижуючи токсичність препаратів для нормальних клітин.

Висновки. Комбінована хіміотерапія із використанням полімерів та наночастинок із лігандами, до складу яких входять синергічні препарати, спряє зменшенню побічних ефектів та дози хіміопрепаратів, а також подоланню множинної лікарської стійкості.

Ключові слова: комбінована нанохіміотерапія; доксорубіцин; куркумін; синергізм; активне націлювання.

UDC 579.2 **https://doi.org/10.15407/biotech16.01.021**

MICROBIAL CO-CULTIVATION: DISCOVERY OF NOVEL SECONDARY METABOLITES WITH DIFFERENT BIOLOGICAL ACTIVITIES

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Received 2022/09/30
Revised 2022/11/10
Accepted 2023/02/28

In recent decades overuse and misuse of antibiotics as well as social and economic factors have accelerated the spread of antibiotic-resistant bacteria, making them a major problem for humanity. One of the most effective approaches to the discovery of new secondary antimicrobial metabolites is co-cultivation of microorganisms, in which the producer of the target products is grown together with competitive microorganisms (inductors), in response to the presence of which silent biosynthetic genes of the producer strain are activated and an increase in the biological activity of the synthesized secondary metabolites and/or even the synthesis of new metabolites is observed. The review summarizes the current literature data on the co-cultivation of antimicrobial substances producers with competitive microorganisms, which results in the synthesis of new metabolites with antimicrobial and cytotoxic activity, not typical for monocultures. During the co-cultivation of fungi, bacteria, and fungi with bacteria, the synthesis of new antimicrobial and anticancer metabolites, which are classified as alkaloids, phenylpropanoids, macrolides, polyketides, cyclopeptides, terpenoids, anthraquinones, and steroids, is observed. These data indicate that the mixed fermentation of microorganisms is a simple, cheap, and quite effective way to obtain new metabolites that are promising for use in medicine.

Key words: co-cultivation; antimicrobial products; anticancer agents.

Nowadays, the number of studies on the development of new antibiotic drugs is decreasing, due to the increasing resistance of pathogenic microorganisms to them due to their excessive use in medicine and agriculture. This situation can lead to dangerous consequences for the world's population, so novel safe natural products are needed [1].

Microorganisms from various terrestrial and marine habitats are a source of new bioactive natural compounds, but one of the problems in the process of discovering new microbial metabolites is the re-isolation of already known compounds. In addition, the biological activity of microbial secondary metabolites depends on the conditions of cultivation of the producers, so the development of approaches that allow to obtain a final product with stable specified properties is a priority in the development of current biotechnology. Recent advances in microbial genomics have clearly demonstrated that the biosynthetic potential of microorganisms as producers of metabolites with unique properties is much higher than expected, because a significant number of microbial gene clusters may remain silent under typical cultivation conditions [2, 3].

At present, both traditional physiological approaches (optimization of cultivation conditions, introduction of exogenous precursors into the culture medium) and methods of genetic and metabolic engineering are being implemented to increase the biosynthetic ability of producers of practically valuable compounds. The application of the above mentioned methods made it possible to effectively activate silent genes as one of the mechanisms for producing new secondary metabolites. An alternative to the chemical

modification of known compounds to increase their antimicrobial activity is the strategy of co-cultivation of microorganisms, which is superior to other approaches in terms of cost and convenience, since it does not require expensive reagents or methods of gene manipulation [4–6]. In addition, the use of co-cultivation methods, in which the producer of the final product is cultivated together with competitive microorganisms (inductors), is a promising approach to increase the activity of existing and/or search for new compounds that are not inherent in axenic cultures (monocultures), metabolites with strong antimicrobial [7, 8], antagonistic [9, 10], and cytotoxic [11] effects.

This review aimed to summarize current literature data on the co-cultivation of antimicrobial compound producers with competitive microorganisms, that results in the synthesis of new biologically active metabolites that are not typical for monocultures.

Novel secondary metabolites with antimicrobial activity

In the works on the co-cultivation of microorganisms published over the past 5–7 years, it has been reported the production of alkaloids [12, 13, 19, 20], phenylpropanoids [14–16], macrolides [7, 12, 27, 28], polyketides [22, 26, 31, 49], cyclopeptides [10], terpenoids [17, 18] and others [21, 23, 24, 29, 30]. It should be noted that these novel synthesized metabolites demonstrate antibacterial and antifungal properties and are not synthesized in monocultures of microorganisms.

The synthesis of new antimicrobial metabolites is reported in the co-cultivation of fungi-fungi [11-18], bacteria-bacteria [26-31, 49], and fungi-bacteria [7, 10, 19-24].

Co-Cultures between Fungal Strains

A new alkaloid, identified as aspergicin, was isolated from a mixed fermentation of *Aspergillus* FSY-01 and *Aspergillus* FSW-02, accompanied by neoaspergic acid and ergosterol [12]. It was found that aspergicin has high antimicrobial activity against bacterial test cultures (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Bacillus dysenteriae*, *Bacillus proteus*, *Escherichia coli*): the minimum inhibitory concentrations (MIC) were 15.62–62.5 μg/ml.

A new nonadride derivative (byssochlamic acid imide) isolated from the co-culture of *Phomopsis* sp. K38 and *Alternaria* sp. E33 was characterized by antifungal activity against *Fusarium graminearum* and *Fusarium oxysporum* with MIC values of 50 and 60 μg/ml, respectively [13].

Phenylpropanoids are a large and structurally diverse group of secondary metabolites characterized by the presence of a C6-C3-phenolic scaffold, that plays a crucial role in a wide spectrum of biological and pharmacological activities. New metabolites of this group were obtained by co-cultivation of fungi of the genus *Penicillium* [14-16]. The metabolites show high antibacterial and antifungal activity. For example, ten citrinine analogs, including a new dimer, secopentitrinol A and pencitrinol L, were isolated from the co-culture of *Aspergillus sydowii* EN-534 and *Penicillium citrinum* EN-535 [14]. The new compounds showed antimicrobial activity against *Vibrio parahaemolyticus* and *Edwardsiella ictaluri*: the minimum inhibitory concentrations were 32-64 μg/ml [14], which is lower than those reported for penixilarins A-C [15]. Penixilarins A-C isolated from the mixed fermentation of *Penicillium crustosum* PRB-2 and *Xylaria* sp. HDN13-24, had antibacterial activity against *Mycobacterium phlei*, *B. subtilis* and *V. parahaemolyticus* (MIC range from 6.25 to 100 μg/ml) [15].

In addition, a new phenylpropanoid, named secopenicillide C, was identified from the co-culture of *Penicillium pinophilum* FKI-5653 and *Trichoderma harzianum* FKI-5655, which was characterized by antimicrobial activity against *E. coli* and *Micrococcus luteus* with MIC values of 16 and 64 μg/ml, respectively [16].

A new terpenoid derivative, asperterrein, was found among the newly synthesized terpenoids by co-cultivation of *Aspergillus terreus* EN-539 and *Paecilomyces lilacinus* EN-531 [17]. The compound showed antibacterial activity against *Alternaria brassicae*, *E. coli*, *Physalospora piricola* and *S. aureus* with MIC values from 4 to 64 μg/ml.

The most effective antimicrobial agents of the new compounds synthesized as a result of co-cultivation of *Penicillium bilaiae* MA-267 and *Penicillium chermesinum* EN-480 were two new meroterpenoid derivatives - chermebilenes A and B [18]. The MIC of chermebilene A against *Ceratobasidium cornigerum* and *Edwardsiella tarda* was 0.5 and 0.25 μg/ml, respectively, which makes this compound perspective for use as an antimicrobial agent in clinical practice.

During the co-cultivation of *Penicillium fuscum* (Sopp) Raper & Thom and *Penicillium camembertii/slavigerum* Thom, five new

16-membered macrolides (berkeleylactones A, B, D, E, G) were synthesized, including berkeleylactone A, which demonstrated the most effective antimicrobial effect compared to the known macrolides: MIC against strains of *S. aureus*, *Bacillus anthracis*, *Streptococcus pyogenes*, *Candida albicans* and *Candida glabrata* were 1-2 μg/ml [11].

Co-Cultures between Fungi and Bacteria

The novel alkaloid compound pulicatin H, isolated from the co-culture of the fungus *P. citrinum* and bacterium *Pantoea agglomerans*, was characterized by high antifungal activity. The MIC values for *P. citrinum*, *Aspergillus niger*, and *C. albicans* were 25, 8.4, and 50 μg/ml, respectively [19]. Also, new alkaloids, dihydrolateropyrone and fusatricinones A-D, were identified from the mixed-fermentation of *Streptomyces lividans* and *Fusarium tricinctum*, and were characterized by antibacterial activity against *S. aureus* and *Pseudomonas aeruginosa* [20], but the authors of this article did not provide the antimicrobial activity of these compounds.

As a result of the co-cultivation of *Streptomyces rochei* MB037 and *Rhinocladiella similis* 35, the macrolides borelidin J and K were obtained, which proved to be effective antimicrobial agents against *S. aureus*: minimum inhibitory concentrations were 0.195 and 1.563 μg/ml, respectively [7].

It is known from the literature that only one new antimicrobial steroid metabolite (ergosterol derivative) was synthesized during the co-cultivation of *Bacillus wiedmannii* Com1 and *Pleosporales* sp. F46 [21]. This compound had antimicrobial activity against *S. aureus*: microbial growth inhibition zone and minimum inhibitory concentration were 71 mm and 100 μg/ml, respectively.

Moderate antibacterial activity against *Streptomyces coelicolor* and *S. lividans* (MIC 1000 and 250 μg/ml, respectively) was demonstrated by a new polyketide fumigermin synthesized by the mixed-fermentation of *Aspergillus fumigatus* with the bacteria *Streptomyces iranensis*, *S. coelicolor, S. lividans*, and *Streptomyces rapamycinicus* [22].

Under co-cultivation of *Bacillus amyloliquefaciens* ACCC11060 and *Trichoderma asperellum* GDFS1009, the synthesis of new cyclopeptides BT1 and BT2 was observed [10], which inhibited the growth of *Bacillus cinerea* by 47.86% and 66.86%, respectively.

New antimicrobial compounds (marcocarpone C, 2-(carboxymethylamino) benzoic acid and (-)-citreoisocoumarinol) were obtained from the co-culture of *B. subtilis* 168 trpC2 and *Fusarium tricinctum* [23]. Macrocarpon C and 2-(carboxymethylamino) benzoic acid are characterized by high antimicrobial activity against bacteria *B. subtilis* 168 trpC2, *S. aureus* ATCC 25697, *Streptococcus pneumonia* ATCC 49619, *E. coli* ATCC 25922, *P. aeruginosa* B 63230 with MIC in the range of 2-64 μg/ml.

During the co-culture of *Cladosporium* sp. WUH1 and *B. subtilis* CMCC (B) 63501, a new compound (trihydroxybutyl ester of 4-carboxydiorcinol) with antibacterial activity was synthesized: MIC against *Klebsiella pneumonia*, *B. subtilis*, *E. coli, S. aureus, S. epidermidis* were 16, 64, 64, and 32 μg/ml, respectively [24].

Co-Cultures between Bacterial Strains

As a result of the co-cultivation of *Tsukamurella pulmonis* TP-B0596 and *S. coelicolor* S-552, a new polyketide alchivemycin A was obtained [31]. The minimal inhibitory concentration of this polyketide against *Micrococcus luteus* TP-B100 was 0.06 μg/ml. The new antimicrobial polyketide glycoside gordonic acid was synthesized in the co-culture of *Streptomyces tendae* KMC006 and *Gordonia* sp. KMC005 [49]. At a concentration of gordonic acid of 10 μg/disc, the growth inhibition zones of *M. luteus* KCCM11548 and *Enterococcus hirae* KCCM11768 were 1.5–2.5 mm.

In 2018, two new polyketides (janthinopolyenemycins A and B) were isolated from a co-culture of two strains of *Janthinobacterium* spp. ZZ145 and ZZ148 [26]. Both polyketides exhibited antifungal activity against *C. albicans* (MIC 15.6 μg/ml). It was found that janthinopolyenemycin congeners are active against methicillin-resistant *S. aureus* and *E. coli*.

In recent years, four new lactams have been discovered as a result of co-cultivation of bacteria [27, 28]. One of these compounds is umezawamide A, synthesized during the co-cultivation of *T. pulmonis* TP-B0596 with *Umezawaea* sp. RD066910 [27]. Umezawamide A is characterized by moderate antimicrobial activity against *C. albicans*: at a concentration of 5 μg/disc, the growth inhibition zone was 1.7 mm. Under the co-cultivation of *Actinosynnema mirum* NBRC 14064 with *T. pulmonis* TP-B0596, antimicrobial mirilactams C, D, E were synthesized [28], and

they exhibited antimicrobial activity against *C. albicans, Bacillus cereus, S. aureus* MSSA (activity parameters are not given).

An effective antimicrobial metabolite is keyicin, synthesized as a result of co-cultivation of *Micromonospora* sp. WMMB-235 and *Rhodococcus* sp. [29], the minimum inhibitory concentrations of keyicin against *Mycobacterium* sp., *Rhodococcus* sp., *B. subtilis, S. aureus* were 2-8 μg/ml.

During the co-cultivation of *T. pulmonis* TP-B0596 with *Streptomyces nigrescens* HEK616, a new compound spirohemiaminal was obtained, which was characterized by antimicrobial activity against the test cultures *B. subtilis, E. coli, S. aureus*: at a concentration of 100 μg/disc, the growth inhibition zones were 2-10 mm [30].

Table 1 summarizes the data on the synthesis of new antimicrobial metabolites during the co-cultivation of fungi, bacteria, and fungi with bacteria. These data indicate that the co-cultivation of microorganisms is a simple, cheap, and quite effective way to obtain new metabolites with significant antimicrobial activity.

Figure 1 illustrated the classes of new antimicrobial compounds synthesized during the co-culture of microorganisms. Metabolites based on co-cultures of bacteria and fungi were identified as alkaloids, anthraquinones, macrolides, phenylpropanoids, polyketides, cyclopeptides, terpenoids, with the largest proportion being macrolides, alkaloids, phenylpropanoids, and polyketides.

Antimicrobial compounds, such as phenylpropanoids and terpenoids, were identified only on the basis of co-cultures of fungi. At the same time, metabolites characterized as alkaloids were synthesized as a result of co-cultivation of both bacterial and fungal cultures.

Novel secondary metabolites with cytotoxic and antimicrobial activity

Studies on the co-cultivation of microorganisms published in the last 5–7 years have reported the production of new alkaloid compounds [32, 33, 39, 42–45], phenylpropanoids [40, 41], macrolides [11], polyketides [35, 48], cyclopeptides [36, 41, 46, 47], terpenoids [34], and compounds of others [11, 34, 37, 38, 41–43]. It should be noted that some of the new metabolites exhibit both cytotoxic and antimicrobial activity [11, 36, 41, 44, 45], and some — only cytotoxic activity [32–35, 37–40, 42, 43, 46–48].

The synthesis of new metabolites with both cytotoxic and antimicrobial activity is reported in the co-culture of fungi-fungi [11, 32–38], bacteria-bacteria [44–48], and fungi with bacteria [39–43].

Co-Cultures between Fungal Strains

Five new prenylated indole alkaloids were isolated from the mixed fermentation of *Aspergillus sulphureus* KMM 4640 and *Isaria felina* KMM 4639: 17-hydroxynotoamide D, 17-O-

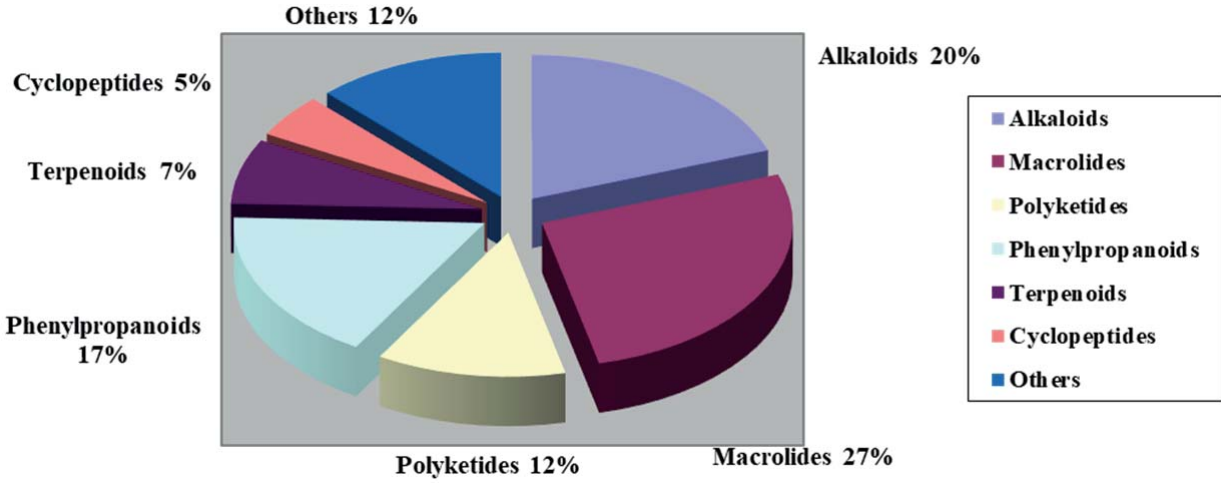

Fig. 1. **The number of new metabolites with antimicrobial activity synthesized as a result of co-cultivation of microorganisms [7, 10–24, 26–31, 49]**

Table 1

Characterization of new antimicrobial compounds synthesized during mixed cultivation of microorganisms

Microorganisms	Carbon source	Novel secondary metabolites		Test-cultures for determining antimicrobial activity	Minimum inhibitory concentration, μg/ml	References
		Compounds	Classes			
Co-cultivation of fungal strains						
Aspergillus FSY-01 + *Aspergillus* FSW-02	Glucose (GYP- broth)	Aspergicin	Alkaloid compound $CH_3COOC_2H_5$	*Staphylococcus aureus*	62.5	[12]
				Staphylococcus epidermidis	31.25	
				Bacillus subtilis	15.62	
				Bacillus dysenteriae	15.62	
				Bacillus proteus	62.5	
				Escherichia coli	31.25	
Penicillium pinophilum FKI-5653 + *Trichoderma harzianum* FKI-5655	Brown rice, glycerin, yeast extract, potato-dextrose broth	Secopenicillin C	Secotype of the penicillin compound $C_{20}H_{22}O_6$	*Escherichia coli*	16	[16]
				Micrococcus luteus	64	
Penicillium fuscum (Sopp) Raper & Thom + *Penicillium camembertii/ clavigerum* Thom	Potato dextrose medium (dextrose, potato broth)	Berkeleylactone A Berkeleylactone B Berkeleylactone D Berkeleylactone E Berkeleylactone G	Macrolide $C_{23}H_{36}O_{10}S$ Macrolide $C_{20}H_{30}O_8$ Macrolide $C_{20}H_{32}O_7$ Macrolide $C_{20}H_{33}O_8$	*Staphylococcus aureus* 13709	4/13/45/24	[11]
				Streptococcus pyogene	119/50/>90/>50	
				Candida glabrata	31/>400/>90/ >50	
				Bacillus subtilis	31/100/ >90/>50	
				Candida albicans	>119/>400/ >90/>50	
				Bacillus anthracis	8/26/>90/24* * — MIC Berkeleylactone B/ Berkeleylactone D/ Berkeleylactone E/ Berkeleylactone G	
Aspergillus sydowii EN-534 + *Penicillium citrinum* EN-535	Rice medium (Rice, corn flour, peptone, monosodium glutamate)	Secopenicitrinol A Penicitrinol L	Citrine dimer Penicillin derivatives $C_{23}H_{26}O_6$ Citrine monomer Penicillin derivatives $C_{14}H_{18}O_5$	*Edwardsiella ictaluri*	64/64*	[14]
				Vibrio parahaemolyticus	32/64* * — MIC Secopenicitrinol A/ penicitrinol L	

Table 1 (Continued)

Microorganisms	Carbon source	Novel secondary metabolites		Test-cultures for determining antimicrobial activity	Minimum inhibitory concentration, μg/ml	References
		Compounds	Classes			
1	2	3	4	5	6	7
Penicillium crustosum PRB-2 + *Xylaria* sp. HDN13-249	Starch, maltose, sucrose, yeast extract	Penicillarin A Penicillarin B Penicillarin C	Alkyl aromatic compounds Penicillin derivatives $C_{33}H_{49}O_6$ $C_{33}H_{49}O_6S$ $C_{32}H_{39}O_5$	*Mycobacterium phlei*	>200/>200/6.25*	[15]
				Bacillus subtilis	>200/100/>200*	
				Vibrio parahemolyticus	>200/>200/12.5* * — MIC of penicillars A/B/C	
Aspergillus terreus EN-539 + *Paecilomyces lilacinus* EN-531	Not given	Asperterrein	A derivative of terrein Cycloalkane $C_9H_{14}O_2$	*Escherichia coli*	32	[17]
				Staphylococcus aureus	32	
Phomopsis sp. K38 + *Alternaria* sp. E33	Glucose, Yeast extract, Pepton	Imide (−)-bys-sochlamic acid	Nonadrenaline derivative Alkaloid $C_{18}H_{21}O_5N$	*Fusarium graminearum*	50	[13]
				Fusarium oxysporum	60	
Penicillium bilaiae MA-267 + *Penicillium chermesinum* EN-480	Rise, Pepton, Corn syrup	Chermebilene A Chermebilen B	Derivatives Meroterpenoids $C_{35}H_{56}O_4Na$ $C_{25}H_{40}O_9N$	*Edwardsiella tarda*	0.25	[18]
				Ceratobasidium cornigerum	0.5	
Co-cultivation of fungi and bacteria						
Fusarium tricinctum + *Bacillus subtilis* 168 trpC2	Rice medium	Macrocarpon C 2-(carboxy-methylamino) benzoic acid	Heterocyclic compound $C_{13}H_{12}O_4$ Derivative of benzoic acid $C_9H_9NO_4$	*Bacillus subtilis* 168 trpC2	8–16*	[23]
				Staphylococcus aureus ATCC 25697	2–8*	
				Staphylococcus aureus ATCC 29213	2–8*	
				Streptococcus pneumonia ATCC 49619	2–8*	
				Escherichia coli ATCC 25922	2–8*	
				Enterococcus faecalis UW 2689	2–8*	

Table 1 (Continued)

1	2	3	4	5	6	7
				Pseudomonas aeruginosa B 63230	> 64* * — macrocarpone C and 2-carboxy-methylamino-benzoic acid exhibit the same antimicrobial activity against the given test-cultures	
Trichoderma asperellum GDFS1009 + *Bacillus amyloliquefaciens* ACCC11060	Meat extract, Pepton	Complex BT1 and BT2	BT1: 4-hydroxybenzoic acid, apigenin, glycine betaine, malic acid and nicotinic acid BT2: indole-3-acetic acid, indole-3-carboxylic acid, phenacillamine, trans-3-coumaric acid and transcinnamic acid	*Botrytis cinerea*	Growth inhibition under the action of BT1 47.86%, under the influence of BT2 — 66.86%	[10]
Pleosporales sp. F46 + *Bacillus wiedmannii* Com1	Rice medium	Not given	Steroid compound	*Staphylococcus aureus*	100	[21]
Rhinocladiella similis 35 + *Streptomyces rochei* MB037	Malt extract, dextrose	Borelidin J Borelidin K	Macrolides $C_{28}H_{45}NO_7$ $C_{29}H_{46}NO_7$	Methicillin-resistant *Staphylococcus aureus* strain	0.195 1.563	[7]
Fusarium tricinctum + *Streptomyces lividans* TK24	Not given	Fusatricinones A-D Dihydrolatero-pyrone	Petroquinone dimers: Fusacitron A $C_{31}H_{24}O_{16}$ Fusacitron B $C_{30}H_{22}O_{16}$ Fusacitron C $C_{32}H_{26}O_{16}$ Fusacitron D $C_{32}H_{26}O_{16}$ A derivative of lateropinone $C_{15}H_{12}O_8$	*Staphylococcus aureus* *Pseudomonas aeruginosa*	all compounds exhibit antimicrobial activity against the test cultures given, activity parameters are not given	[20]

Table 1 (Continued)

1	2	3	4	5	6	7
Cladosporium sp. WUH1 + *Bacillus subtilis* CMCC(B) 63,501	Potato broth, dextrose, yeast extract, peptone	Trihydroxybutyl ester of 4-carboxydiorcinol	Ester $C_4H_8O_3$	*Bacillus subtilis*	32	[24]
				Escherichia coli	64	
				Staphylococcus aureus	64	
				Staphylococcus epidermidis	32	
				Klebsiella pneumoniae	16	
				Pseudomonas aeruginosa	>64	
Penicillium citrinum + *Pantoea aggolomerans*	Potato broth, dextrose, yeast extract, peptone	Pulicatin H Pulicatin F	Siderophore derivatives Alkaloid $C_{13}H_{13}NO_3S$ $C_{10}H_8O_2N_2S$	*Penicillium citrinum*	25/53*	[19]
				Pantoea aggolomerans	>200/>200*	
				Aspergillus niger	8.4/22.6*	
				Candida albicans	>50/>50* * — MIC pulicatin H/ pulicatin F	
Aspergillus fumigatus + *Streptomyces rapamycinicus* + *Streptomyces iranensis* + *Streptomyces coelicolor* + *Streptomyces lividans*	Lactose, Glucose, arginine	Fumigermin	Microbial α-pyrone (polyketide) $C_{11}H_{15}O_3$	*Streptomyces coelicolor*	1000	[22]
				Streptomyces lividans	250	
Co-cultivation of bacterial strains						
Streptomyces endus S-552 + *Tsukamurella pulmonis* TP-B0596	Starch, glycerin, glucose, yeast extract	Alchivemycin A	Polyketide $C_{35}H_{53}NO_{10}$	*Bacillus subtilis* ATCC 6633	40	[31]
				Escherichia coli NIHJ JC-2	>50	
				Staphylococcus aureus 209P JC-1	>50	
				Micrococcus luteus TP-B100	0,06	
				Candida albicans TP-F0176	>50	
				Saccharomyces cerevisiae TP-F0176	>50	

Table 1 (End)

1	2	3	4	5	6	7
Umezawaea sp. RD066910 + *Tsukamurella pulmonis* TP-B0596	Starch, glycerin, glucose, yeast extract	Umezawamide A	Polycyclic tetramate macrolactam $C_{29}H_{40}N_2O_6$	*Candida albicans*	Growth inhibition zone of 1.7 mm (5 μg/disc)	[27]
Actinosynnema mirum NBRC 14064 + *Tsukamurella pulmonis* TP-B0596	Starch, glycerin, glucose	Mirilactam C Mirilactam D Mirilactam E	Monocyclic polyene macrolactams $C_{27}H_{37}NO_6$	*Candida albicans*	all compounds exhibit antimicrobial activity against the test cultures given, activity parameters are not given	[28]
				Bacillus cereus		
				Staphylococcus aureus MSSA		
Streptomyces tendae KMC006 + *Gordonia* sp. KMC005	Malt extract	Gordonic acid	Polyketide glycoside $C_{24}H_{36}NO_6$	*Micrococcus luteus* KCCM11548	Growth inhibition zone of 2.5 mm (10 μg/disc)	[49]
				Enterococcus hirae KCCM11768	Growth inhibition zone of 2.5 mm (10 μg/disc)	
Streptomyces nigrescens HEK616 + *Tsukamurella pulmonis* TP-B0596	Not given	Spirohemiaminal	Lipid [5,5]-spiroge mimetics $C_{18}H_{34}NO_2$	*Bacillus subtilis*	Growth inhibition zone of 12 mm (30 μg/disc)	[30]
				Escherichia coli	Growth inhibition zone of 12 mm (30 μg/disc)	
				Staphylococcus aureus	Growth inhibition zone of 12 mm (30 μg/disc)	
Janthinobacterium spp. ZZ145 + *Janthinobacterium* spp. ZZ148	Rice medium	Janthino-polyenemycin A Janthino-polyenemycin B	Polyketides $C_{26}H_{36}O_3$	*Candida albicans*	15.6* * — compounds exhibit the equal antimicrobial activity against the test-culture	[26]
Micromonospora sp. WMMB-235 + *Rhodococcus* sp. WMMA185	Yeast extract, malt extract, dextrose	Keyicin	Nitroglycosylated anthracycline Anthraquinone $C_{75}H_{108}N_4O_{34}$	*Mycobacterium* sp.	activity parameters are not given	[29]
				Rhodococcus sp.		
				Bacillus subtilis	8	
				Staphylococcus aureus MSSA	2	

ethylnotoamide M, 10-O-acetylsclerotiamide, 10-O-ethylsclerotiamide, and 10-O-ethylnotoamide R [32]. The compound 17-O-ethylnotoamide M inhibited the growth of human prostate cancer cells 22Rv1 at concentration of 10 μM. The first natural 1,2,4-oxadiazin-6-one (sclerotiorumin C) and aluminiumneohydroxyaspergillin were isolated from the co-culture of fungi *Aspergillus sclerotiorum* and *P. citrinum* [33]. Aluminiumneohydroxyaspergillin exhibited high cytotoxicity against human histiocytic lymphoma U937 cell line (IC_{50} = 4.2 μm) and strong toxicity towards brine shrimp (LC_{50} = 6.1 μm).

New macrolides were synthesized after co-cultivation of *P. fuscum* (Sopp) Raper & Thom and *P. camembertii/slavigerum* Thom, including berkeleylactones A, C, F and A26771B [11]. The compounds exhibited cytotoxic activity against K-562, RPMI-8226, and CCRF-CEM leukemia cells with IC_{50} values of 10 μM and drastically reduced the viability of cancer cells by 38-85%.

Eight newly induced secondary metabolites were isolated during the co-cultivation of *Armillaria* sp. with *Epicoccum* sp. YUD 17002, including five protoiludane-type sesquiterpenoids and three aryl esters [34]. One of aryl ester exhibited moderate cytotoxicity against five human cancer cell lines (HL-60, A549, MCF-7, SMMC-7721, and SW480) with IC_{50} values ranging from 15.80 to 23.03 μM [34]. The newly synthesized polyketides, in particular, nafuredin B, identified from a co-culture of *Talaromyces aculeatus* and *Penicillium variabile*, exhibited higher activity against human tumor cell lines [35]. Nafuredin B demonstrated high cytotoxicity against human tumor HeLa, K562, HCT-116, HL-60, A549, and MCF-7 cell lines with IC_{50} values in the range of 1.2–9.8 μM, respectively. At the same time, a new cyclopeptide, lateritin, was identified after co-cultivation of *Ovadendron sulphureoochraceum* MIC 5759, *Ascochyta pisi* MIC 5620, *Emericellopsis minima* MIC 5835, *Cylindrocarpon destructans* MIC 5638, *F. oxysporum* MIC 5789, were characterized by cytotoxic activity against human tumor cell lines (BXPC-3, MCF-7, CNS SF268, NSC H460, KM20L2 and DU-145) with half maximal inhibitory concentration in the range of 1.7-2.0 μg/ml [36]. In addition to human tumor cell lines, the compound inhibited mouse leukemia P388 cells (IC_{50} = 1.8 μg/mL).

High cytotoxic activity is typical for the compound diorcinol J, synthesized as a result of the co-cultivation of *Aspergillus sulphureus* KMM 4640 and *Isaria felina* KMM 4639 [37]. The IC_{50} value for mouse Ehrlich carcinoma cells was 37.6 μM. It was found that diorcinol J is able to affect the expression of the heat shock protein Hsp70 in Ehrlich ascites carcinoma cells. It is well known that the heat shock protein 70 (HSP70) was frequently overexpressed in tumor cell lines as an ATP-dependent molecular chaperone and played a significant role in refolding misfolded proteins and promoting cell survival under stress. Thus, compounds that could inhibit HSP70 had great potential in tumor therapy [37].

Under the co-cultivation of *Aspergillus fischeri* NRRL 181 and *Xylaria flabelliformis* G536, a new compound wheldone was obtained [38], that was characterized by cytotoxic activity against breast cancer cells MDA-MB-231, ovarian cancer cells OVCAR-3, human melanoma cells MDA-MB-435 with IC_{50} values of 7.6, 3.8 and 2.4 μM, respectively.

Co-Cultures between Fungi and Bacteria

As a result of co-cultivation of *Saccharomonospora* sp. UR22 and *Dietzia* sp. UR66 obtained a new compound saccharomonosporin A with cytotoxic activity against human colon adenocarcinoma NT-29 and human promyelocytic leukemia HL-60: IC_{50} values of 3.6 and 2.8 μM, respectively [39].

During the co-cultivation of *Trichoderma* sp. 307 and *Acinetobacter johnsonii* B2, one new depsidone, botryorodin H, was synthesized together with three known analogues (botryorodins C, D, and G) [40]. Botryorodins H, C, D showed α-glucosidase inhibitory activity with IC_{50} ranging from 8.1 to 11.2 μM, and botryorodin H exhibited potent cytotoxicity against rat prolactinoma MMQ and rat pituitary adenoma GH3 cell lines (IC_{50} = 3.09 and 3.64 μM).

Under co-cultivation of *Aspergillus versicolor* and *B. subtilis*, the synthesis of the cyclic pentapeptide cotteslosin C, aphaquinolone, 22-epi-aflaquinolone B, two anthraquinones and the known isoversicolorin B and O-demethylsterigmatocystin, sterigmatocystin, sterigmatine was observed [41]. O-demethylsterigmatocystin, sterigmatocystin, sterigmatin inhibited rat lymphoma cell lines L5178Y, with IC_{50} values ranging from 2.2 to 5.8 μM.

The new compounds, ochraspergillic acids A and B, and the known viomellein and ochratoxin B were obtained from the co-culture of *Aspergillus ochraceus* and *B. subtilis* [42]. Viomellein and ochratoxin are characterized by high cytotoxic activity against the A2780

human ovarian carcinoma cells with IC_{50} values of 5.0 and 3.0 μM, respectively.

As a result of co-cultivation of *Bionectria* sp. and *S. lividans* TK24, a new alkaloid, 1,2-dihydrophenopyrrozin, was obtained together with five known analogues, including penicolinate A. Penicolinate A exhibited potent cytotoxic activity against the human ovarian cancer cell line A2780 with an IC_{50} value of 4.1μM [43].

Co-Cultures between Bacterial Strains

In recent years, two new alkaloid compounds with cytotoxic activity have been identified as a result of co-cultivation of bacteria [44, 45]. One of these compounds is the alkaloid BE-13793C, synthesized by co-cultivation of *Streptomyces* sp. MA37 and *Pseudomonas* sp. [44]. BE-13793C exhibited strong antiproliferative activity against human colon carcinoma HT-29 cells (ATCC HTB-38), with an IC_{50} value of 3.16 μM, but did not cause toxic effects on normal lung cells (ATCC CCL-171). During the cultivation of *Actinokineospora* sp. EG49 with *Nocardiopsis* sp. RV163, 1,6-dihydroxyphenazine was synthesized, which, in addition to cytotoxic (IC_{50} against human parasite Trypanosoma brucei TC 221 was 19 μM), also showed antimicrobial activity (growth inhibition zones of *Bacillus* sp. P25, *Actinokineospora* sp. EG49 were 11-15 mm) [45].

An effective anti-cancer compound was a novel cyclic peptide, dentigerumycin E, synthesized as a result of co-cultivation of *Streptomyces* sp. JB5 and *Bacillus* sp. GN1 [46]. Experiments have shown that dentigerumycin E demonstrated antimetastatic activity against cancer cells. Thus, the moderate cytotoxicity against cancer cell lines A549 (lung cancer), HCT116 (colorectal cancer), MDA-MB-231 (breast cancer), SK-HEP-1 (liver cancer) and SNU638 (gastric cancer) with half maximal inhibitory concentration (IC_{50}) in the range of 27–39 μM.

Two new isomers of heterocyclic peptides (catenulobactins A and B) were synthesized under cultivation of *Catenuloplanes* sp. RD067331 with *T. pulmonis* TP-B0596 [47]. Catenulobactin B exhibited Fe(III)-chelating activity and moderate cytotoxicity against P388 murine leukemia cells (IC_{50} = 22.4 μM). New metabolites (chojalactones A and B) identified after co-cultivation of *Streptomyces* sp. CJ5 and *T. pulmonis* TP-B0596 also had cytotoxic activity against P338 murine leukemia cells [48]. Thus, the IC_{50} values of chojalactone A stereoisomers were 28–37, and those of chojalactone B stereoisomers were 17–18 μM.

Table 2 summarizes the data on the synthesis of new metabolites with antimicrobial and cytotoxic activity during the co-cultivation of fungi, bacteria, and fungi with bacteria. These data indicate that the largest number of new metabolites with potent cytotoxic activity was identified as a result of the co-cultivation of fungi.

Figure 2 illustrated the classes of new metabolites with anticancer activity synthesized during the co-cultivation of

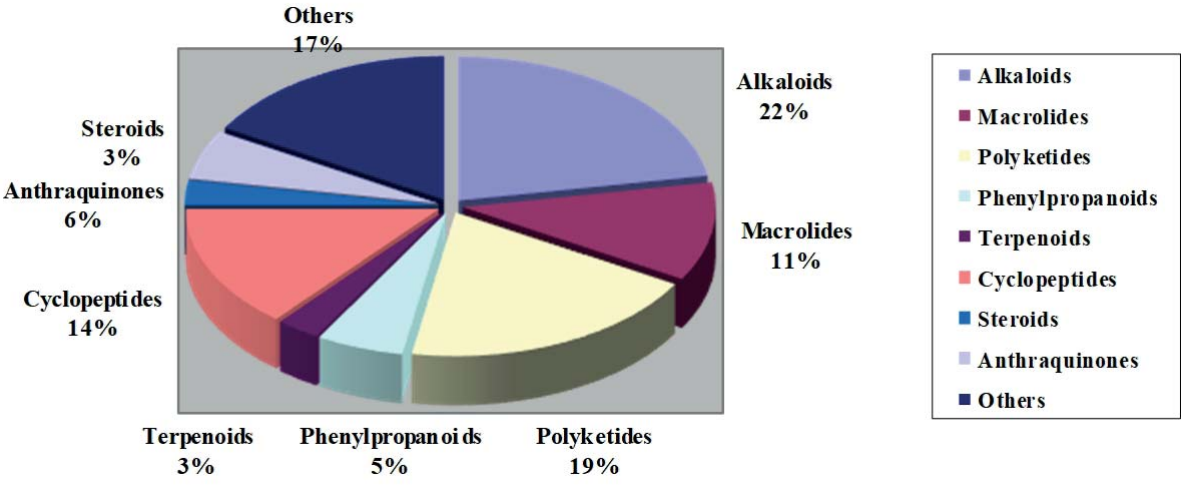

Fig. 2. **Number of new metabolites with cytotoxic activity synthesized as a result of co-cultivation of microorganisms [11, 32–48]**

Table 2

Characterization of new compounds with antimicrobial and cytotoxic activity, formed as a result of combined cultivation of microorganisms

Microorganisms	Carbon source	Compounds	Cytotoxic and antitumor activity	Test-cultures for determining antimicrobial activity	Minimum inhibitory concentration, μg/ml	References
1	2	3	4	5	6	7
Co-cultivation of fungal strains						
Ovadendron sulphureoochraceum MIC 5759 + *Ascochyta pisi* MIC 5620 + *Emericellopsis minima* MIC 5835 + *Cylindrocarpon destructans* MIC 5638 + *Fusarium oxysporum* MIC 5789	Malt extract, Maltose, Dextrose, Yeast autolysate	Lateritin N-methylated peptide	Cytotoxic activity against human tumor cell lines (BXPC-3, MCF-7, CNS SF268, NSC H460, KM20L2 and DU-145) with IC_{50} in the range of 1.7-2.0 μg/mL and against mouse leukemia P388 cells 1.8 μg/mL	*Candida albicans* ATCC 90028	4–8	[36]
				Micrococcus luteus Presque Isle 456	2–4	
				Staphylococcus aureus ATCC 29213	4–8	
				Enterococcus faecalis ATCC 29212	8	
				Streptococcus pneumoniae ATCC 6303	8-16	
Penicillium fuscum (Sopp) Raper & Thom + *Penicillium camembertii/ clavigerum* Thom	Potato-dextrose medium	Berkeleylactone A Monocyclic macrolide $C_{19}H_{32}O_7S$ Berkeleylactone A26771B macrolide $C_{20}H_{30}O_7$ Berkeleylactone C macrolide $C_{20}H_{30}O_8$ Berkeleylactone F macrolide $C_{16}H_{28}O_5$	The IC_{50} value for the leukemia cell line K-562 was 10 μM, causing inhibition (85%) and lethality (2.4%) of the cells The IC_{50} value of RPMI-8226 leukemia cells was 10 μM, and caused inhibition of cells (48%) The IC_{50}value of CCRF-CEM leukemia cells was 10 μM, causing (48%) of cells, as well as inhibition (46%) of K-562 leukemia cells The IC_{50} value of CCRF-CEM leukemia cells was 10 μM, causing inhibition (38%) of cells	*Staphylococcus aureus* 13709	1/3/6/19	[11]
				Streptococcus pyogene	3/48/26/150	
				Candida glabrata	6/48/26/>300	
				Bacillus subtilis	13/12/26/>300	
				Candida albicans	26/96/50/>300	
				Bacillus anthracis	3/6/6/75 * — MIC Berkeleylactone A / A26771B / C / F	

Table 2 (Continued)

1	2	3	4	5	6	7
Aspergillus fischeri NRRL 181 + *Xylaria flabelliformis* G536	Oatmeal medium	Wheldone macrolide $C_{25}H_{34}O_6$	Cytotoxic activity against breast cancer cells MDA-MB-231, ovarian cancer cells OVCAR-3, human melanoma cells MDA-MB-435 with IC_{50} values of 7.6, 3.8 and 2.4 μM	Does not exhibit antimicrobial activity		[38]
Aspergillus sulphureus KMM4640 + *Isaria felina* KMM4639	Rice medium	Diorcinol J Diphenyl ether $C_{19}H_{22}O_4$	The IC_{50} value for mouse Ehrlich carcinoma cells was 37.6 μM	Does not exhibit antimicrobial activity		[37]
Aspergillus sulphureus KMM4640 + *Isaria felina* KMM4639	Rice medium	17-O-ethylno-toamide M Alkaloid $C_{28}H_{35}N_3O_5$	The IC_{50} value for human prostate cancer cells 22Rv1 was 10 μM	Does not exhibit antimicrobial activity		[32]
Epicoccum sp. YUD 17002 + *Armillaria* sp.	Potato dextrose medium	Epicoterpene A-E Sesqui-terpenoids Armilliphatic A Aryl ester $C_{23}H_{28}O_5Cl$	Armilliphatic A had cytotoxicity against five human cancer cell lines (HL-60, A549, MCF-7, SMMC-7721, and SW480) with IC_{50} values ranging from 15.80 to 23.03 μM	Does not exhibit antimicrobial activity		[34]
Talaromyces aculeatus + *Penicillium variabile*	Maltose medium	Penitalarins A–C Polyketides Nafuredin B Polyketide $C_{22}H_{32}O_3Na$	The IC_{50} values of six human cancer cell lines (HeLa, K562, HCT-116, HL-6.0 A549, MCF-7) were in the range of 1.2 — 9.8 μM for nafuredin B	Do not exhibit antimicrobial activity		[35]
Aspergillus sclerotiorum SCSGAF 0053 + *Penicillium citrinum* SCSGAF 0052	Starch, Glucose, Peptone	Sclerotiorumin A Alkaloid $C_{14}H_{14}O_5$ Aluminium-neohydroxy-aspergillin $C_{36}H_{57}AlN_6O_9$	The IC_{50} value of human histiocytic lymphoma U937 cell line was 4.2 μM for aluminiumneohydroxyaspergillin	Do not exhibit antimicrobial activity		[33]

Table 2 (Continued)

1	2	3	4	5	6	7
Co-cultivation of bacterial strains						
Actinokineospora sp. EG49 + *Nocardiopsis* sp. RV163	ISP 2 medium	1,6-dihydroxy-phenazine Alkaloid $C_{12}H_8N_2O_2$	The IC_{50} value of human parasites Trypanosoma brucei TC 221 was 19 μM	*Bacillus* sp. P25	Growth inhibition zone of 11 mm (10 μg/disc)	[45]
				Actinokineo-spora sp. EG49	Growth inhibition zone of 15 mm (10 μg/disc)	
Streptomyces sp. CJ5 + *Tsukamurella pulmonis* TP-B0596	Not given	Chojalactone A Contains 2-hydroxy-3-methyl-γ-butyrolactone fragment $C_{13}H_{16}O_4$ Chojalactone B Contains 2-hydroxy-3-methyl-γ-butyrolactone $C_{13}H_{14}O_4$	IC_{50} values for murine leukemia cells P338 of two stereoisomers of choyalactone A was 37 and 28 μM IC_{50} values for murine leukemia cells P338 of two stereoisomers of choyalactone B was 18 and 17 μM	Do not exhibit antimicrobial activity		[48]
Streptomyces sp. MA37 + *Pseudomonas* sp.	Yeast extract, malt extract, glucose	Indole carbazole alkaloid BE-13793C $C_{20}H_{11}N_3O_2$	Antiproliferative activity against human colon carcinoma HT-29 cells (ATCC HTB-38), with an IC_{50} value of 3.16 μM	*Enterococcus faecalis* ATCC 29,212	>140	[44]
				Staphylococcus aureus ATCC 25,923	>140	
				Streptococcus B. ATCC 12,386	>140	
				Escherichia coli ATCC 25,922	>140	
				Pseudomonas aeruginosa ATCC 27,853	>140	

Table 2 (Continued)

1	2	3	4	5	6	7
Streptomyces sp. JB5 + *Bacillus* sp. GN1	Yeast extract, malt extract, glucose	Dentigerumycin E cyclic peptide $C_{39}H_{63}N_9O_{16}$	IC_{50} values of cancer cell lines, A549 (lung cancer), HCT116 (colorectal cancer), MDA-MB-231 (breast cancer), SK-HEP-1 (liver cancer) and SNU638 (gastric cancer) were 38, 28, 28, 27, 39 μM, respectively	Does not exhibit antimicrobial activity		[46]
Catenuloplanes sp. RD067331 + *Tsukamurella pulmonis* TP-B0596	Starch, Soybean flour, Yeast extract	Catenulobactin A Heterocyclic Peptide $C_{27}H_{31}N_4O_9$ Catenulobactin B $C_{27}H_{31}N_4O_9$	The IC_{50} value of P388 mouse leukemia cells was 22.4 μM	Do not exhibit antimicrobial activity		[47]
Co-cultivation of fungi and bacteria						
Dietzia sp. UR66 + *Saccharomonospora* sp. UR22	Malt extract, dextrose, yeast extract	Saccharo-monosporin A Brominated oxoindole alkaloid $C_{19}H_{15}O_2N_2Br$	Antiproliferative activity against human colon adenocarcinoma NT-29 and human promyelocytic leukemia HL-60 (IC_{50} = 3.6 and 2.8 μM, respectively)	Does not exhibit antimicrobial activity		[39]
Trichoderma sp. 307 + *Acinetobacter johnsonii* B2	Malt extract, dextrose, yeast extract	Botryorodin H Depsidone Phenylpropanoid $C_{22}H_{18}O_6$	IC_{50} values of rat prolactinoma cell lines MMQ and rat pituitary adenoma GH3 were 3.09 and 64 μM, respectively	Does not exhibit antimicrobial activity		[40]
Aspergillus versicolor + *Bacillus subtilis*	Rice medium	Coteslosin C Cyclopeptide 22-epi-afla-chinolone B Phenylpropanoid Versicolorin B Anthraquinones 6,8-O-dimethyl-bipolarin Anthraquinones Diorcinol D Diorcinol G Diorcinol I	The IC_{50} value of rat lymphoma cell lines L5178Y was 5.8, 2.2, 2.3 μM for O-demethylsterigmatocystin, sterigmatocystin, sterigmatin	*Staphylococcus aureus* ATCC 29213	25/12.5/50*	[41]
				Enterococcus faecalis ATCC 29212	50/12.5/50*	
				Enterococcus faecalis ATCC 51299	50/12.5/50*	
				Enterococcus faecalis ATCC 35667	12.5/12.5/25* * - MIC Diorcinol D/ G/ I	

Table 2 (End)

1	2	3	4	5	6	7
Aspergillus ochraceus + *Bacillus subtilis*	Rice medium	Ochraspergillic acids A, B Viomellein Ochratoxin B	The IC_{50} value of human ovarian carcinoma A2780 cells was 5.0 and 3.0 μM for viomellein and ochratoxin B, respectively	Do not exhibit antimicrobial activity		[42]
Bionectria sp. + *Streptomyces lividans* TK24	Rice medium	1,2-dihydro-pheno-pyrazine Alkaloid $C_{13}H_{16}NO_2$ Penicolinate A Steroid $C_{24}H_{32}O_4N_2$	The IC_{50} of human ovarian cancer cell line A2780 4.1 μM for penicolinate A	Does not exhibit antimicrobial activity		[43]

microorganisms. Metabolites synthesized from co-cultures of bacteria and fungi were identified as alkaloids, cyclopeptides, phenylpropanoids, polyketides, macrolides, steroids, terpenoids, anthraquinones, with alkaloids, cyclopeptides, and polyketides taking the largest part.

As a result of the co-cultivation of microorganisms, a large number of new biologically active secondary metabolites have been obtained. In particular, 77 new metabolites that are not typical for monocultures have been identified (see Tables 1, 2). 29 compounds were isolated as a result of co-cultivation of fungi; 31 compounds were isolated from co-culture of fungi and bacteria, and a total of 17 compounds were isolated from co-culture of bacteria. The largest group (41% of all metabolites) was compounds identified after co-cultivation of fungi and bacteria. The largest number of novel metabolites was found as alkaloids (≥42%), and the smallest (<3%) as steroids. Most of the new compounds of different chemical structures were found as a result of co-cultivation of *Aspergillus* spp. and *Penicillium* spp. fungi with various bacterial strains.

The synthesis of most of the novel compounds is based on a protective mechanism, which results in the activation of silent genes for their biosynthesis. At the same time, it is impossible to predict which clusters of biosynthetic genes will be expressed or what types of molecules will be synthesized during co-cultivation of microorganisms.

The methods of co-cultivation of fungi and bacteria mentioned in this review certainly limit the variety of novel compounds synthesized. Therefore, increasing the diversity of microorganisms used, for example, by using amoebas or phages for co-cultivation, may be a promising step in future research. In addition, in order to understand the complex regulation of secondary metabolism and to determine the possibilities of genetic engineering to induce or enhance the synthesis of target secondary metabolites, it is necessary to establish all the mechanisms that ensure the formation of new compounds during co-cultivation of microorganisms.

Financing

The work was carried out within the framework of the State budget scientific topic of the Department of Biotechnology and Microbiology of the National University of Food Technologies "Biotechnological potential of microorganisms of natural and man-made ecosystems (2019–2023, State registration number 0119U001485).

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КОМБІНОВАНЕ КУЛЬТИВУВАННЯ МІКРООРГАНІЗМІВ: УТВОРЕННЯ НОВИХ МЕТАБОЛІТІВ З РІЗНОЮ БІОЛОГІЧНОЮ АКТИВНІСТЮ

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Останнім часом через надмірне та необґрунтоване використання антибіотиків антибіотикорезистентність стала найгострішою проблемою людства. Одним з ефективних підходів до відкриття нових вторинних антимікробних метаболітів є спільне культивування мікроорганізмів, за якого продуцент цільового продукту вирощується разом із конкурентними мікроорганізмами (індукторами), у відповідь на наявність яких відбувається активація мовчазних біосинтетичних генів штаму-продуцента і спостерігається підвищення біологічної активності синтезованих вторинних метаболітів та/або навіть утворення нових сполук. В огляді наведено сучасні дані літератури щодо спільного культивування продуцентів антимікробних сполук з конкурентними мікроорганізмами, результатом якого є синтез нових, не характерних для монокультур, метаболітів з антимікробною та цитотоксичною активністю. Під час спільного культивування грибів, бактерій, а також грибів з бактеріями спостерігається утворення нових антимікробних та протипухлинних метаболітів, які належать до алкалоїдів, фенілпропаноїдів, макролідів, полікетидів, циклопептидів, терпеноїдів, антрахінонів, стероїдів. Наведені дані свідчать про те, що комбіноване культивування мікроорганізмів є простим, дешевим та достатньо ефективним способом отримання нових метаболітів, перспективних для використання у медицині.

Ключові слова: спільне культивування; антимікробні препарати; протипухлинні агенти.

UDC 631.147.99 https://doi.org/10.15407/biotech16.01.040

MAIN ASPECTS OF THE MANUFACTURER OF ORGANIC PRODUCTS IN UKRAINE

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Received 2022/12/29
Revised 2023/01/20
Accepted 2023/02/28

The article is devoted to highlighting the state and prospects for the development of organic production in Ukraine. The main requirements for the production, classification and labeling of organic products of animal and plant origin are presented. The current legal norms governing their certification and circulation are emphasized. The key provisions regarding evaluation and regulation in this field of activity are reflected. The stages of improvement of the domestic legislative framework of organic production are shown on the way of adaptation to European standards.

Purpose. To highlight the state and prospects for the development of organic production in Ukraine and the improvement of the legislative framework of organic production on the way to adaptation to European standards.

Materials and methods. Methodical analysis and abstract-logical method for summarizing the criteria for evaluating the formation, development and integration of domestic organic production into the structure of the world production of safe products of animal husbandry and crop production.

Results. The article describes in detail the development of the organic movement, which is a promising lever for food security in Ukraine. Therefore, the work on the legal regulation of the activities of domestic producers of organic products does not stop. The legislation is improving in the direction of introducing effective state support in this area at the regional and national levels. Of course, organic feed production, animal husbandry and crop production will continue to exist in parallel with non-organic production. However, the principles and relationship of these systems will depend significantly on the availability of energy sources, plant protection products, fertilizers, soil fertility, care for the preservation of the natural environment, ensuring welfare population and its needs in healthy nutrition. In addition, for the restoration of agricultural lands, demining and bioremediation with the use of bacterial and phytoremediation of soil and water resources should be applied. For this, after the liberation of our state, a return to the peaceful management of the national economy is necessary. We believe in the victory and restoration of Ukraine with the help of allied states and people of good will.

Key words: organic products; certification; marking; European standards; legal principles of organic market regulation.

One of the conditions for Ukraine's accession to the European Union is compliance with the requirements of international standards in the production of agricultural products [1]. For the certification of any organic product, safety criteria must be taken into account, while legal acts are used, which establish requirements for state regulation in the field of its production [2]. Legal norms are often overlooked and state regulation of the production of organic products relies on business standards, which are easier to apply and harmonize with international requirements. In this regard, in 2014 the Verkhovna Rada of Ukraine adopted the Law "On Standardization", and in 2017 — the Law "On State Control of Compliance with the Legislation on Food Products, Feeds, Byproducts of Animal Origin, Health and animal welfare" [3]. According to these Laws,

the effect of current national and international standards on agricultural products was simultaneously recognized in our country, along with this, their harmonization took place — in accordance with European norms, the basic principles in the field of agricultural production and criteria for control over the quality and safety of products were defined, and the requirements were regulated regarding environmental protection.

The quality of products of animal origin is definitely affected by the standards relating to feed, compound feed and feed additives. The quality of agricultural products depends on the machines and equipment used in the production process, as well as on the use of fertilizers, technologies for growing, harvesting, transporting and storing the crop, methods of its processing and sale [4, 5]. In this regard, technical committees for standardization constantly update the requirements for evaluating quality indicators and technological characteristics of manufactured products, make corrections in the division into commodity varieties, classes or groups. When developing standards for livestock products, the effect of biological, chemical, and soil factors must be taken into account [6].

The increase in requirements encourages manufacturers to reorient themselves to international standards, to produce higher quality and safer products, along with this, the share of farms engaged in organic production is growing. However, the development of this segment of production faces certain difficulties, the biggest problem is the low purchasing power of the population and the level of consumer awareness of the range of organic products, therefore domestic production facilities are mainly oriented towards export [7, 8]. This is facilitated by the conclusion of the Association Agreement with the European Union in June 2014 and the introduction of a free trade zone between Ukraine and the EU in January 2016, and with Canada in August 2017. It should be noted that in January 2021, the "Agreement on political cooperation, free trade and partnership between Ukraine and the United Kingdom of Great Britain and Northern Ireland» entered into force. It is known that Great Britain is the TOP importer of Ukrainian organic products and is currently introducing its own organic certification system, which accordingly increases the requirements for Ukrainian products [9].

Obviously, in the future, the volumes of organic production will not only grow in the world, but will also occupy a significant share in the agro-industrial complex of our country. Thus, the combination of traditional and organic production systems with science-based technologies will contribute to the development of Ukraine's economy.

To highlight the state and prospects for the development of organic production in Ukraine and the improvement of the legislative framework of organic production on the way to adaptation to European standards.

Materials and Methods

Methodical analysis and abstract-logical method for summarizing the criteria for evaluating the formation, development and integration of domestic organic production into the structure of the world production of safe products of animal husbandry and crop production.

Results and Discussion

According to research by the European Crop Protection Association (ECPA), 45% of respondents prefer to purchase organic products, but only 28% have an idea of what is actually included in the term «organic» and what fertilizers and plant protection agents are used in organic production [10]. According to the definition of the International Federation of Organic Agricultural Movement (IFOAM — Organics International), "organic agriculture is a production system that supports the condition of soils, ecosystems and people. It depends on ecological processes, biodiversity and natural cycles that are characteristic of local conditions, but avoid the use of non-renewable resources. Organic agriculture combines traditions, innovations and science to improve the state of the environment, to develop relationships and ensure a decent standard of living" [7].

In general, all organic production is based on obtaining ecologically safe products, using substances and processes of natural origin without genetically modified organisms (GMOs) and non-specific chemical elements, and animal rations fully include organic feed [11]. This usually requires rational forms of production management, the introduction of resource-saving technologies, the use of harmless processing methods that ensure the preservation of the organic integrity and nutritional value of the product at all stages of the production process, the acquisition of the latest equipment, the use of microbiological preparations, the creation of highly productive varieties and hybrids of plants, new breeds of animals and birds [4, 12, 13].

The production of organic products involves methods that:

• provide for the use of food products, fodder, additives, fertilizers, plant and soil protection preparations, seeds, microorganisms and livestock products free from GMOs and their derivatives;

• exclude preservatives, chemically synthesized dyes, flavorings, stabilizers, flavor enhancers, hormones, antibiotics and growth stimulants;

• not related to the use of ionizing radiation treatment of raw materials or feed;

• use living organisms and mechanical production;

• provide plant nutrition through the soil system;

• include preventive measures [2].

Products that contain at least 95% by weight of organic ingredients (not including the share of water and kitchen salt) and up to 5% of permitted inorganic components in the maximum permissible quantities are considered organic, and this is confirmed by a certificate, a state logo with the inscription «organic product» (Fig. 1) [5, 6]. The logo of organic products is not allowed to label products obtained through non-organic production [14]. Transportation of such products takes place in packaging, containers or vehicles, which makes it impossible to replace them without damage to the seal, they are stored in warehouses separately from inorganic ones.

In the countries of the European Union, the official logo "Eurolist" has been adopted to denote organic products (Fig. 2). Products of animal husbandry, beekeeping, aquaculture, crop production, forest products, etc., are subject to labeling, provided there is a certificate of conformity issued by certification bodies [10]. In order to inform the consumer, the packaging is marked in accordance with the legally defined standards. The label contains information on the code number of the control body, the method of organic production, and data on the country of origin of the organic product [15].

In addition to the fact that organic production is based on the principles of public health protection, it provides for the protection of the environment, cares for the preservation of the soil structure, and promotes the development of flora and fauna [16]. In general, organic and ecological certification systems are fixed at the legislative level and are interconnected, although they have different requirements for assessment bodies and criteria for determining the conformity of products. The logo of the environmental certification body, which is used to mark goods and services, is a "green crane" (Fig. 3) [6].In organic production, it is very important to adhere to current canons and produce products according to standards. In various countries of the world, it is customary to use the following private logos (Fig. 4) [17] to denote organic products.

In the transition period before the full implementation of the system, 85% of feed for ruminants and 80% of feed for non-ruminant animals must be produced according to organic production standards with the use of approved additives and substances [11]. During the processing of livestock products, it is allowed to use antioxidants, dyes and flavorings of natural origin, enzymes, probiotics and microorganisms are not prohibited. Marking of such products is carried out with the state logo "product at the stage of transition to organic production" [18].

It should be noted that for the first time domestic organic products appeared on the shelves relatively recently. This was facilitated by the creation in 2007 of the Ukrainian accredited body — "Organic Standard", which certifies organic products [7, 19]. Until now, the certification of Ukrainian enterprises working in this sector of production was carried out by foreign organizations. Therefore, Organic Standard Limited Liability Company (LLC) became the first and only domestic certification company in Ukraine with its own logo (Fig. 5).

Fig. 1. **Ukrainian logo for marking organic products**

Fig. 2. **Logo of the European Union for labeling organic products**

"The Organic Standard united the leading Ukrainian organizations working in the field of organic production".

The mission of "Organic Standard" LLC is work related to the certification of organic agriculture and animal husbandry. The company provides producers and consumers of organic products with professional certification and information services, promotes the development of domestic and foreign organic markets, ensures their financial stability and profitability [19]. With this in mind, international experience is implemented for certification on a permanent basis.

In general, the creation of standardization bodies in Ukraine became possible thanks to the international technical and financial support of Switzerland, in particular the State Secretariat for Economic Affairs (SECO) and the conclusion in 1997 between the governments of both countries of the Treaty on Cooperation, according to which the development of the joint Ukrainian-Swiss project "Development organic market in Ukraine" [20]. Since 2019, Ukraine has been participating in the implementation of the joint program with Switzerland "Development of trade with higher added value in the organic and dairy sectors of Ukraine" (Quality FOOD Trade Program) [17].

Today, "Organic Standard" LLC is included in the official list of certification bodies that are recognized not only in Switzerland, but also in a number of European Union states, is a member of the Association of Accredited Certification Bodies (IASC) and the European Association of Organic Certification Bodies (EOCC). According to ISO 65 standards, it is accredited by the International Federation of Organic Agricultural Movements (IOAS) [30, 36]. The responsibilities of "Organic Standart" LLC include the certification of products of animal husbandry, beekeeping, aquaculture, crop production, wild plants, processing and marketing products, plant protection products and fertilizers, which are allowed to be used in organic production and according to the assessment meet the requirements of Ukrainian standards "BIOLan" and international standards: Switzerland, USA, Japan, and EU countries [15, 22, 23].

It should be noted that «BIOLan» standards regarding organic production and labeling of products appeared due to the adoption by the Council of the European Union of Resolution No. 834/2007, which concerns organic production and labeling of organic products [9, 10]. At the same time, the basic standards of the International Federation of Organic Agriculture and Bio Suisse Organic Standards from the Association of Swiss Organic Producers were taken as a basis [24]. Also, the Ministry of Economic Development, Trade and Agriculture has developed a regulatory framework for organic production, which was formed with the technical support of Germany. Considering that Germany is the leader in Europe and the second country in the world in terms of sales of organic products, in 2016 the joint Ukrainian-German project "Organic Agriculture" was initiated, thanks to which a platform was developed for the training of specialists in this field [25]. Since then, the German Federal Ministry of Food and Agriculture has initiated financial support in Ukraine for five cooperation projects aimed at introducing modern technologies in this field.

Obviously, the further development of the organic movement was facilitated by the adoption in September 2013 of the Law of Ukraine 425-VII "On the Production and Circulation of Organic Agricultural Products and Raw Materials", which entered into force in January 2014. The document regulated

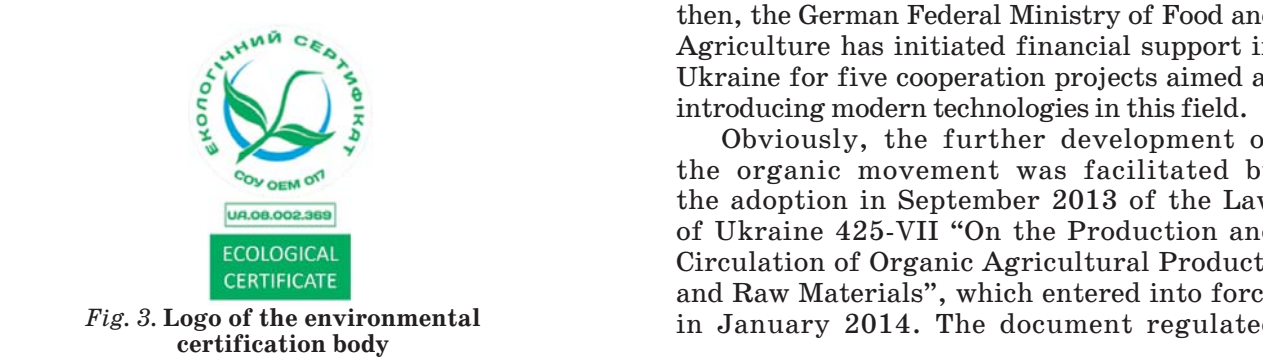

Fig. 3. **Logo of the environmental certification body**

Fig. 4. **Logos of regional, national and private organic labeling systems**

Fig. 5. **Logo of the first Ukrainian accredited standardization body**

the basics of conducting organic agriculture, defined the requirements for cultivation , production and processing of organic products, their transportation, storage, labeling, certification and sale [26]. However, the ineffective system of protecting consumers from low-quality and dangerous food products, the imperfection of certification bodies, as well as the difficulties of state bodies in monitoring the quality of products and detecting counterfeits — significantly slowed down the progress of the Ukrainian organic movement, and created great competition between producers and sellers of pseudo-organic products , it was used in the domestic market and entered foreign markets, which discredited the domestic industry at the international level.

An important aspect in the growth of the organic market is the development of a legal framework to ensure the activities of domestic producers in accordance with organic standards and under the control of certification bodies. Until August 2019, the provisions of the Law of Ukraine "On the Production and Circulation of Organic Agricultural Products and Raw Materials" adopted in 2013 were in force. However, its obvious imperfection and the desire to adapt Ukrainian legislation to the requirements of the legal regulation of the European Union prompted specialists in this field, with the support of the public sector and the executive power, to simultaneously develop and adopt in July 2018 Law of Ukraine 2496-VIII "On the Basic Principles and Requirements for Organic production, circulation and labeling of organic products" [27]. European Union directives on organic production were written into the new Law, which made it possible to better implement European criteria into the domestic legal framework.

This Law regulated the norms of labeling and circulation of organic products. Its provisions improved the principles of certification and changed the requirements for certification and inspection bodies, established responsibility for violations of legislative principles in the field of production, circulation and labeling of organic products. Instead, articles were removed from the legislation in accordance with international legal norms, which were supposed to carry out a preliminary assessment of the suitability of land for organic production. Objects of organic crop production (seed production and nursery production), livestock production (poultry farming, beekeeping), mushroom production (including yeast production), aquaculture, seaweed, food products (wine production) and fodder are covered by the Law [3]. The activities of the certification bodies have extended to the primary production of organic products, including their harvesting, harvesting, preparation, processing, processing, mixing, restoration, filling and packaging. The law regulated relations in the field of production of organic products that are in circulation, imported or exported through the customs territory of Ukraine.

In this context, in 2020, the State Service of Ukraine for Food Safety and Consumer Protection (State Food and Consumer Service) launched the List of foreign certification bodies [28, 29]. Central executive bodies were entrusted with the responsibilities of forming and implementing state regulation of the safety and quality of organic products, maintaining the List of foreign bodies and the Register of domestic certification bodies, exercising control over organic market entities. According to the current Law, the subject of certification of organic production, circulation and labeling, whose organic products meet the standards recognized by other states or international organizations, can additionally conclude an agreement with foreign certification bodies available in the List [27]. At the same time, it is allowed to conclude a contract for one branch of organic production with only one domestic certification body. The certificate is valid for 15 months from the date of issue, and 6 months before the expiration date, the manufacturer informs the certification body of the intention to renew it. Distributors of such products must have originals or copies of certificates that allow establishing their origin and compliance with the requirements of legislation in the field of organic production.

Organic market operators (producers) undergo annual monitoring, scheduled and unscheduled control by the State Production and Consumer Service regarding compliance with organic production standards [28]. The activity of operators is periodically checked by taking samples of finished products or raw materials for laboratory tests. If it does not meet the requirements of the legislation, the supervisory

body issues a prescription to eliminate violations or acts (resolutions, decisions) regarding the recall and/or removal of such products.

According to the Law, for the sale or introduction into circulation of products that do not have a certificate of compliance with the requirements of organic production, circulation or labeling, as well as for violators who did not fulfill or untimely fulfilled the prescriptions or administrative acts of state supervisory bodies — legal entities are subject to a fine in the amount of eight, and five minimum wages for individual entrepreneurs [3, 9]. For the absence, untimely provision of information or false information regarding the volumes of organic products put into circulation, fines are also provided — for legal entities five, and for individual entrepreneurs — in the amount of three minimum wages.

Certification bodies are also responsible in the field of production, circulation and labeling of organic products: for failure to provide, untimely provision or inaccurate information about issued certificates — a fine of five, for non-fulfillment or untimely fulfillment of prescriptions or administrative acts to eliminate violations of legal requirements — eight , for illegal issuance of a certificate — sixteen, and for repeated illegal issuance of a certificate — in the amount of twenty-four minimum wages [9].

As the analysis showed, the volume of consumption of organic products increases every year, accordingly, organic production grows (Fig. 6), and the pace of its development in Ukraine is 4.9 times higher, in accordance with the world and 5.5 times more than in Europe [30]. In general, the domestic Ukrainian consumer market of organic products is estimated at almost 18 million euros, while per capita consumption did not exceed 0.5 euros. On the other hand, the export potential of our country in this sector of the economy is about 50 million euros and according to the estimate of "Organic Standard" LLC, it may reach 150 million euros in the future [19].More than 400 types of organic products are produced in Ukraine, 10% of the sales volume is on the domestic market and 90% is on export [31]. According to the data of "Organic Standart" LLC, the main importing countries of Ukrainian organic products are: the Netherlands, Germany, Great Britain, Austria, Poland, Italy, Switzerland, Belgium, the Czech Republic, Bulgaria, France, Denmark, the USA and Canada, in total more than 200 countries of the world [19]. Ukraine ranks 4th in the world in terms of export of organic products to the European Union, after Ecuador, the Dominican Republic and China [7].

The main exported products are: grains (wheat, spelled, barley, rye, oats, millet), oilseeds (sunflower, corn, rapeseed), legumes (soy, peas), nuts, seeds of pumpkins, sunflower, flax, mushrooms, berries, fruits, medicinal herbs, spices, a total of over 80 names of organic products [8, 32]. About 99% of organic wheat grain goes to the EU countries. According to the data of the independent international Research Institute of Organic Agriculture (FiBL), there is a dynamic trend towards an increase in the share of semi-finished and processed products: cereals, flour, pasta, dairy and meat products, juices, concentrates, jam, oils, tea, chocolate [20].

In 2017, Ukraine exported organic honey for the first time, and in the following years, the volume of its supply amounted to more than 300 tons [30]. On the European market, the main exporter of organic sugar is LLC "Deddens Agro", its sales volume exceeds 800 tons. The supply of organic raspberries to the European Union has reached 400 tons in recent years, and the export of blueberries, elderberries, and blueberries has been established [7, 8].

Another driver of the organic market of Ukraine was the Law "On Amendments to the Law of Ukraine "On State Support of the Agriculture of Ukraine" and other laws of Ukraine regarding the functioning of the State Agrarian Register and improvement of state

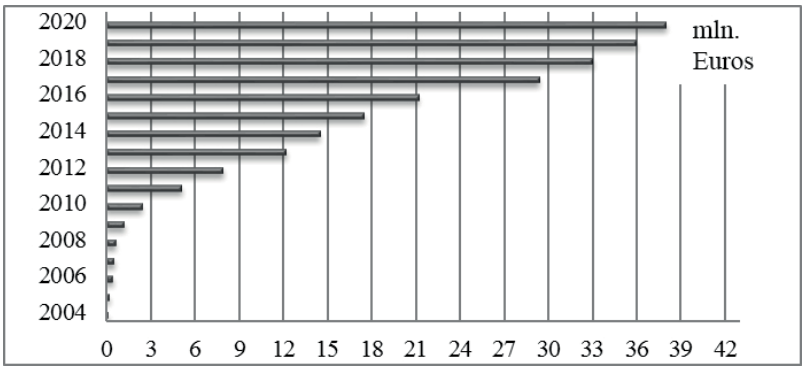

Fig. 6. **According to the data of IFOAM and the Federation of Organic Movement of Ukraine**

support for producers of agricultural products» adopted in November 2020 [9]. According to the Law, producers of organic products can apply for state subsidies for cultivated land and keeping cattle. Compensation of up to 30% of costs for certification of organic production is provided at the expense of budget funds. State support also refers to reimbursement of up to 30% of the purchase price allowed in this area of plant protection products, seed material, fertilizers and feed.

Today, the structure of Ukrainian organic livestock production is dominated by milk production, in particular, a prominent place in the domestic market is occupied by the certified line of lactose-free products of the company "Organis milk "O" [29]. It should be noted that high production costs make organic meat an expensive product, correspondingly less popular among Ukrainian consumers. In this segment, beef and veal are the most represented — 41%, as well as pork — 28%, chicken has smaller production capacity, because poultry farms traditionally specialize in the production of egg products [31]. However, in this regard, everything is not so clear, organic egg in Ukraine is not among the top five most consumed food products, while in Switzerland it occupies a leading position in terms of consumption [20]. This is due to the small number of producers in the field of organic poultry farming, mostly concentrated in Zhytomyr, Odesa and Khmelnytskyi regions.

Although organic production is in the trend of increasing agricultural land, Ukraine ranks 11th in Europe and 20th — among world countries — among the leaders of the organic movement in terms of the area of organic land [30]. On the other hand, in the Eastern European region, our country ranks first in terms of the area of certified organic arable land, which is primarily used in the cultivation of grain, leguminous and oil crops. More than 48% of Ukrainian certified land is under grain crops, which ranks 7th among organic grain-producing countries, 16% of land is occupied by oil crops — 5th place, and 4.5% by legumes — 7th place in the world [31]. Vegetables are grown on 2% of certified land — this is Ukraine's 10th place in the world, fruits — on 0.6%, and grapes — on 0.1% of land [12]. In general, the area of Ukrainian certified land, which specializes in the production of various organic products, is more than 430 thousand hectares, which is 1% of all agricultural land, while in the countries of the European Union it is more than 6% [9].

According to the report of IFOAM and the Federation of the Organic Movement of Ukraine, there is a positive trend in the growth of areas under organic production, while the number of organic market operators is increasing [7]. The vast majority of Ukrainian operators producing products for export and domestic consumption are certified according to organic standards equivalent to EU Regulations No. 834/2007 and No. 889/2008 [10, 22, 31, 33].

According to the Resolution «On the Approval of the National Economic Strategy for the Period Until 2030» approved by the Cabinet of Ministers of Ukraine in March 2020, it is planned to expand the area of land with organic status to 3% of the land, i.e. to 1.3 million hectares [9]. This corresponds to the key principles of the European Union laid down in the "Farm to Fork" Strategy, which foresee an increase by 2030 of the number of agricultural areas under organic production to 25% and reflected in the Ukrainian Agricultural Sector Project "Ukrainian Green Way from Farm to Fork: Step step by step", which is aimed at the development of rural communities [1].

Official statistics of the Reform Support Office and data from Organic Standart LLC show that in Ukraine over the past 20 years, the number of certified organic farms has increased 17 times and currently totals more than 600 [19]. This was undoubtedly facilitated by the growth in the level of domestic consumption of organic products, mainly juices, oils, honey, jams, syrups, cereals, flour, teas, medicinal herbs, dairy and meat products [8, 32]. According to the Federation of the Organic Movement, the Ukrainian consumer market for organic products has grown 40 times over the past 10 years [30]. Emphasis on the consumption of safe organic products was observed in the years leading up to the pandemic, when the call for sustainable food emerged. During the war, there were significant changes in the agricultural sector of Ukraine in the direction of a decrease in organic production, because many organic farms were concentrated in the Kherson, Zhytomyr, and Kyiv regions.

In contrast to European colleagues, where organic production is mostly inherent to small private farms, in Odesa, Vinnytsia, Poltava, Zakarpattia, Ternopil and Lviv regions there are organic farms that own agricultural land with an area of several to thousands of hectares. They specialize not only in fruit and vegetable and berry products, but also in growing legumes, keeping and breeding farm animals and poultry. The largest area of land (16,000 ha) is owned by the Arnica company [7].

Given the high cost of organic products, greater consumer demand for them is usually

observed among the urban population, which is mainly concerned with the consumption of safe products and has a higher level of income. It should be noted that the price policy in the field of organic production is determined by the high cost of production and processing, which takes into account additional costs for environmental protection, cultivation of agricultural crops, improvement of animal husbandry conditions, measures for the development of rural areas [34]. According to forecasts of the Food and Agricultural Organization of the United Nations (FAO), the profitability of this sector, without setting high prices, will be low, because if organic producers refuse to use traditional means of plant protection and fertilizers in intensive agriculture, they will suffer losses of 30–40%, or even more crop yield due to damage by pests, due to the spread of diseases and in connection with excessive weeding of crops [10, 23]. If during the first five years of the transition to organic farming, the yield of crops decreases, then the cost of maintaining such a field increases by 20–30%, and the cultivation of certain types of plants — by 50%. For some cultures, the return of the invested funds can be expected within 10 years, however, the increase in the price of organic products up to 60% allows to somewhat level the losses [7].

Ukrainian agricultural producers claim that during the transition from an inorganic farming system to an organic one, a high level of crop yield reduction is also caused by a lack of Nitrogen, therefore care should be taken to feed plants, especially to provide organic fertilizers, in particular manure, which, given the real state of animal husbandry today, is problematic [34, 36]. The use of siderates, natural minerals, and compost can improve the situation. Crop contamination is eliminated by introducing soil-protective crop rotations, mulching, and growing cover crops [34]. Chemical means of plant protection should be replaced by biological methods of control, the use of traps, beneficial insects, birds, and safe substances, which at the same time increases the frequency of spraying, correspondingly increases the costs of using fuel, tillage, increases the amount of material and technical means and human resources involved [36]. In addition, taking into account the rate of growth of the population's needs for food products and the absence of unused lands in the field of agriculture, as well as the destruction of the arable fund of Ukrainian lands by the attacks of the aggressor, the prospect of a full transition to organic production is impossible.

A common opinion of consumers in favor of organic products is that producers under the traditional system apply an excessive amount of pesticides to the fields, but this is not the case, because chemicals can not only harm plants, but also have an effect on the following crops in the crop rotation [34]. In fact, Ukrainian agricultural producers use two to three times less plant protection products compared to European ones, this is due not only to their cost, concern for the condition of plants, higher soil fertility — Ukraine is home to a tenth of the world's chernozems (this is about 45% of our territory), but and climatic conditions [29]. Winters that are colder than in some European countries help to reduce the number of pathogens, therefore the need for additional plant protection is eliminated, and therefore safer products are obtained [1, 34].

On the other hand, organic products protect people from pesticides, and farmers from the danger of poisoning during crop spraying [31]. Organic farming promotes the development of soil microflora by more than 30%.

The advantages of organic production also include:

- lack of dependence on mineral fertilizers and toxic chemicals;
- increase of jobs for the rural population;
- creation of local markets for the sale of safe products;
- better taste qualities of produced products;
- conservation of biological diversity;
- minimal impact on the soil;
- reducing the level of air, ground and surface water pollution;
- prohibition of veterinary drugs and food additives for feeding animals, which have a negative effect on health;
- more comfortable conditions for keeping farm animals [13, 35].

Public events significantly stimulate organic production. Thanks to the Federation of the Organic Movement of Ukraine with the support of FiBL, the First All-Ukrainian Fair of Organic Products was held in Lviv in 2009, since then this event has become traditional [36]. In 2020, the Specialized Exhibition-Fair of Organic Products and Technologies "ORGANIC-2020" and the XII All-Ukrainian Fair of Organic Products took place [19]. Exhibitors from more than 80 countries of the world, including 37 companies from Ukraine, presented their products and took part in Nuremberg in the main event of 2021 — the BIOFACH2021 training exhibition [7].

In 2020, the transition to the "online era" took place, a number of conferences and forums were held partly in this format, including the "Organic Ukraine 2020" Congress, the

"Organic: Knowledge, Experience, Results" conference, the VII International Conference "Organic Processing and trade 2020" [7]. In January 2020, the conference "Organic berry business: mistakes 2019 — prospects 2020" was held as part of the International Berry Forum "S-FRUIT TRANSFORMATION", the world's attention was drawn to the workshop "Ancillary products in organic agricultural production" and the report of the FAO Program [19].

At the independent international competition "Favorite Food&Drinks", which was held in Ukraine in 2021, the nomination "Organic products" was introduced for the first time and the victory was won by the domestic certified product Liqberry [30]. In April 2021, at the initiative of the Federation of the Organic Movement of Ukraine and the Ministry of Economic Development, Trade and Agriculture, a round table was organized on the topic: "Supporting organic production in Ukraine: the mechanism of 2021 and new promising directions", at which, in particular, the key directions of implementation were discussed The project "German-Ukrainian cooperation in the field of organic agriculture".

In 2020, Ukrainian exporters of organic products enlisted the support of the State institution "Export Promotion Office of Ukraine", in the same year, with the assistance of FiBL-IFOAM, a number of informational materials and books were published, in particular, "The World of Organic Agriculture. Statistics and new trends 2020" [30]. The source that annually publishes information on accredited entities of organic production is the edition of FiBL — "Organic Business Directory of Ukraine". The Ministry of Economy developed the Organic Map of Ukraine on the basis of operational monitoring data [19].

Since the spread of organic products is facilitated by wide promotion, in this context, on March 25, 2021, the European Parliament, the Council of Europe and the European Commission introduced the observance of September 23 "EU organic day". European Commissioner for Agriculture Janusz Wojciechowski noted during this event that "Organic production is a sustainable type of agriculture, in which food products are produced taking into account the laws of nature, preservation of biodiversity and animal welfare" [9]. On April 4, 2018, the Law of Ukraine "On State Control of Compliance with the Legislation on Food Products, Fodder, Animal By-products, Animal Health and Welfare" entered into force, which provides for the regulation of the legal and organizational principles of state control, which is carried out in order to verify compliance by market operators of the legislation on food products, feed, animal health and welfare, as well as on by-products of animal origin during the importation (forwarding) of such by-products to the customs territory of Ukraine.

Conclusions

The development of the organic movement is a promising lever of Ukraine's food security, accordingly, the work on the legal regulation of the activities of domestic producers of organic products does not stop, the legislation is being improved in the direction of introducing effective state support in this area at the regional and national levels. Today, the main problem of actively implementing the system and joining the Organic movement on the way to adapting to European standards is the conduct of hostilities and the aggressor's mining of Ukrainian agricultural lands, the destruction of livestock farms and agricultural animals, and the killing of farmers during rocket attacks. Therefore, the Ukrainian organic movement needs the support of the world community in order to speed up plans to restore and expand the area of land with organic land status and renew organic production.

Of course, organic feed production, animal husbandry and crop production will continue to exist in parallel with non-organic production, but the principles and relationships of these systems will depend significantly on the availability of energy sources, the availability of plant protection products, fertilizers, soil fertility, care for the preservation of the natural environment, ensuring welfare population and its needs in healthy nutrition. Also, for the restoration of agricultural lands, demining and bioremediation with the use of bacterial and phytoremediation of soil and water resources should be applied, and for this, after the liberation of our state, a return to the peaceful management of the national economy is necessary. We believe in the victory and restoration of Ukraine with the help of allied states and people of good will.

This article contains no human or animal research conducted by any of the authors.

The work was performed within the framework of the salary of an associate professor for the lecture course "Modern application of biotechnological methods" and the departmental fundamental topic of the NDR/TBSFB-20/2019-2023 "Implementation of methodological developments in the educational process of biotechnology and pharmacy" (NU LP); state registration 0119U102350.

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ОСНОВНІ АСПЕКТИ ВИРОБНИЦТВА ОРГАНІЧНОЇ ПРОДУКЦІЇ В УКРАЇНІ

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Стаття присвячена висвітленню стану й перспектив розвитку в Україні органічного виробництва. Представлено основні вимоги до виготовлення, класифікації та маркування органічної продукції тваринного і рослинного походження, наголошується на чинних правових засадах, якими керуються за її сертифікації та обігу, відображено ключові положення щодо оцінювання й регулювання у цій сфері діяльності, показано етапи удосконалення вітчизняної законодавчої бази органічного виробництва на шляху адаптації до європейських стандартів.

Мета. Висвітлити стан та перспективи розвитку в Україні органічного виробництва й удосконалення законодавчої бази органічного виробництва на шляху адаптації до європейських стандартів.

Матеріали та методи. Методичний аналіз й абстрактно-логічний метод для узагальнення критеріїв оцінки становлення, розвитку та інтегрування вітчизняного органічного виробництва в структуру світового виробництва безпечної продукції тваринництва і рослинництва.

Результати. Детально описано розвиток органічного руху, який є перспективним важелем продовольчої безпеки України, і тому, відповідно, не припиняється робота з правового врегулювання діяльності вітчизняних виробників органічної продукції, вдосконалюється законодавство у напрямку запровадження на регіональному та національному рівнях ефективної державної підтримки цієї сфери. Звичайно, органічне виробництво кормів, тваринництво та рослинництво продовжуватимуть існувати паралельно з неорганічним виробництвом, але принципи та взаємозв'язки цих систем суттєво залежатимуть від наявності джерел енергії, наявності засобів захисту рослин, добрив, родючості ґрунту, догляду на збереження навколишнього природного середовища, забезпечення добробуту населення та його потреб у здоровому харчуванні. Також для відновлення агротехнічних угідь, очевидно, слід буде застосувати розмінування та біоремедіацію із застосуванням бактеріальної та фіторемедіації ґрунтів і водних ресурсів, а для цього після звільнення нашої держави необхідно повернення до мирного ведення народного господарства. Віримо у перемогу та відновлення України за допомогою союзних держав і людей доброї волі.

Ключові слова: органічна продукція; сертифікація; маркування; європейські стандарти; біоремедіація; правові засади регулювання органічного ринку.

UDC 664.785.86 https://doi.org/10.15407/biotech16.01.051

ULTRASOUND-ASSISTED AND ENZYMATIC-BASED METHOD FOR ISOLATION OF β-GLUCANS FROM OAT BRAN

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Received 2022/01/11
Revised 2022/02/12
Accepted 2023/02/28

β-Glucans are a group of non-starchy polysaccharides, or (1,3),(1,4)-β-D-glucans, that can be found in the cell walls of several species of bacteria, algae, lichens, fungi, and cereal grains. These carbohydrates are extensively used in food industry, cosmetics, pharmaceuticals and healthcare, therefore optimization of the extraction and isolation of β-glucans from grain sources has an especial importance in various fields of biotechnology, drug design, food science and technology.

The aim of the study was to develop an optimized technological scheme for isolation of β-glucans from oat bran based on ultrasonic and enzymatic processing of raw material.

Materials and methods. β-Glucans were isolated from grinded oat cereals during multi-stage process, which includes extraction of grain fats, hydrobarothermic processing, ultrasonification, enzymatic hydrolysis of concomitant starch and proteins, precipitation of β-glucan fraction by ethanol, centrifugation, and dry-freezing. Yield of β-glucans from raw material and its concentration in the final product were determined after hydrolysis by sulfuric acid or enzymatic cleavage by endo-1,3(4)-β-glucanase.

Results. As shown by acidic hydrolysis of the final product, the yield of β-glucans was 10.8 ± 0.23% and concentration was 79.6 ± 3.89%, while enzymatic hydrolysis gave 8.7 ± 0.82% and 65.1 ± 4.72%, respectively. Thus, the use of hydrobarothermic and ultrasound pre-treatment of raw material in combination with proteolytic digestion of ballast lipids and proteins allowed producing oat β-glucans in amounts comparable with those in case of acid- or alkali-based procedures.

Conclusions. The described technological scheme of β-glucan isolation from oat bran based on sequential hydrobarothermic processing, ultrasonification, and enzymatic removing starch and proteins can be widely used for routine β-glucan production for various purposes in food technology, pharmacological industry, and medicine.

Key words: β-glucan; oat; hydrobarothermic processing; ultrasonification; enzymatic hydrolysis.

β-Glucans are a group of non-starch polysaccharides, in which D-glucose residues are linked by β-(1–4) and β-(1–3) glycosidic bonds. Single β-(1–3) linkages are generally separated by 2 or 3 β-(1–4) linkages but the ratio between β-(1–4) and β-(1–3) linkages differs between various species (Fig. 1) [1].

These polysaccharides are present in the cell walls of many natural sources including bacteria, yeasts, lichens, fungi, algae, edible mushrooms, and cereal grains such as oats, barley, wheat, and rye. β-Glucans are referred to as a type of dietary fibers, which are classically used to boost the immune system and to treat hyperlipidemia [2]. Results of the studies conducted during the past two decades support the suggestion that regular intake of oat β-glucan at daily doses of at least 3 g may reduce plasma total and low-density lipoprotein (LDL) cholesterol levels by 5–10% in normocholesterolemic or hypercholesterolemic subjects. As an effective food supplement with no side effects even at a higher consumption, β-glucans have been reported as generally recognized as safe (GRAS) by U.S. Food and Drug Administration (FDA) [3]. β-Glucans have numerous healthcare properties and have found a variety of applications in human

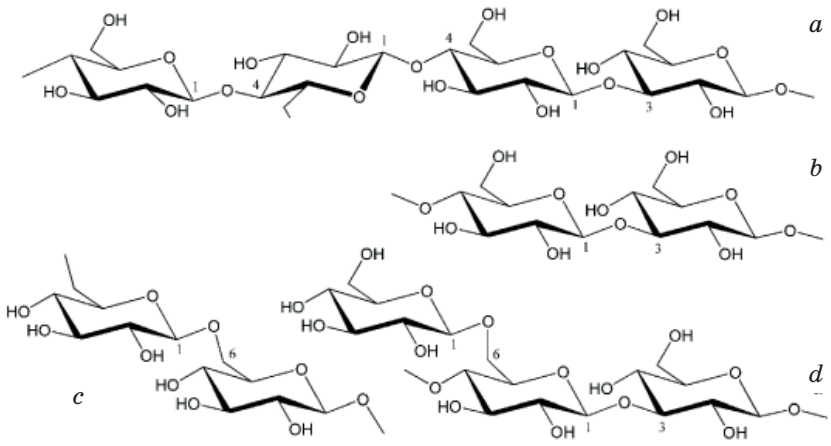

Fig. 1. **Structure of various β-D-glucans:**
a — cereal mixed-linkage (1-3) (1-4)-β-D-glucan, *b* — microbial (1-3)-β-D-glucan,
c — 1-3-β-D-glucan from lichens, *d* — branched fungal or seaweed (1-3) (1-6)-β-D-glucan

and veterinary medicine, pharmaceutical, cosmetic and chemical industries, food and feed production. β-Glucans can be incorporated into various products, such as bread, muffins, pasta, noodles, salad dressings, beverages, soups, and reduced-fat dairy and meat products [4]. Mushroom- and cereal-based foods containing these polysaccharides have been reported to be beneficial for health to display anticarcinogenic, antiviral, anti-inflammatory, prebiotic, antioxidant, neuroprotective, and immunostimulatory properties [5].

The oat (*Avena sativa*) is a well-known cereal and one of the first cultivated plants by humans. This crop is used extensively and currently world oat production is about 22 million tons per year, however less than barley at 170 million tons per year and much less than the 772 million tons of wheat in 2021. Oat groats are rich in protein (usually 13-20% of dry weight), a source of unsaturated fatty acids and contain natural antioxidants such as tocopherols, tocotrienols, sterols, and phenolic acids. Quantitative analysis have shown that β-glucan content in whole oat grains and oat bran products is typically in the range of 2% to 8.5% and from 6% to 12%, respectively [6]. β-Glucans are present in the aleurone cell wall, but their amount is small compared with that in the underlying starchy endosperm, which is the primary storage site of starch, protein, lipid, and β-glucans. β-Glucans are concentrated mainly in bran during the processing of grain crops into flour and cereals [7]. Therefore, oat bran can be used as a raw material for the industrial production of β-glucan concentrates and isolates for food manufacturing, pharmaceutical industry, and cosmetology. This justifies the need for isolating β-glucans in maximally pure form. Thus, studies on the extraction and isolation of β-glucans from oat bran remain of tremendous interest and have outstanding applied significance.

There are different extraction methods for the isolation of β-glucans from grain sources mainly oats and barley [8]. Four classes of extraction methods are described as follows:

i) water extraction,
ii) alkaline extraction,
iii) acidic extraction, and
iv) enzymatic extraction.

As considered, extraction methods for isolating β-glucans involve the use of acid or alkali. Meanwhile, these substances may cause corrosion of equipment, are dangerous in producing process, and toxic to humans. Therefore, the development of technology using softer and more efficient methods of β-glucan extraction is an urgent problem. Further, presence of starch and protein in minor quantities in the final product will not be harmful for human organism as being extracted from edible source. On the contrary, their presence at higher levels may decrease the viscosity of β-glucans and consequently exerts adverse effect on biological activity of these polysaccharides. Hence, it is a challenge for researchers to obtain high yield of the produced component with high purity via removing undesirable impurities preferentially by enzymatic processing of raw material. Thus, the aim of the presents study was to elaborate an optimized method for β-glucan isolation based on ultrasonic and enzymatic processing of the oat bran.

Materials and Methods

Oat bran obtained by processing naked grain oats into flour according to the state

standard (DSTU 4963:2008 Oat Technical Conditions) was used in the study. The bran was preliminarily ground into flour with a particle size of 0.5 mm. For grain fat extraction, oat flour (150 g) was suspended in 50% ethanol (1 l) during 1 h on the water bath at 60 ^{O}C. Then, ethanol extract was separated from defatted bran by centrifugation at 16,000 g for 30 min at 4 ^{O}C. Sedimented bran was resuspended in distilled water (1:5) and heated up to 115 ^{O}C for 1 h. Hydrobarothermic processing of crude material results in gelatinization of oat starch, formation of dextrins, and dissolution of water-soluble proteins of aleurone grains, as well as microbiological sterilization. After cooling, the suspension was sonicated with a density of acoustic energy of 0.5 W/cm^3 for 10 min. Ultrasonification is carried out in a mode that allows generating multi-scale acoustic flows directly in the zone of the mass transfer process, and as a result, a developed system of flows appears in the extractor. It includes such flows from the sizes comparable to the scales of the containing capacity of the extractor to the scales of the hydrodynamic boundary layer (1–10 μm). In this case, ultrasonic action is accompanied by cavitation and, consequently, the appearance of many local impact waves with pressures up to hundreds and thousands of atmospheres. Such an impact on the solid phase leads to a decrease in diffusion resistance inside the solid particle, removes diffusion resistance at the solid phase-liquid interface, and significantly increases the efficiency of the extraction process. During ultrasonic processing, β-glucan is extracted from the cellulose matrix in combination with nutrients, vitamins, microelements and other biologically active compounds. β-Glucan-containing extract forms a gel that prevents the reverse sorption of the extracted substances. As a result, a suspension of cellulose particles with extracted substances is formed.

After hydrobarothermic and sonification procedures, extract of oat bran was incubated with a thermostable α-amylase (EC 3.2.1.1) (Amylase®AG XXL, Novozymes, Sweden, 3,000 Units/ml) for 9 hs at 60 °C, pH 7.0–7.4, and constant agitation for starch degradation (liquefaction). Starch degradation was monitored by measuring glucose concentration with glucose oxidase glucometer until reaching the maximal plateauing concentration of the monosaccharides. Then, fermented mixture was cooled at 40–45 °C and incubated with alkali protease (EC 3.4.21.62) (Protease Subtilisin A, Novozymes, Sweden, 8 Units/mg protein) for 9 hs at pH 8.0 and constant agitation in order to digest plant proteins. Rate of proteolysis was monitored by determining free amino acids by ninhydrin method until saturating concentration [9]. After proteolytic treatment, hydrolysate containing starch and protein monomers and oligomers and cellulose particles was separated from liquid phase by centrifugation at 16,000 g for 30 min at 4 °C. β-Glucan was precipitated from supernatant by ethanol (1:3) during 12 hs at 4 °C, sedimented by centrifugation as mentioned above, and freeze-dried. Determination of β-glucan concentration in the final product was made by acidic hydrolysis with 2 M H_2SO_4 for 30 min followed by measuring reducing carbohydrates as described elsewhere [10]. As an alternative approach, β-glucan concentration was evaluated by enzymatic hydrolysis with non-specific endo-1,3(4)-β-glucanase (EC 3.2.1.6) (E-LICACT, Novozymes, Sweden, 186 Units/mg) for 6 hs at 40 °C, pH 6.5.

The principal technological scheme for β-glucan production from oat bran is presented in Fig. 2.

Procedures of β-glucan isolation were made in triplicate. Quantitative results were expressed as Mean ± SEM (Standard Error of Mean).

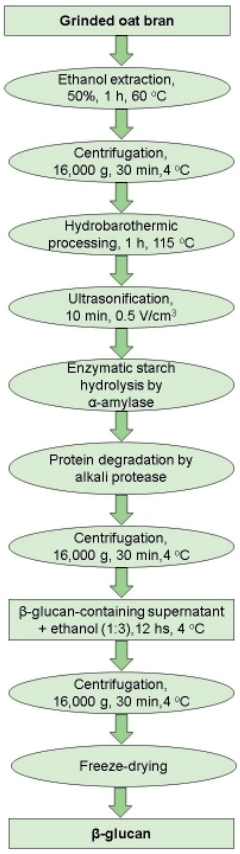

Fig. 2. **Technological scheme for β-glucan isolation from oat bran by ultrasound-assisted and enzymatic processing method**

Results and Discussion

Raw material and final product are depicted in Fig. 3. The yield and concentrations of β-glucans isolated from oat bran are presented in the Table. Some differences in β-glucan concentrations measured after H_2SO_4 hydrolysis and enzymatic degradation can be explained by the fact that acidic hydrolysis could provide total hydrolysis of β-glucans as compared with cleavage by endo-1,3(4)-β-glucanase, which is preferentially is endo-acting enzyme. The content of β-glucan in the final product was obtained as high as ~ 80% that is comparable with β-glucan abundance produced by various methods based on alkalic or acidic treatment of raw material [5, 11]. Moreover, the final product contains less of 5% monosaccharides and trace amount of proteins and amino acids. Thus, combination of hydrobarothermic and ultrasound pre-treatment of raw material followed by its enzymatic processing was shown to be effective strategy for β-glucan extraction and isolation from oat bran. Small amounts of monosaccharides and amino groups make this product potentially usable for many applications.

Summarizing obtained results, it can be assumed that hydrobarothermic treatment of raw material promotes starch gelatinization and dextrinization and contributes to a more complete dissolution of the proteins of the aleurone layer, which makes them more accessible for enzymatic hydrolysis. Ultrasonification step allows extracting β-glucans from cell wall, thus increasing its yield to the final product. In the future experiments, some principal parameters of β-glucans produced by the described methods should be tested because the processing of oats has a crucial impact on the main parameters of β-D-glucans, such as molecular weight (Mw) and viscosity. It has been reported in literature that viscosity of β-glucans in the gut is mainly responsible for its cholesterol lowering effects [7, 12]. Water-solubility and Mw of β-glucans are considered to control availability to immune cells or other biological or biomolecular targets [5, 13].

A *B*

Fig. 3. Oat bran as raw material (*A*) and isolated and freeze-dried β-glucans as a final product (*B*)

Considering the prices of many products, containing β-D-glucans, it should be taken in mind that oats belong to the cheapest grains available in the world. Therefore, oat β-glucans may have extensive industrial applications (foods, medicines, cosmetics, feeds, etc.). Consumption of oat β-glucans has well-documented health benefits [2, 5, 6] (Fig. 4).

β-Glucan is used classically to boost the immune system and to treat hypercholesterolemia [7, 12, 13, 14]. For example, β-glucan can modulate the autoimmune mechanisms directed to pancreatic islets and inhibit the development of diabetes mellitus [15, 16]. It is of interest that in the central nervous system, β-glucans activate microglial cells, which act as scavengers of the brain cell debris and play a protective role in Alzheimer's disease, AIDS, ischemia injury, and multiple sclerosis [5, 16, 17]. Using an atherosclerosis model, Gao et al. [18] have shown that dietary oat fiber had an anti-neuroinflammatory effect and

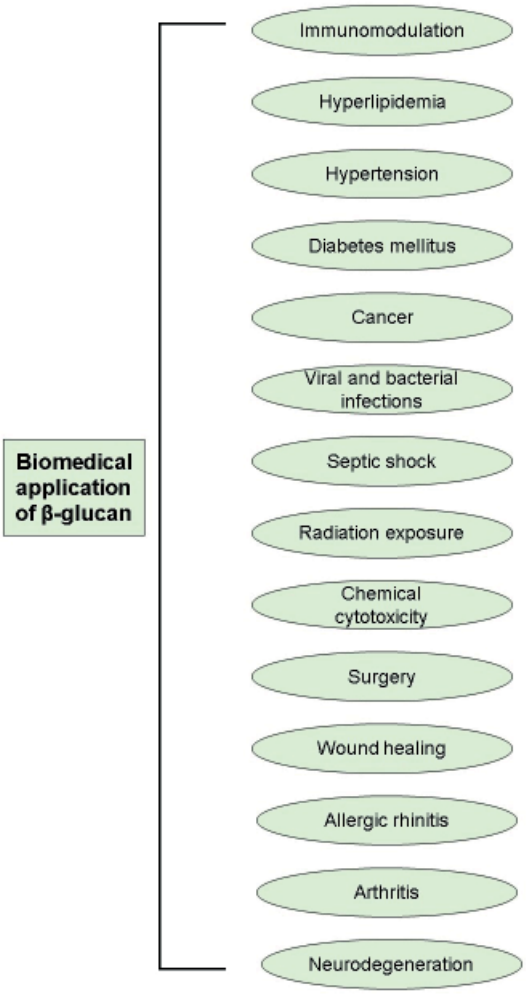

Fig. 4. **Prospective biomedical applications of oat β-glucan**

Evaluation of β-glucan concentration in the final product (n = 3)

Parameter, %	Method for determination of reducing carbohydrates	
	H_2SO_4 hydrolysis	β-glucanase hydrolases
β-glucan yield	10.8 ± 0.23	8.7 ± 0.82
β-glucan concentration in the final product	79.6 ± 3.89	65.1 ± 4.72
Monosaccharide concentration in the final product	3.48 ± 0.55	
Protein/amino acid concentration in the final product	0.24 ± 0.03	

reduced expression of reactive astrocytosis marker, glial fibrillary acidic protein (GFAP), in the cortex and hippocampus of rat supplied by high cholesterol diet. As potent immunomodulators, β-glucans orally administered may be useful as adjuvants, improving the effectiveness of various vaccines currently marketed against SARS-CoV-2 [19]. Purified β-glucan as a substrate for β-1,3-glucanase enzyme assays are widely applied in the research of plant pathology and adversity physiology as well as in routine analysis of microbial β-glucanase activities in their industrial production [20].

Conclusion

Taken together, elaboration of ultrasound-assisted and enzymatic-based method for isolation of β-glucans from oat bran represents highly reproducible and cost-effective approach for β-glucan production. Isolated oat β-**glucans** are free of harmful impurities and suitable for various industrial and biomedical applications.

Conflict of interests. Authors declare no conflict of interest.

Funding. The present research was conducted without any external financial support.

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УЛЬТРАЗВУКОВИЙ ТА ЕНЗИМАТИЧНИЙ МЕТОД ОТРИМАННЯ β-ГЛЮКАНІВ З ВІВСЯНИХ ВИСІВОК

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β-Глюкани — група некрохмалистих полісахаридів, або (1,4),(1,3)-β-D-глюканів, які входять до складу клітинних стінок деяких видів бактерій, водоростей, лишайників, грибів і зерна злакових рослин. Ці вуглеводи широко використовуються в харчовій промисловості, косметиці, фармацевтиці та сфері охорони здоров'я, тому оптимізація методів виділення β-глюканів із зернових має особливе значення для розвитку різних галузей біотехнології, дизайну ліків, харчових технологій.

Метою роботи було розробити оптимізовану технологічну схему виділення β-глюканів з вівсяних висівок із застосуванням ультразвукової та ензиматичної обробки сировини.

Методи. β-Глюкани було виділено з подрібнених вівсяних злаків в ході багатостадійного процесу, який включав екстракцію жирів зерна, гідробаротермічну та ультразвукову обробку, ензиматичний гідроліз супутніх крохмалю та протеїнів, осадження фракції β-глюкану етанолом, центрифугування та ліофільне висушування. Вихід β-глюканів із сировини та його концентрацію в кінцевому продукті визначали після гідролізу сірчаною кислотою або ензиматичного розщеплення ендо4)1,3-)-β-глюканазою.

Результати. Кислотний гідроліз кінцевого продукту дозволив встановити, що вихід β-глюканів склав 10,8 ± 0,23%, а концентрація — 79,6 ± 3,89%, тоді як ензиматичний гідроліз дав відповідні величини 8,7 ± 0,82% і 65,1 ± 4,72%. Таким чином, використання попередньої гідробаротермічної та ультразвукової обробки сировини в комбінації із застосуванням протеолітичниого розщеплення баластних ліпідів і протеїнів дозволило отримувати β-глюкани вівса в кількостях, порівнянних з такими, що отримуються при використанні кислотної або лужної обробки сировини.

Висновки. Описана технологічна схема виділення β-глюкану з висівок вівсяного зерна на основі послідовної гідробаротермічної та ультразвукової обробки, ензиматичного видалення крохмалю та протеїнів може бути широко використана для рутинного виробництва β-глюкану для різних потреб харчової технології, фармакологічної промисловості та медицині.

Ключові слова: β-глюкан; овес; гідробаротермічна обробка; ультразвук; ензиматичний гідроліз

UDC 571.27; 612.017.11; 612.112.3; 616.894-053.8
https://doi.org/10.15407/biotech16.01.057

MICROGLIA PHAGOCYTIC ACTIVITY IN RATS WITH DIFFERENT MODELS OF ALZHEIMER'S DISEASE

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Received 2022/03/01
Revised 2023/02/18
Accepted 2023/02/28

Neuroinflammation is a key feature of Alzheimer's disease (AD), a progressive neurodegenerative disorder. Microglia, the resident immune cells of the central nervous system, are involved in the AD pathogenesis and are principal players of neuroinflammation. Enhanced phagocytic activity is one of the main features of microglial cells mediated neuroinflammation. Correct reproduction of neuroinflammation in animal models is one of the main methodological approaches for studying AD pathogenesis and pathophysiology. The aim of the study was to conduct a comparative assessment of the microglia phagocytic activity of in rats with AD induced by intrahippocampal injection of amyloid β (Aβ) 1–40 and Aβ25-35.

Methods. Male Wistar rats were used in the study. Intact and sham-operated animals were used as controls. The development of the disease was confirmed by the assessment of cognitive impairment in the Barnes maze behavioral test, as well as by the level of dopaminergic neurons (DN) loss. The microglia phagocytic activity, as well as oxidative metabolism and the expression of phenotypic markers CD86 and CD206 were determined by flow cytometry.

Results. In animals with Aβ1-40-induced AD, significant impairment of cognitive activity and DA loss were registered, microglia was characterized by an increase in the proportion of phagocytic cells with up-regulated endocytic activity along with increased oxidative metabolism and overexpression of CD86 and CD206. In animals with Aβ25–35-induced AD, moderate impairment of cognitive activity was observed, microglia was characterized only by an increase in the number of phagocytizing cells without changes in endocytic activity, oxidative metabolism, and expression of phenotypic markers of phagocyte polarized activation.

Conclusion. Thus, in animals with Aβ1-40-induced AD, the proinflammatory metabolic profile of microglia, which is characteristic for neuroinflammation in the clinical course of the disease, is more adequately reproduced.

Key words: Alzheimer's disease; microglia; phagocytosis; inflammation.

Alzheimer's disease (AD) is the leading progressive neurodegenerative disorder associated with memory loss and disability, which affects millions of people worldwide. It ranks seventh among the leading causes of death in people aged ≥ 65 [1–3]. AD is characterized by the accumulation of extracellular senile plaques of abnormally folded amyloid β (Aβ) and intracellular deposits of tau protein, causing the neurons loss and cognitive impairment [4]. Neuroinflammation, involving the microglia proinflammatory activation, reactive astrogliosis, the expression of proinflammatory cytokines, and the release of reactive oxygen and nitrogen species, is considered one of the key mechanisms of the AD pathogenesis, which underlies the initiation and progression of neurodegeneration [5, 6]. The main effectors of neuroinflammation are microglial cells — specialized resident macrophages in the central nervous system (CNS), which respond to tissue damage and the presence of pathogens by removing cellular debris, misfolded protein aggregates, damaged

neurons as well as foreign inviders through the process of phagocytosis [7].

Microglia is activated in response to Aβ deposition and are thought to play a dual role in the AD pathophysiology. On the one hand, it participates in phagocytosis and clearance of Aβ, and on the other hand, it can release pro-inflammatory mediators that can increase neuronal damage and promote disease progression [8]. Resting microglia has a small cell body and very thin, highly ramified processes, and maintains an anti-inflammatory state with low expression of pro-inflammatory mediators and low phagocytic activity [9, 10]. Recognition of Aβ causes a change in the microglia morphology with the acquisition of a rounded cell shape with almost completely absent processes (dendrites), and stimulates phagocytic activity for amyloid clearance. Microglial cells express several phagocytic receptors involved in Aβ clearance: scavenger receptors (SR-AI/II), CD36, RAGE (receptor for advanced glycosylation end products), Fc receptors, TLRs (toll-like receptors) [11]. Removal of Aβ from the extracellular space by phagocytosis is thought to limit its accumulation. AD occurs when the formation of Aβ exceeds its removal by microglia [12].

The exact mechanisms underlying impaired microglial phagocytosis of Aβ remain a subject of active research and debates. Recent data indicate that the AD development is associated with phagocytic dysfunction of microglia [10, 13]. It is noted that the presence of large heterogeneous intracellular inclusions indicates that increased engulfment, but inefficient phagolysosomal degradation of the phagocytosed material may be associated with aging of microglia and, as a result, with ineffective Aβ clearance [12]. A decrease of phagocytic activity in the brain of AD patients is also associated with genetic defects of microglial, as well as astroglial cells [14].

On the other hand, there are also data on the increased microglia phagocytic activity, which correlates with cognitive impairment both in AD patients and in animals with a model of this disease [15]. Recent studies have shown that in AD there is an increase in microglial phagocytosis simultaneously with an increase in the level of production of reactive oxygen species (ROS) by these cells, which is known to lead to increased inflammation and neuron damage [16].

However, all authors agree that the microglia phagocytic activity plays a decisive role in the pathogenesis of neuroinflammation in AD and requires more thorough research.

One of the methodological approaches used to study the AD pathogenesis and search for new therapeutic targets are interventional models based on intracerebral administration of Aβ [17]. In this study, two most commonly used AD animal models based on intrahippocampal Aβ administration: the Aβ1-40-induced model and the Aβ25-35-induced were compared. Senile plaques in AD patients are usually composed of Aβ1-42 and Aβ1-40. The AD animal model based on intrahippocampal administration of Aβ1-40 is a classic interventional model of this disease and is accompanied by the development of progressive neuroinflammation. However, although Aβ1-40 is the form of amyloid most prone to aggregation [18], the ability to cause cognitive impairment is inherent not only to Aβ1–40, but also to some fragments, in particular the undecapeptide Aβ25–35. This fragment, located at the C-terminus of the molecule, is the functional domain of Aβ, required for both neurotrophic and neurotoxic effects. Taking this into account, Aβ25-35 is often chosen as AD model for in-depth study of the effects of Aβ-mediated neurotoxicity. More pronounced cognitive disorders in experimental animals are observed when aggregated Aβ25–35 is administered [19]. According to literature data, intrahippocampal administration of Aβ25-35 causes the development of neuroinflammation with increased synthesis of neurotoxic reactive oxygen and nitrogen species by microglial cells. Data on the phagocytic activity of microglia, which is assigned a significant role in the process of neurodegeneration, in these two AD models are absent in the literature.

The aim of the study was to conduct a comparative assessment of the phagocytic activity of microglia in rats with AD induced by intrahippocampal administration of Aβ1-40 and Aβ25-35.

Materials and Methods

Animals and study design. 14-month-old male Wistar rats (300–500 g) bred in the vivarium of the Educational and Scientific Center “Institute of Biology and Medicine” of Taras Shevchenko Kyiv National University were used in the experiment. Animals were kept under standard conditions with access to water and food ad libitum. The animal maintenance protocol was approved by the University’s Bioethics Committee in accordance with the Animal Protection Act. All animal studies were conducted in accordance with the norms established by the Law of Ukraine No. 3447-

IV "On the Protection of Animals from Cruelty", as well as in accordance with the standards of the Convention on Bioethics of the Council of Europe "European Convention for the Protection of Vertebrate Animals Used in Experimental and Other Scientific Research goals" (1997), general ethical principles of work with experimental animals approved by the First National Congress on Bioethics of Ukraine (September 2001) and other international agreements and national legislation in this field. Before the experiment, the animals were randomly divided into 4 groups: I ($n = 10$) — intact animals kept in standard vivarium conditions and not subjected to any manipulations; II ($n = 10$) — sham-operated (placebo) rats; III ($n = 10$) — rats with Aβ1-40 induced AD; IV ($n = 10$) — rats with Aβ25-35 induced AD. Randomization was performed using the "RAND ()" function in Microsoft Excel.

Surgery and Aβ1-40 and Aβ25-35 AD induction were performed as described by Mudò et al., 2019 and Schimidt et al., 2019 correspondingly [20, 21]. Rats were anesthetized with a mixture of ketamine (75 mg/kg, Sigma, USA) and 2% xylazine (100 µl/rat, Alfasan International B.V., The Netherlands) intraperitoneally in the volume of 1 ml. After this, animals were placed in a stereotaxic apparatus (SEJ-4, Ukraine), and were scalped from the point of intersection of the sagittal suture with the bregma (zero point): 2 mm distally, 2 mm laterally, and 3.5 mm deep, and a burr hole was made with an injection needle directly into the hippocampus. Next, animals received unilateral intra-hippocampal injections of Aβ1-42 or Aβ25-35. The suspension volume was 10 µl per animal, infusion was carried out for 5 minutes at a rate of 0.5 µl/min (every 15 s). After administration of Aβ, the tip of the microinjector remained in the brain tissue for 4 min. After that, the microinjector was removed, and the soft tissues of the head were sutured. The sham group was intra-hippocampal-injected with 10 µl of sterile ddH2O.

Degeneration of hippocampal dopamineric neurons (DN) was assessed using immunohistochemical staining (IHC) with antibodies to tyrosine hydroxylase (TH) [22]. The intensity of TH-positive staining was assessed on a semi-quantitative scale using quantitation methods (as described by Quantitative Scoring Methods [http://www.ihcworld.com/ihc_scoring.htm]), taking into account the number of positive (stained) cells and staining intensity (Table 1). The results were calculated by multiplying the percentage of positive cells (P) by the intensity (I) and presented as a quick estimate (Q): $Q = P \times I$.

Spatial learning and memory of rats were assessed via navigational ability in Barnes maze [23]. The aim of the test is to assess the ability to learn and remember the location of the escape box by placing visual tips on the walls surrounding the apparatus. The Barnes maze is a round table with 16 holes. On the walls of the room, as peripheral visual cues, black marks were placed (a triangle on one wall and two parallel stripes on the other) for better orientation of the experimental animals. A box (ESCAPE BOX) was attached to one of the holes in the table, into which a standard animal filler was poured. The rest of the holes remained closed. The test consisted of 4 days of training (4trial/day on day 1, 2, 3 and 4 of the experiment), and in each trial, rats were given 180 s to find the ESCAPE BOX. On day 5, rats were placed in the maze's center and explored for 90 s for assessing initial (pre-surgery) short-term memory, and on day 9 — for assessing initial (pre-surgery) long-term memory. Post-surgery short- and long-term memory was assessed on day 23 and 27 after the intrahippocampal Aβ injection correspondingly. Test endpoints (in seconds): 1) the time required for the animal to find the entrance to the ESCAPE BOX (spatial learning and spatial memory — related to the function of the hippocampus); 2) the time spent near the entrance to closed hole (cognitive flexibility — related to the function of the frontal cortex of the brain).

The concentration of the soluble form of beta-amyloid and Tau-protein in the homogenates of the hippocampus of rats with AD was determined by ELISA (Cloud-Clone Corp Co., Ltd. Houston, TX, USA) according to the manufacturer's recommendations.

Table 1

Semi-quantitative scale for assessing the intensity of TH-positive staining by quantification methods

Score	0	1	2	3	4
Percentage of positive cells (P)	<10%	10–25%	25–50%	50–75%	>75%
Staining intensity	no	weak	moderate	high	–

To prevent proteolytic degradation of beta-amyloid in the homogenate, a complex of protease and phosphatase inhibitors was used.

Microglia cells isolation. Microglia cells were isolated using a Percoll density gradient as described previously [24]. Purity of isolated microglia cell fraction was assessed by flow cytometry using FITC-conjugated mouse anti-rat CD11b (BD Pharmingen™) and phycoerythrin (PE) mouse anti-rat CD45 (BD Pharmingen™). The percentage of CD11b + CD45+ cells was 88.9 ± 3.7. Cell viability was estimated by Trypan blue exclusion test. The percentage of viable cells was ≥93.

Microglia cell function assessment. Phagocytic activity, oxidative metabolism and phenotypic marker expression level were determined by flow cytometry as described previously [18]. Briefly, ROS generation was assessed using 2′7′-dichlorodihydrofluorescein diacetate (H2DCFDA, Invitrogen). Reactivity reserve of the oxidative metabolism was assessed by the modulation coefficient (MC). MC was estimated after the treatment of microglial cells with phorbol 12-myristate 13-acetate (PMA) (protein kinase C activator) [25] *in vitro* and was calculated using formula: $MC = ((S - B)/B) \times 100$, where S — level of ROS generated after treatment with PMA *in vitro*; B — ROS value of untreated cells (basal value). Phagocytic activity was studied with the use of FITC-labeled heat-inactivated *Staphylococcus aureus* Cowan I bacteria (collection of the Department of Microbiology and Immunology of the ESC "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv) as an object of phagocytosis. The results were recorded as the percentage of cells emitting fluorescence (phagocytosis percentage, PhP) and as the phagocytosis index (PhI) — the mean fluorescence per cell, which is proportional to the number of phagocytosed bacteria. Phycoerythrin (PE)-labeled anti-CD206, and Alexa Fluor anti-CD86 antibodies (Becton Dickinson, Farmingen, USA) were used for phagocyte phenotyping. Samples were analyzed on a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA). Data were analyzed using CELLQuest software (BD; Franklin Lakes, NJ, USA).

Statistical analysis. All data are presented as mean ± SD, and Statistica.12 applied for statistical analysis. Data were tested using the Kolmogorov–Smirnov test for a normal distribution before other statistical tests. Statistical differences were calculated using ANOVA with post-hoc Tukey's multiple-comparison test. Differences were considered significant at $P < 0.05$.

Results and Discussion

According to the results of our research, intrahippocampal administration of Aβ1-40 and Aβ25-35 was not accompanied by statistically significant changes in the weight of animals and their eating behavior (data are not presented), cognitive impairment was more pronounced in rats with Aβ 1-40-induced AD (Table 2).

In the Aβ1–40 AD group, the time period to search for the "ESCAPE BOX" 1 day after the end of training, which characterizes short-term spatial memory, was on average 3 times longer, while in the group with Aβ25–35-induced model — 2 times as compared to intact and sham-operated animals. When assessing long-term spatial memory, impairments were observed only in rats with the Aβ1-40-induced model: 5 days after the training, the search time for "ESCAPE BOX" in these animals was increased by 50% compared to intact and sham-operated animals.

To check short-term and long-term cognitive flexibility, the duration of the animal's stay at the entrance to the closed hole was determined 24 hours and 5 days after training. In rats with Aβ1–40-induced AD, the values of this indicator exceeded those in both control groups, which indicates the uncertainty of the animal regarding the correctness of the selected entry option. In rats with Aβ25–35-induced AD only impairment of long-term cognitive flexibility was found. Indicators of short-term cognitive flexibility in this model were similar to animals without the disease (intact and sham-operated groups).

Additional criteria for the AD development were the number of TH-positive neurons in the hippocampus, as well as the concentration of Aβ and Tau protein in the hippocampus homogenate. TH is a marker of DN. In rats, the number of TH-positive neurons is significantly reduced with age. AD in human is also characterized by a decrease in the number of these neurons [26, 27]. Significant loss of DNs was found in rats with the Aβ1–40-induced model, whereas only moderate loss of these neurons was observed in animals with Aβ25-35-induced AD.

A threefold higher concentration of Aβ was observed in the homogenate of the hippocampus of rats with both Aβ1-40- and Aβ25-35-induced AD. A 3-3.5 times increased concentration of Tau protein was also registered in both

Table 2

Criteria for the development of AD induced by intrahippocampal injections of Aβ1-40 and Aβ25-35 in rats

Criterium	Intact animals, $n = 10$	Sham-operated, $n = 10$	Aβ1-40 indused AD, $n = 10$	Aβ25-35 indused AD, $n = 10$
Spatial memory				
Short-term post-surgery (the time required for the animal to find the entrance to the ESCAPE BOX 24 h after the training, c)	5.7 ± 1.3	4.5 ± 1.1	$17.0 \pm 7.2^{1, 2}$	$11.4 \pm 4.3^{1, 2, 3}$
Long-term post-surgery (the time required for the animal to find the entrance to the ESCAPE BOX 5 days after the training, c)	9.9 ± 4.3	11.2 ± 7.4	15.2 ± 3.3^{2}	10.3 ± 3.7^{3}
Cognitive flexibility				
Short-term post-surgery (the time spent near the entrance to closed hole 24 h after the training, c)	18.0 ± 2.2	21.8 ± 6.7	$26.8 \pm 12.2^{1, 2}$	17.4 ± 1.9^{3}
Long-term post-surgery (the time spent near the entrance to closed hole 5 days after the training, c)	16.3 ± 2.3	24.0 ± 6.3	$28.3 \pm 7.8^{1, 2}$	$28.4 \pm 5.4^{1, 2}$
The number of TH-positive neurins in the hippocampus (% of intact animals/% of sham-operated animals)	100	116.7	$38.9^{1, 2}$/ $33.3^{1, 2}$	$88.92,3/76.2^{1, 2, 3}$
Concentration of Aβ in the homogenate of hippocampus, pg/μg protein	18.8 ± 8.1	21.3 ± 15.2	$62.2 \pm 18.3^{1, 2}$	$66.5 \pm 21.0^{1, 2}$
Concentration of Tau-protein in the homogenate of hippocampus, pg/ml	22.0 ± 9.1	38.8 ± 10.6	$86.9 \pm 32.1^{1, 2}$	$92.5 \pm 28.5^{1, 2}$

Notes: 1 — $P < 0.05$ as compared to intact animals; 2 — $P < 0.05$ as compared to sham-operated animals; $P < 0.05$ as compared to animals with Aβ1-40-induced AD

groups. Accumulation of Aβ and Tau protein in the hippocampus indicates that microglia are unable to clear these substances in both models, which, nevertheless, was associated with varying degrees of neurodegeneration and the development of cognitive impairments characteristic for the disease [28].

The study of microglia phagocytic activity showed an increase in the proportion of phagocytic cells in animals with both AD models by an average of 2 times compared to control animals. At the same time, the endocytic activity of microglial cells was increased (more than 5 times) as compared to the intact control and by 2 times in comparison with sham-operated rats only in animals with Aβ1–40-induced AD. In animals with Aβ25–35-induced AD, this indicator did not differ from controls (Fig. 1). As we reported previously [29, 30], sham surgery significantly affects microglia metabolism even in the far terms after the placebo neurosurgical manipulations, indicating the necessity the use of placebo control groups in the experiments concerning neurodegenerative disease modelling in order to evade the influence of these effects on the analysis of study results

According to the literature data, a comprehensive analysis of the transcriptome and metabolome of immune cells of the CNS in neurodegenerative conditions revealed Disease-Associated Microglia (DAM), a subpopulation of microglia that concentrates in areas of neurodegeneration and is characterized by unique phenotypic and functional properties, one of which is significantly increased phagocytic activity [31].

Another functional feature of DAM, in addition to enhanced phagocytic activity, is increased antigen-presenting ability associated with up-regulated expression of histocompatibility molecules and costimulatory molecules CD80/86 [32]. According to the results of our research, in animals with Aβ1–

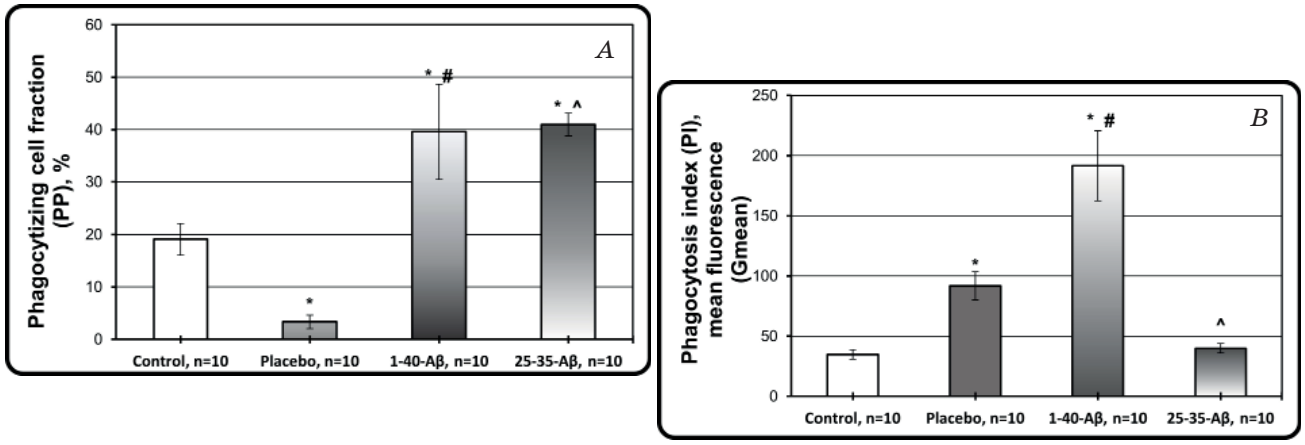

Fig. 1. **Phagocytic activity of microglial cells in rats with AD induced by injections of Aβ 1-40 and Aβ 25-35**
Phagocytizing cell fraction, PP (*A*) and phagocytosis index, PhI (*B*). Data are presented as Mean ± SD. Statistical differences are calculated using ANOVA with Tukey's post-hoc test. * and # indicate significant ($P < 0.05$) differences as compared to the values in intact and sham-operated animals correspondingly, ^ — $P \leq 0.05$ compared with the rats with AD induced by injections of Aβ1-40.

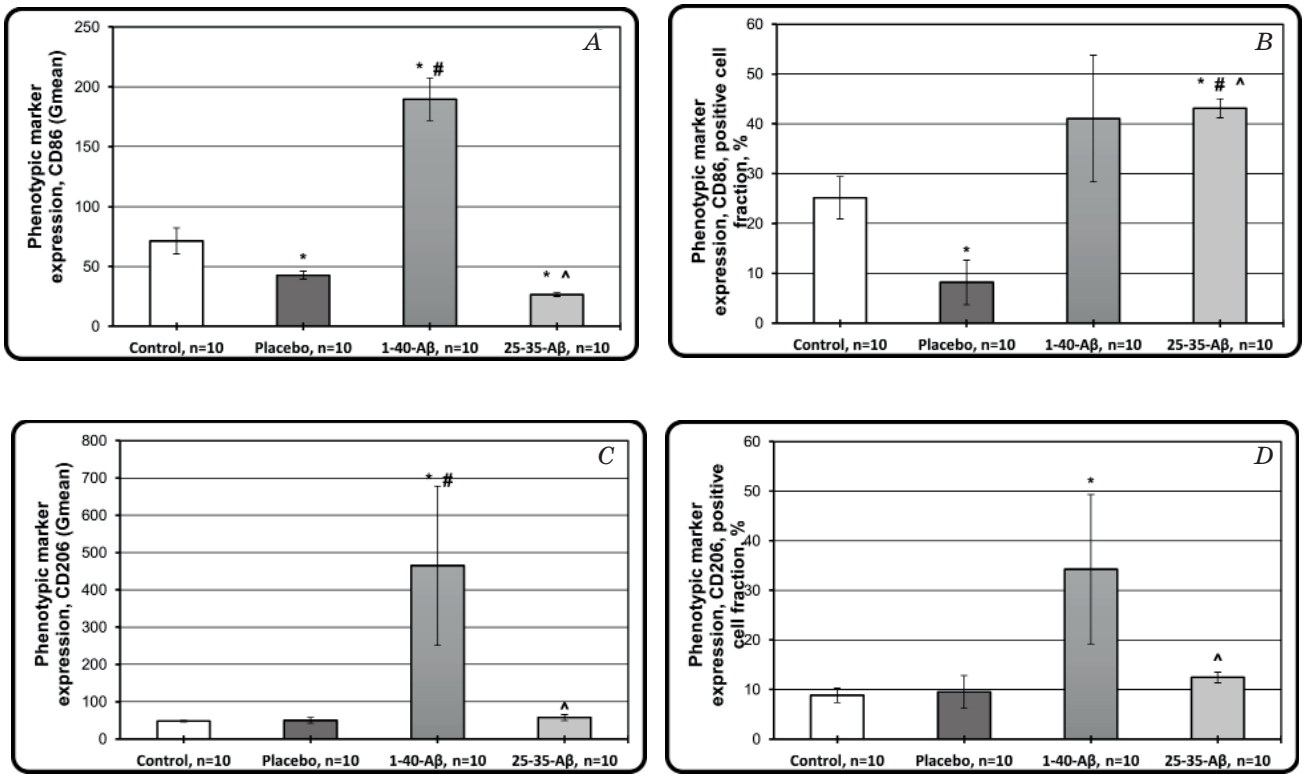

Fig. 2. **Membrane expression of CD86 — pro-inflammatory phenotype marker (*A*, *B*) and CD206, anti-inflammatory phenotype marker (*C*, *D*) in rats with AD induced by injections of Aβ 1-40 and Aβ 25-35**
Data are presented as Mean ± SD. Statistical differences are calculated using ANOVA with Tukey's post-hoc test. * and # indicate significant ($P \leq 0.05$) differences as compared to the values in intact and sham-operated animals correspondingly, ^ — $P \leq 0.05$ compared with the rats with AD induced by injections of Aβ 1-40.

40-induced AD, the number of CD86+ cells was 1.6 times higher, and the level of expression of this marker was 2.5 times higher compared to control animals (Fig. 2). In animals with Aβ25–35-induced AD, the number of CD86+ cells was also significantly higher than in controls. However, the expression level of this marker was significantly lower than the control values.

One of the phenotypic markers of DAM, which is detected in brain preparations of AD patients, is the overexpression of the mannose receptor CD206 [33]. The role of the mannose receptor in the pathogenesis of taupathies, including AD, remains unclear. Contrary to the fact that increased expression of CD206 is considered a marker of alternative (anti-inflammatory) metabolic polarization of macrophages [34], it has a special role in the assessment of polarized activation of microglia. It is known that mannose-binding lectins, including mannose receptors, are able to bind to Aβ, which causes a pro-inflammatory metabolic shift of cells of the immune system, including microglia [35, 36]. In animals with Aβ1–40-induced AD, the quantitative indicators of CD206+ cells were 3.5 times higher, and the expression level was 5 times higher as compared to the groups of control animals. In animals with Aβ25–35-induced AD, the expression indicators of this marker did not differ from those in animals in the control groups.

The concomitant increase in CD86+/CD206+ expression of animals with Aβ 1–40-induced AD may indicate an intermediate nature of microglial polarized activation, showing a mixed proinflammatory and anti-inflammatory phenotype (M1/M2) typical for DAM. In AD, microglia of intermediate polarization are involved in chronic inflammation and neurodegeneration. These microglial cells are thought to both contribute to the formation of toxic Aβ oligomers and are responsible for the clearance of Aβ plaques.

An important component of neuroinflammation is increased oxidative metabolism of microglia. As mentioned above, recent studies have shown that in AD, increased microglial phagocytosis is associated with an increase in the synthesis of ROS [37]. The development of AD, according to the results of our research, was accompanied by a significant increase in microglia oxidative metabolism (by 5 times as compared to the control) in animals with Aβ1–40-induced model (Fig. 3). In addition, treatment of cell samples from this group with PMA *in vitro* caused sharp drop of ROS level. Negative MC value –60,7 (which mirrors the residual cell ability to perform given metabolic reaction under stress) indicates extremely high activation of oxidative metabolism or cell metabolic exhaustion caused by persistent inflammation [38].

Unlike this, in animals with Aβ25–35-induced AD, the level of ROS generation was not significantly different from groups of control animals.

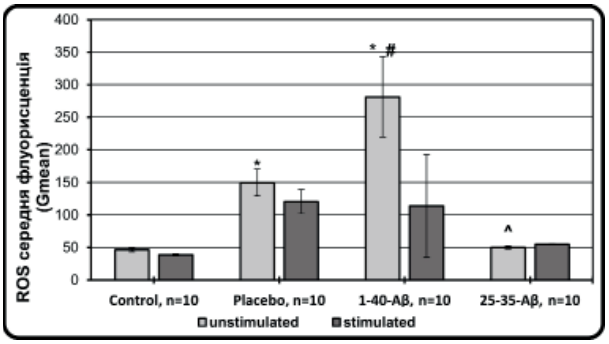

Fig. 3. **Oxidative metabolism of microglial cells in rats with AD induced by injections of Aβ1-40 and Aβ25-35**

Data are presented as Mean ± SD. Statistical differences are calculated using ANOVA with Tukey's post-hoc test. * and # indicate significant ($P < 0.05$) differences as compared to the values in intact and sham-operated animals correspondingly, ^ — $P < 0.05$ compared with the rats with AD induced by injections of Aβ1-40.

Conclusions

Comparative assessment of the microglia phagocytic activity in animals with different AD models revealed an increase in this indicator in animals with Aβ1-40-induced AD. Enhanced microglia phagocytic activity in these animals was associated with the presence of other phenotypic and functional characteristics typical for co-called DAM — the subpopulation of microglial cells that concentrates in foci of neurodegeneration in AD patients, as well as with distinct cognitive impairments. The functional profile of microglial cells in rats with Aβ25-35-induced AD indicates their moderate proinflammatory activation associated with moderate cognitive impairment. The obtained data suggest that full-length Aβ is a more powerful trigger of neuroinflammation, and the AD model induced by this Aβ is more appropriate for studying the role of neuroinflammation in the disease pathogenesis and pathophysiology.

Acknowledgement

We would like to commend bachelor and master students of ESC "Institute of Biology and Medicine", which took part in this study, as well as Dr.Vitalina Svyatetskaya for the assistance in surgical manipulations.

The study was supported by a project No.19DP036-03.

Conflicts of Interest

Authors declare no conflict of interest.

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ФАГОЦИТАРНА АКТИВНІСТЬ МІКРОГЛІЇ У ЩУРІВ З РІЗНИМИ МОДЕЛЯМИ ХВОРОБИ АЛЬЦГЕЙМЕРА

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Нейрозапалення є ключовою ознакою хвороби Альцгеймера (ХА), нейродегенеративного розладу, що прогресує. Мікроглія, резидентні імунні клітини центральної нервової системи, беруть участь у патогенезі ХА і є основними ефекторами нейрозапалення. Посилена фагоцитарна активність є однією з головних особливостей мікрогліальних клітин, що опосередковують нейрозапалення. Коректне відтворення нейрозапалення на тваринних моделях є одним із основних методичних підходів до вивчення патогенезу та патофізіології ХА. Метою дослідження було провести порівняльну оцінку фагоцитарної активності мікроглії у щурів з ХА, індукованою інтрагіпокампальним уведенням амілоїду β (Аβ) 1-40 та Аβ25-35.

Методи. У дослідженні використовували самців щурів лінії Wistar. Як контроль використовували інтактних і хиброоперованих тварин. Розвиток захворювання підверджували оцінкою когнітивних порушень у поведінковому тесті лабіринт Барнса, а також за рівнем загибелі дофамінергічних нейронів (ДН). Фагоцитарну активність мікроглії, а також оксидативний метаболізм та експресію фенотипових маркерів CD80 і CD206 визначали методом проточної цитометрії.

Результати. У тварин з Аβ1-40-індукованою ХА зареєстровано значне порушення когнітивної активності та втрату ДН, мікроглія характеризувалася збільшенням частки фагоцитувальних клітин із підвищеною ендоцитарною активністю, посиленням окисного метаболізму та надекспресією CD86 та CD206. У тварин з Аβ25-35-індукованою ХА спостерігалося помірне порушення когнітивної діяльності, мікроглія характеризувалася лише збільшенням кількості фагоцитувальних клітин без змін ендоцитної активності, окисного метаболізму та експресії фенотипових маркерів поляризованої активації фагоцитів.

Висновки. Таким чином, у тварин з Аβ1-40-індукованою ХА більш адекватно відтворюється прозапальний метаболічний профіль мікроглії, характерний для нейрозапалення в клінічному перебігу захворювання.

Ключові слова: хвороба Альцгеймера; мікроглія; фагоцитоз; запалення.

UDC 664.9; 639.21 https://doi.org/10.15407/biotech16.01.067

NUTRITIONAL VALUE OF CAVIAR OF SIBERIAN STURGEON IN UKRAINE

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Received 2022/12/23
Revised 2023/01/26
Accepted 2023/02/28

Aim. The purpose of the work was to study the nutritional value of *Acipenser baerii* siberian sturgeon caviar, grown in aquaculture conditions in the Dnieper reservoirs of Ukraine, and to compare its quality indicators with this one of the products produced abroad.

Methods. There are identified organoleptic properties of siberian sturgeon caviar, and its energetic value, composition of amino acids in albumens and content of fatty acids in lipids of the product. The data obtained in the research were compared with the same indices of quality of caviar of sturgeons bred abroad.

Results. The organoleptic properties of Siberian sturgeon caviar bred in Ukraine (appearance, color, consistency, taste and aroma) conformed to its standardized indices of quality. The caviar contained all eight essential amino acids and belonged to category of products rich by albumen (21.54±2.13%), and fats (13.20±0.93%) eicosapentaenoic and docosahexaenoic ω-3 acids (3.46% and 11.2%, respectively) (13.20±0.93%). The big content of fat, especially of polyunsaturated fatty acids and ω3 acids (the eicosapentaenoic and docosahexaenoic ones — 3.46% and 11.2%, respectively) is one more factor, which enables to identify the siberian sturgeon caviar as the product of high biological value. It was shown that the caviar of siberian sturgeon produced in Ukraine is in close coincidence with those that were produced in other countries.

Conclusions The totality of studied characteristics of caviar of siberian sturgeon produced in Ukraine witnesses its high nutritional value. Therefore this product may be recommended in prophylactics of numerous illnesses and strengthening of state of health.

Key words: Siberian sturgeon caviar; nutritional value; organoleptic properties essential amino acids; fatty acids.

The nutritional value of caviar of hydrobiontes was defined by their unique composition, and balanced content of substances necessary for live organisms in their growing, development and resistivity to influence of negative factors of environment [1–3]. It was shown that composition of metabolites of caviar of sturgeons is three time better of respective characteristics of constitutes of muscles tissue. However the number of sturgeons in wild nature continuously decreases, what called the need of cutting down of norms of their catching, and the Convention on International Trade by Species of Wild Fauna and Flora, Which are Under the Treat of disappearance of 1997 claims to limit volumes of trade by caviar of all of sturgeons [4]. This statement called the need of theoretical substantiation and development of norms of work in sphere of aquaculture, especially development of norms of industrial breeding of sturgeons.

To reach the economic effectiveness of sturgeons breeding, there were developed and introduced in practice the technology of reusable (up to ten times) taking of caviar during the whole reproductive life of sturgeons of 10 and more years [5], as well as optimized the technology of preservation of frozen sperm [6]. The quantity of caviar produced annually by this technology is now of 350-450 tons [7]. The biggest quantity of sturgeons caviar taken in the world belongs to races of siberian sturgeon of A*cipenser baerii* (31% of total volume of production) and russian sturgeon of A*cipenser gueldenstaedtii* (20%).

The nutritional and biological values is now the object of extensive research [2, 3, 8–11].

It was shown that chemical composition and biochemical properties of sturgeon's caviar differed by all indices of quality and depended of climatic conditions of fish breeding, its age and kind of nutrition. The bigger caloricity and biological value have the sturgeons caviar, which live in natural conditions, as compared with those that are bred by men because of their balanced ration [8]. Presently, Ukrainian businessmen actively breed sturgeons too [12]. However, the nutritional and biological values, as well as safety of caviar produced at fish farms is not studied comprehensively [2, 3]. Meantime, the demand for sturgeons caviar as the product of prophylactic and medicinal prescription increases each year because of its delicious taste and richness by biologically active substances — irreplaceable amino acids, ω3 polyunsaturated fatty acids, vitamins, micro- and macroelements. Therefore there exist the crucial need for studying of nutritional value of siberian caviar sturgeon produced in Ukraine in conditions of aquaculture and its comparing with the analogous index of caviar in other conditions of breeding.

Purpose of the work was to studying the organoleptic properties, determination of energetic value and indices of biological value of albuminous and lipidic components of siberian caviar sturgeon bred in conditions of aquaculture in water of Dnieper in Ukraine, as well as comparing of said characteristics with those ones of sturgeons caviar bred abroad.

Materials and Methods

The object of research was siberian caviar sturgeon of A*cipenser baerii* kind. The samples of siberian caviar sturgeon were taken in fish-breeding complex fed by water taken from the Dnieper river. The caviar was taken by method of "Cesarean section" of 9 years old female of siberian sturgeon. The taken samples, was rinsed during 30 seconds to remove grumes, crushed berries and pieces of films by water cooled to 5–10 °C in ratio of caviar and water of 1:2. The rinsed caviar was placed then onto the sieve to remove residues of water, treated 3 minutes by water heated to 60 °C, added 5 % of kitchen salt, mixed carefully, packed into 50 cm^3 glass flakes, and hermetically packed.

The organoleptic properties of the caviar was evaluated by expert commission of 5 persons by norms of standard of DSTU GOST 7442-2004 [13].

The mass parts of lipids and albumens were determined in the specialized laboratory of National University of Life and Environmental Sciences of Ukraine. The content of lipids was determined by the extracting and weighing method of Soxhlet in use of apparatus of "Soxtex SOX 406 Fat Analyzer" ("Hanon Instruments", China). It was taken 20 grams of caviar weighed with precision of ±0.001 g, mixed with 60 g of waterless Na_2SO_4 and carefully grinded in porcelain mortar. The milled mix was put in pack of filter paper, weighed with precision of ±0.001 g, placed in the Soxhlet extractor and treated in it by ethyl ether during 5–6 hours. The pack with deprived of fat material was placed then onto the glass plate for preliminary evaporation of ether and dried finally to constant weight in weighed flask at 100–105 °C. The content of fat in the caviar (on dry substance) was determined by difference of masses of pack before and after extraction in taking into consideration of mass of empty pack.

The content of albumen was determined by Kjeldahl method, which consists in preliminary mineralization of amides contained in the sample at 360–370 °C in digester in form of $(NH_4)_2SO_4$ in presence of concentrated H_2SO_4. To speed up the process, it was added into the mix the catalyst consisted of mix of $CuSO_4$, K_2SO_4 and Se. The content of mineral salt of $(NH_4)_2SO_4$ obtained in this process was determined by titration of resulting solution, and the total quantity of albumens in the sample was recalculated in use of obtained result with coefficient of 6.25.

The content and composition of fatty acids were determined by the method of liquid chromatography in the Palladin Institute of biochemistry of the National Academy of Sciences of Ukraine in use of the instrument of HRGC 5300. The extract of lipids prepared by the method described in article [14] treated as follows. The extract dissolved in benzene, placed into the flask closed by glass cork, and stored at temperature of minus 18 °C. The aliquot of 0.5 cm^3 of extract of lipids placed in glassy ampoule, added 1.5–2.0 cm^3 of 1N solution of HCl on methyl alcohol, sealed the ampoule hermetically and boiled it 50 minutes at water bath. After finishing of heat treatment opened the ampoule added the same volume of water, extracted the organic component by distilled hexane, cleansed by water, and dried by waterless sodium sulfate. The dried extracts were evaporated at rotary evaporator, dissolved the obtained methyl esters of fatty acids in benzene putted the preparation on glassy plates covered by KSK silica gel and evaporated the solvent. The layer of purified esters was taken off the glassy plate and rinsed

by hexane at the No. 4 Shott filter. To obtain pure mix of esters, the residues of solvent were secondly removed out from the preparation at rotary evaporator, dissolved in hexane and analyzed in use of chromatographic columns of 3.5 meters long filled by sorbent of Chromosorb W/HP impregnated by liquid phase of Silar 5CP at chromatograph HRGC 5300 (Italy) at 140–250 °C in rising of temperature in speed of rising of temperature by 2 °C in a minute. Identification of individual fatty acids was carried out in accordance with the standards of Sigma-Aldrich firm. The content of each fatty acid was expressed in dimensionality of percent of its total quantity.

The mass parts of essential amino acids were determined by liquid chromatography at automatic analyzer of T-339 (Czech republic) in Palladin Institute of biochemistry. There was carried out hydrolysis at 110 °C during 24–36 hours of samples of siberian sturgeon caviar of 1–5 milligrams mass mixed with 6 N hydrochloric acid. Identification of individual amino acids was carried out in accordance with the standards of Sigma-Aldrich firm. Determining of content of amino acid of tryptophan was done in Dokuchayev Kharkov national university using GOST 13496.21-2015 standard method [15].

The content of table salt was found by the method of [16]. 10 grams of milled Sturgeon caviar was placed into 100 cm^3 volumetric flask, and added to it 75 cm^3 of water, stirred the mix and heat at water bath at 80 °C during 30 minutes and cooled it at periodical stirring to room temperature. Then there was added water into the flask to the mark, mixed the solution and filtered it through the paper filter. The final operation was adding to 20 cm^3 of filtrate of 1 cm^3 of solution of $KMnO_4$ and titration of resulting solution by 0.1 N solution of $AgNO_3$ till reaching by it of nonvanishing coloration. The mass part of sodium chloride was calculated by known formula.

Results and Discussion

The organoleptic properties of siberian caviar sturgeon bred in Ukraine conform to norms of DSTU 7442-2004 standard [13] (Table 1).

It could be seen from the Table 1 that all indices of quality of caviar bred in Ukraine conform to norms of DSTU 7442-2004 standard "Grain sturgeon caviar. Specifications".

The chemical composition and energetic value of siberian caviar sturgeon compared with those ones of caviar produced abroad are given in Table 2.

The energetic value of Ukrainian caviar of 210.94 ccal/100 g differs of this parameter of caviar produced in other counties. The biggest caloricity of 271.45 ccal/100 g has the sturgeon caviar of *A. gueldenstaedti* from Rumanian [8], and the lowest — of 202.94 ccal/100 g — the caviar of *A. baerii* from of French. According to the protein content (from 21.54±2.13 to 29.32±0.92 g/100 g), sturgeon caviar species belongs to high-protein and high-fat products [17].

The biological value of albuminous component of the product is determined by correspondence of their parameters of quality and quantities of essential amino acids to norms of FAO/WHO standard of ideal albumen [18], and recommendations of the European Food Safety Authority (EFSA) [19]. Results of evaluation of conformity of quality of sturgeons caviar produced in different countries to recommended FAO/WHO parameters are given in Table 3.

It is clear that albumen of caviar produced in Ukraine contains all essential amino acids, which contents sum is sufficiently bigger of recommended by FAO/WHO level, and of quantities that meets human needs: 43.90±0.50% as compared with 36.00% and 26.20%, correspondingly. At the same time the content of essential amino acids in caviar of

Table 1

Results of evaluation of conformity of organoleptic properties of siberian caviar sturgeon bred in Ukraine to norms of standard of DSTU 7442-2004

Index	Norms of DSTU 7442-2004	Characteristic
Appearance	Uniform size and shape	Conforms
Color	Uniform, proper to caviar of this kind fish, varies from light grey to gray	Conforms
Consistency and state	Grains are safe and separate one of other	Conforms
Aroma and taste	Proper to caviar of this kind fish. Absence of foreign smell and flavor	Conforms
Mass of table salt, %	2.5–5.0	3.60
Presence of foreign particles	Absence	Absent

Table 2

The characteristics of chemical composition and energetic value of caviar of sturgeons produced in Ukraine and abroad

Object	Content, g/100 g of the product		Energetic value, ccal/100 g
	albumen	fat	
A. baerii, (Ukraine)*	21. 54±2.13	13.20±0.93	210.94
A. ruthenus, (Korea) [9]	25.43	13.21	220.61
A. baerii, (France) [9]	26.21±1.14	10.90±0.07	202.94
A. gueldenstaedti, (Romania) [9]	29.32±0.92	17.13±0.76	271.45
A. baerii, (China) [10]	23.98±0.78	14.23±0.71	223.99

* — results of own investigation

Table 3

The content of essential amino acids in sturgeon siberian caviar produced in Ukraine and abroad (g/100 g of albumen) and their correspondence to parameters of ideal albumen

Amino acid	Race of sturgeon			Ideal albumen parameter [18]	EFSA recommended content [18]
	A. baerii, Ukraine *	*A. baerii*, China [11]	*A. baerii*, Korea [10]		
Valine	4.28±0.95	2.95±0.06	5.7	5.00	3.90
Isoleucinele	3.92±0.05	2.62±0.07	5.5	4.00	3.00
Leucine	8.52±0.23	4.57±0.11	9.6	7.00	5.90
Lysine	8.04±0.54	4.43±0.14	11.3	5.50	4.50
Metphionine + Cysteine	4.88±0.89	2.57±0.26	6.0	3.50	2.20
Threonine	5.44±0.32	2.58±0.12	3.9	4.00	2.30
Phenylalanine + Tyrosine	7.76±0.97	4.18±0.08	16.80	6.00	3.80
Tryptophan	1.06±0.09	0.49±0.03	-	1.00	0.60
In total	43.90±0.50	24.39±0.09	58.8	36.00	26.20

* — results of own investigation.

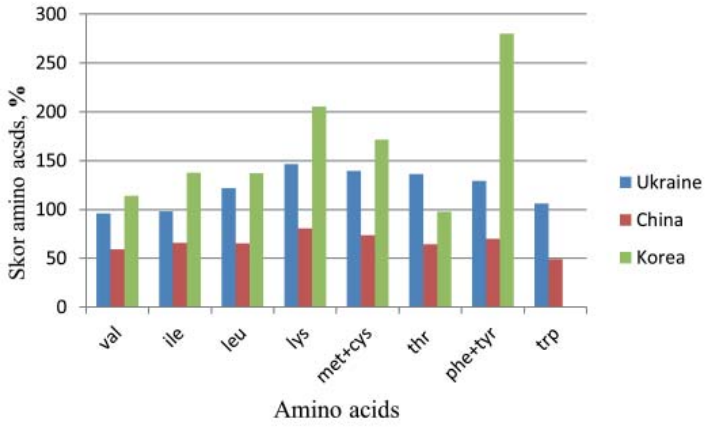

Fig. **Scores of essential amino acids in roes of siberian sturgeons bred in Ukraine and abroad**

this race sturgeon is the least in sturgeons bred in China (24.39±0.09%) and the biggest — in Korea (58.8%).

The analysis of scores of essential amino acids in roes produced in said countries is given in Figure.

The results we obtained show that the albumen of siberian sturgeon bred in Ukraine has two limiting amino acids, namely valine and isoleucine, which score is 96 % and 98%, respectively. At the same time, all irreversible amine acids contained in caviar produced in China are of limiting character, and the Korean caviar contains only treonine as the limiting one. At the same time, albumen of siberain sturgeon caviar produced in Ukraine contains all essential amino acids in quantities that are bigger of those ones recommended by the European Food Safety Authority [19].

The composition of lipids in analyzed samples of siberian sturgeon caviar, is represented by saturated (26.19%), monounsaturated (34.05%), and polyunsaturated (38.92%) fatty acids (Table 4).

The quota of saturated fatty acids in lipids recommended by FAO/WHO is 20.00 %, what

Table 4

The content of fatty acids in Siberian sturgeon roes produced in Ukraine and abroad (% of their total quantity)

Code of the acid	Place of breeding of a sturgeon				Recommended quantity [18]
	Ukraine*	Korea [8]	China [9]	France [7]	
1	2	3	4	5	6
Saturated fatty acids					
14:0	0.66	1.59	0.86	–	–
15:0	0.17	0.29	0.15	–	–
16:0	16.19	22.46	20.80	–	–
17:0	0.40	0.44	0.11	–	–
18:0	3.82	0.19	2.82	–	–
20:0	0.69	0.22–	–	–	–
21:0	3.27	–	–	–	–
22:0	0.84	0.41	–	–	–
22:3	–	1.82	–	–	–
24:0	0.15	–	–	–	–
In total	26.19	27.42	24.74	–	20.00
Monounsaturated fatty acids					
16:1ω7	5.81	7.51	4.39	–	–
17:1	0.39	0.81	0.16	–	–
18:1 ω9	23.59	33.67	33.19	32.9±3.2	–
20:1 ω7	3.83	1.15	–	–	–
20:1 ω9	–	–	1.19	–	–
In total	34.05	43.14	38.93	–	35.00
Polyunsaturated fatty acids					
18:2 ω6	11.65	10.19	13.13	5.4±0.1	–
18:2 ω7	1.71	–	–	–	–
18:3 ω3	1.99	0.85	–	–	–
18:3 ω6	–	–	1.31	–	–
20:2 ω9	1.46	0.26	–	–	–
20:2 ω6	–	–	0.25	–	–
20:3 ω6	1.68	0.40	0.31	–	–
20:4 ω6	4.91	–	1.66	1.1±0.4	–

Table 4 (End)

1	2	3	4	5	6
20:5 ω3	3.46	4.69	4.63	4.9±1.1	–
22:2	0.01	–	0.34	–	–
22:3	0.07	–	–	–	–
22:5 ω3	0.61	1.83	1.21	–	–
22:6 ω3	11.20	11.39	12.78	13.1±2.6	–
In total	38.92	29.66	35.62	–	6.00
The sum of ω6 fatty acids	18.24	10.59	16.66	7.4±0.8	–
The sum of ω3 fatty acids	17.26	17.91	18.62	20.7±5.2	–
The ω6:ω3 ratio [18]	1.05 : 1.00	1.00 : 1.69	1.00 : 1. 14	1.00:3.98	10 : 1-5

* — results of own investigation.

is less of their real content in siberian caviar sturgeon bred in different world regions (24.74% to 27.42%). The most abundant in this group acids is the 16:0 palmitic one. However, its minimal content in the caviar produced in Ukraine is of 16.19%, what is less of respective parameter in caviar produced in China (20.80 %), and Korea (22.46 %).

The quota of monounsaturated fatty acids in the siberian sturgeon caviar produced in all studied cases differs of its recommended value (35.00 %) and constitutes 34.05 % in Ukrainian, 38.93 % in Chinese, and 43.14 % in Korean products. The most abundant one in this group of acids is irreplaceable ω9 oleic acid, which physiological value consists in regulation of variations of composition of cell membranes, activity of receptors on their surfaces, and normalization of metabolic processes [21].

The quota of polyunsaturated fatty acids in analyzed caviar is sufficiently greater of recommended FAO/WHO value (6.0%), and is of 38.92 % for Ukraine, 29.61% for Korea, and 35.62 % for China.

The last time valuable parameter is the ratio of ω3:ω6 fatty acids, and as it was shown in this investigation, the quantity of polyunsaturated fatty acids of ω3 group in the caviar produced in Ukraine is of 17.26 %, what is less of respective value for Korea (17.91%), and China (18.62 %). It was found that their ratio optimal in support of normal state of organism is about of 1.0 : 1–5 [20]. At the same time it was shown (Table 4) that the real ratio of such acids is too less for all kinds of studied Siberian surgeon caviar (1.05:1.00; 1.00:1.69 and 1.00:1.14 for Ukraine, China and Korea, respectively), what witnesses that the real content of ω3 in these caviar lipids is too bigger of recommended parameter.

It was shown also that the men's ration is too scarce by essential docosahexaenoic (22:6 ω3) and eicosapentaenoic (22:5 ω3) fatty acids critically necessary in normalization of metabolism of lipids in the organism as substances that support the immune system in normal state, assist in medical treating of cancer, and treating of cardiovascular diseases [21]. Nevertheless, lipids of all studied siberian sturgeon caviar are rich by ω3 and ω6 fatty acids, so are too valuable from the viewpoint of their biological activity.

The siberian sturgeon caviar of *A. baerii* produced kind in the Dnieper river water in borders of Kyiv region by indices of appearance, color, consistency, taste and aroma conforms to norms of the national standard [13].

It was found that the energetic value of siberian caviar sturgeon bred in Ukraine and other countries differs of this one of sturgeon caviar bred in nature [8]. The caviar obtained in natural conditions contained 31.10% of albumens and 19.40% of fat, what gave in sum 299.0 ccal/100 g [8]. At the same time the energetic value of caviar produced in Ukraine was 210.94 ccal/100 g, and reached the maximum value of 271.45 for caviar bred industrially in the product bred in Rumania [8]. Such differences may be explained as consequence of influence of numerous factors, especially age of female sturgeons, type and quality of forage, and quality of water in places of their breeding

The caviar relates to category of products rich by albumens and fat, what conforms

the results we obtained previously [2, 3]. As compared with the FAO/WHO and European commission by food safety recommendations, the siberian caviar sturgeon from Ukraine contains more albumens – 43.0 against of 36.0 and 26.2% correspondingly [18]. However, the albumen of caviar produced in Ukraine is character by insufficient quantities of essential valine and isoleucine as compared with content of ideal albumen. It differs its content of composition of albumen of caviar from Chinese deficit by all irreplaceable amino acids, and Korean one, where all essential amino acids are present in quantities character for ideal albumen [10].

However composition of ideal albumen is the conventional characteristic of albuminous component of foodstuffs. So this characteristic is often by notion of "adequate" and "maximally permissible" contents of essential amino acids in albumens capable to satisfy in its consumption optimal conditions of biosynthesis of albumens in the organism [19]. Taking this notion into consideration, one may affirm that content of all essential amino acids in siberian sturgeon caviar produced in Ukraine is bigger of recommended levels [19], what permits to consider it as the biologically valuable capable to ensure proper conditions synthesis of albumen in the human body. It [s seen in use of these criteria that albumen of caviar produced in Ukraine contains the limiting amino acids as well (valine 2.95% against of normalized content of 3.9%, isoleucine — 2.62% and 3.00%, leucine 4.57% and 5.90%, lysine 4.43% and 4.50%, and threonine 0.49% and 0.60%). At the same time it is clear that albumen of caviar produced in Korea satisfies to all norms established by the European Food Safety Authority.

The amino acids obtained by organism with foods were classified until the very recent times as essential and replaceable ones. The key element in this classification was supposition that the men's organism is capable to synthesize all essential amino acids in quantities capable to satisfy its needs in synthesis of own albumens only [22, 23], in ignoring of their regulatory functions. Meantime the set of data obtained last time enable to formulate new conception of role of functional amino acids in regulation of key metabolic processes directed on bettering of state of health, surviving, development and reproduction of live organisms. Therefore, the concept of "ideal albumen" has to be amended from viewpoint of taking into consideration of content in albumen of both type amino acids.

The lipids contained in siberian caviar sturgeon ensure more of half of its energy value independently of conditions of breeding of sturgeons. The dominating part of fatty acids in lipids of siberian caviar sturgeon, same as in lipids of other types of organisms, which live in water, belongs to long-chain (more of C_{20}) substances. The distinctive feature of lipids of siberian caviar sturgeon is its richness by palmitic acid (16.19–22.46%) active in regulation of physical properties of cell' membranes and state of skin, as well as means of prophylactics of metabolic syndrome. The approved norm of its consumption is about of 10 % of general caloricity of foods [20]. So lipids of siberian caviar sturgeon are one of known sources of consumption of palmitic acid.

The big part of monounsaturated acids contained in siberian caviar sturgeon constitutes oleic acid C 18:1ω9 (23.59–33.67%), which physiological role in men's organisms consists in regulation of composition of cell membranes, activity of their receptors, and normalization of metabolic processes [24].

The lipids of siberian caviar sturgeon are the source of long-chain essential ω3 and ω6 eicosapentaenoic, and docosahexaenoic fatty acids, which play the key role in normalization of metabolism of lipids, optimization of functioning of cardiovascular and immune systems, as well as decreasing of probability of beginnings of cancer [25–27]. The results of our research agree with the data obtained in earlier studying of composition of lipids of organisms, which live in water and witness their big biological value.

There exist differences in chemical composition, content of amino acids in albumens, and content of lipids of siberian caviar sturgeon produced in different countries, what may be explained, probably, by differences in age of female sturgeons, climatic conditions of their breeding, and type of feeding.

Conclusions

It was studied the nutritional and energetic values, composition and indices of quality of siberian caviar sturgeon of *Acipenser baerii* kind bred in Dnieper water in borders of Kyiv province. The comparative analysis of content in it of essential amino acids and fatty acids showed only minor differences of these parameters of the same of caviar produced in other countries. The principal conclusion made in it is those that the siberian caviar sturgeon bred in Ukraine is the valuable

foodstuff, which can be recommended for the prevention of many diseases and health promotion. The results obtained in this work are important in progress of trade by this product internationally and confirming of its competitiveness with analogous products produced abroad.

Financing of this work was done by the National University of Life and Environmental Sciences of Ukraine according the theme "Scientific substantiation of use of raw materials of animal origin in purposes of development of enterprises by producing of products of sanative nutrition" (DR #0110U003586).

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ХАРЧОВА ЦІННІСТЬ ІКРИ СИБІРСЬКОГО ОСЕТРА ЗА УМОВ АКВАКУЛЬТУРИ УКРАЇНИ

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Мета. Дослідити харчову цінність ікри сибірського осетра *Acipenser baerii*, вирощеного за умов аквакультури України, та порівняти отримані результати з даними літератури щодо виробництва цієї продукції в аквакультурі інших країн.

Методи. Використано органолептична оцінка якості ікри, визначено загальний хімічний склад та енергетичну цінність, склад незамінних амінокислот протеїну та жирнокислотний склад ліпідів ікри сибірського осетра за умов аквакультури України. Проведено порівняне результатів досліджень з даними джерел літератури цих показників ікри осетрових за умов інших країн.

Результати. Органолептичні показники ікри сибірського осетра, вирощеного з виведеної в умовах аквакультури в Україні (зовнішній вигляд, колір, консистенція, смак і аромат), відповідали її стандартизованим показникам якості. Ікра містила всі вісім незамінних амінокислот і належала до категорії продуктів, багатих протеїном (21,54±2,13%), а також жирами ейкозапентаєновою та докозагексаєновою ω3–кислотами (3,46 та 11,2% відповідно) (13,20±0,93%). Великий вміст жиру, особливо поліненасичених жирних кислот та ω3 кислот є ще одним фактором, що дозволяє ідентифікувати ікру сибірського осетра як продукт високої біологічної цінності. Показано, що вироблена в Україні ікра сибірського осетра дуже збігається з ікрою, виробленою в інших країнах.

Висновки. Сукупність досліджених характеристик в умовах аквакультури України ікри сибірського осетра свідчить про її високу харчову цінність. Тому цей продукт можна рекомендувати для профілактики багатьох захворювань і зміцнення самопочуття.

Ключові слова: ікра сибірського осетра; харчова цінність; органолептичні властивості; незамінні амінокислоти; жирні кислоти.