ISSN 2313–5891 (Online)
ISSN 2304–974X (Print)

Ukrainian Food Journal

Volume 13, Issue 3
2024

Kyiv **Київ**

2024

Contents

From production to regulation: the comprehensive role of hyaluronic acid in the food and cosmetic industry

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Keywords:

Hyaluronic acid
Food
Microbial synthesis
Regulatory
Cosmetic
Nanotechnology

Article history:

Received 12.02.2024
Received in revised form 10.07.2024
Accepted 30.09.2024

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DOI: 10.24263/2304-974X-2024-13-3-5

Abstract

Introduction. Hyaluronic acid (HA) is now approved as a food additive in many countries of Europe, Asia and America and is used as an important element in the food industry. Also, HA is one of the most popular cosmetic ingredients. The aim of this research was to review the comprehensive role of hyaluronic acid in the food industry, from production methods to regulatory aspects.

Materials and methods. The study utilized international and domestic scientific publications from leading periodicals and specialized global journals, focusing on hyaluronic acid applications in the food and cosmetic industry and suitable producers. Scientific articles were sourced using global scientometric databases such as Google Scholar and PubMed.

Results and discussion. The review revealed that hyaluronic acid has broad applications in the food industry, including as a modifier for dairy and starch-based products, a natural flavor enhancer, and a salt reducer. In the cosmetic industry, HA is used as an anti-aging component that promotes skin hydration. Various microbial strains for HA production were compared, with *Corynebacterium glutamicum* showing the highest yield (32 g/l over 60 hours). Moreover, international regulations are essential in ensuring the quality and safety of HA-containing products. In the European Union, Regulation (EC) No 1223/2009 sets the standards for the use of hyaluronic acid in cosmetic products, outlining guidelines for product safety, labeling, and permissible concentrations. Globally, HA used in cosmetics and food products must comply with various international standards such as the Codex Alimentarius, which governs food additives, and the ISO 22716:2007 for Good Manufacturing Practices (GMP) in cosmetics production, ensuring consistency in product quality and consumer safety

Conclusions. Hyaluronic acid presents significant potential for use in the food and cosmetic industry, with promising production methods using GRAS-status (Generally Recognized As Safe) microorganisms. Adherence to regulatory requirements is crucial for manufacturers and importers of HA-containing products. Further development of the regulatory framework is expected as technologies and research in HA application continue to advance.

Introduction

Hyaluronic acid is a natural substance that plays a crucial role in maintaining skin health and youthfulness. While it is best known for its use in cosmetics, hyaluronic acid has recently been gaining wider application in the food industry.

The commercial use of hyaluronic acid in baked goods as a substitute for egg whites was discovered in 1942, long before its initial use in medical and cosmetic products in 1960 and 1979, respectively. In 2011, the Japan Association for Healthy Nutrition and Food, and in 2014, the Korean Ministry of Food and Drug Safety officially approved HA for use as a food additive. Subsequently, China and the European Union also made decisions allowing its use as a food ingredient. Currently, HA is actively promoted as a food additive in countries like the USA, Canada, Italy, and Belgium (Joshi et al., 2024).

Since 2021, China has permitted the use of HA as a food additive in common foods such as yogurt, fruit juice, green tea, carbonated beverages, as well as in snacks, including soft candies and jelly. These products are marketed as having health benefits, including weight control, relaxation, liver protection, stomach support, skin whitening, and antioxidant effects. Studies have confirmed that oral intake of HA can improve skin hydration, elasticity, reduce roughness, and wrinkle depth, and help in treating osteoarthritis. Pharmacokinetic studies have shown that after oral administration, HA is degraded in the gastrointestinal tract, and its bioavailability is significantly influenced by the gut microbiota (Hu et al., 2023).

This unique molecule has the ability to retain a large amount of water, making it a valuable ingredient for improving the texture and consistency of various food products. Moreover, beyond its primary function, hyaluronic acid is used as an emulsifier, thickener, and stabilizer. It helps extend the shelf life of products, enhances their taste, and gives them a more appealing appearance. Additionally, hyaluronic acid can be used to create functional foods aimed at supporting joint and skin health. It is added to beverages, yogurts, confectionery, and other products to increase their nutritional value (Cheng et al., 2023).

Furthermore, research is ongoing into the use of hyaluronic acid in food products, including dietary supplements, food safety testing, food packaging, food delivery systems, and food quality enhancers. Overall, hyaluronic acid has great potential in the food industry for improving the functional properties of food products.

In addition, hyaluronic acid is a unique glycosaminoglycan (GAG) and stands apart from other GAGs because it is non-sulfated, has a remarkably large molecular size, and is synthesized in the cytoplasm of cells rather than within the Golgi apparatus (Figure 1). Its structure and properties make it essential in a variety of biological processes, especially in maintaining the skin's hydration. Hyaluronic acid's ability to retain large amounts of water is one of its most notable features, which contributes significantly to the moisture content and elasticity of the skin. This exceptional water-binding ability makes HA a key molecule not only in cosmetic applications but also in medical treatments aimed at improving skin quality and healing (Mendoza-Muñoz et al., 2023).

In terms of its role in the body, hyaluronic acid plays several vital functions. It is involved in cell adhesion, where it assists cells in attaching to the extracellular matrix (ECM), and in fixing cells to specific locations within the skin. These processes are crucial for maintaining skin structure and integrity, as well as for ensuring that cells function correctly within their given environment. HA also plays a significant role in cellular communication, growth, and migration. It acts as a signaling molecule in a variety of cellular activities, including cell motility, inflammation, and tissue repair. Furthermore, it participates in more complex processes like cancer metastasis, where cell movement is critical, and in the regulation of cell metabolism, where it influences cellular energy use and repair mechanisms (Mendoza-Muñoz et al., 2023).

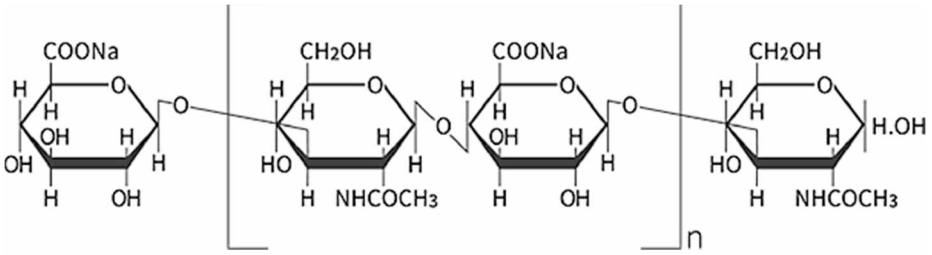

Figure 1. Structural formula of hyaluronic acid

Within the skin, hyaluronic acid is found in different concentrations depending on the layer. It is present in much higher amounts in the dermis, where its concentration can reach 0.5 mg per gram of tissue, compared to 0.1 mg per gram in the epidermis. This uneven distribution is significant because the dermis is the thicker layer of the skin, providing structural support, while the epidermis serves as the protective barrier. Because of this, HA's moisturizing properties are especially important in the dermis. It serves as a popular ingredient in dermal fillers used in aesthetic medicine to smooth wrinkles and restore volume to the face. These fillers, such as Hylaform®, Restylane®, and Dermalive®, typically contain HA in concentrations ranging from 0.025% to 0.050%, ensuring that they provide long-lasting moisture retention and volume to the treated areas. Topically applied HA can also improve the function of the outer layer of the skin by restoring its barrier function, which is crucial for protecting the skin from external stressors and preventing moisture loss (Kanlayavattanakul & Lourith, 2015).

Another important factor that determines the biological effects of hyaluronic acid is its molecular weight. HA molecules can vary greatly in size, with molecular weights ranging from 2 × 10^5 to 10^7 Daltons. This broad range of sizes allows HA to perform a diverse array of functions within the body, depending on whether it is in its high-molecular-weight form or low-molecular-weight form.

High-molecular-weight hyaluronic acid (HMWHA) primarily works through passive mechanisms. One of its most well known properties is its ability to retain water, making it highly effective in promoting tissue hydration. This property is essential not only in maintaining skin moisture but also in protecting joint cartilage. In joints, HMWHA acts as a lubricant, ensuring smooth movement and protecting cartilage from wear and tear. It also helps maintain osmotic balance in tissues and contributes to the stabilization of the extracellular matrix, which provides structural support to cells. While HMWHA cannot easily penetrate cells due to its large size, it can still influence cellular activity by interacting with receptors on the cell surface. Through these interactions, HMWHA can activate signaling pathways that regulate processes like cell proliferation (the growth and division of cells) and angiogenesis (the formation of new blood vessels). Angiogenesis is a critical process for both wound healing and the growth of tumors, as new blood vessels are needed to supply tissues with nutrients and oxygen. HMWHA, however, acts as an inhibitor of angiogenesis, which helps prevent the uncontrolled growth of blood vessels associated with cancer progression. This inhibitory effect can be beneficial in preventing tumor growth but may also have a downside, as excessive inhibition of angiogenesis can slow down wound healing and tissue regeneration. Thus, while HMWHA offers protective benefits, its overuse

could potentially interfere with the body's ability to repair itself after injury (Juncan et al., 2021).

Low-molecular-weight hyaluronic acid (LMWHA), on the other hand, behaves quite differently. Unlike HMWHA, which primarily inhibits angiogenesis, LMWHA actually promotes it. This angiogenic activity is useful in situations where tissue repair is needed, such as in wound healing or after an injury. However, it can also contribute to tumor progression, as new blood vessels can supply a growing tumor with the resources it needs to expand. Despite this potential risk, LMWHA also has anti-inflammatory properties, which can be beneficial in reducing inflammation and promoting tissue healing. This dual role makes LMWHA a molecule of interest in both therapeutic and cosmetic applications. By carefully controlling its concentration and molecular weight, LMWHA can be used to manage both tissue repair and inflammation without encouraging undesirable side effects such as tumor growth (Juncan et al., 2021).

Overall, hyaluronic acid is a highly versatile molecule with a wide range of applications in both medical and cosmetic fields. Its ability to influence hydration, tissue repair, inflammation, and even cancer progression underscores its importance in modern skincare and therapeutic treatments. As research into HA continues, new applications and formulations are likely to emerge, making it an even more indispensable component in health and beauty products.

Since hyaluronic acid has such a wide range of applications in the cosmetic, medical, pharmaceutical, and food industries, there is a pressing need to examine the regulatory documentation that governs this glycosaminoglycan. Considerable attention is paid to documentation that is relevant for Ukraine, while there are no hyaluronic acid production facilities, but national companies do use HA as a raw material in their manufacturing processes (Kumar, 2024).

Materials and methods

This review utilized scientific publications from leading periodicals and specialized global journals, focusing on the application of hyaluronic acid in the food and cosmetics industry with a consideration of suitable producers.

Results and discussion

Hyaluronic acid in nanotechnology and encapsulation

In a study by Guo et al. (2018), nanocapsules were developed to improve the stability and effectiveness of poorly soluble antioxidants in products such as juices, yogurts, and dietary supplements. These nanocapsules are created based on a special polymer that combines molecules of hyaluronic acid and curcumin. The inner part of the capsules is filled with curcumin and resveratrol, antioxidants beneficial for cardiovascular diseases.

Nanocapsules have the ability to form spherical particles of very small size - approximately 134.5 nanometers. Due to this, they can penetrate cells more effectively. Another important characteristic of these capsules is their stable electric charge (zeta potential), which is -29.4 mV at pH 7.4. This ensures the stability of nanocapsules in the gastric environment, where they are able to maintain their properties and gradually release active substances.

During testing in conditions simulating the stomach environment, these nanocapsules demonstrated high stability and the ability to gradually release curcumin and resveratrol. In addition, they showed higher activity in neutralizing free radicals compared to other known formulations and liposomes. Therefore, it would be good to use this development in health food products.

In another study (Wang et al., 2024), the authors also used HA in combination with gelatin to create a multilayer coating for probiotics to improve their delivery and effectiveness. Layer-by-layer (LBL) technology allowed obtaining encapsulation with an efficiency of 78%–92%, which was confirmed by FT-IR (Fourier transform infrared spectroscopy) and XRD (X-ray diffraction) analysis methods. Multilayer microcapsules demonstrated high biocompatibility, lack of immunogenicity and toxicity, significant improvement in probiotic survival in adverse conditions such as high acidity and temperature. In addition, multilayer microcapsules with HA showed improvement in antioxidant properties and viability of probiotic cells compared to non-encapsulated forms. These results confirm the effectiveness of LBL technology and HA not only in protecting probiotics, but also in potentially enhancing their beneficial properties.

Hyaluronic Acid Coatings for Food Preservation

As it turned out, HA-based coatings are effective not only for probiotics but also for many food products, as they allow increasing their shelf life. Thus, the authors (Al-Hilifi et al., 2024) managed to create an edible coating with HA, chitosan, and gelatin for strawberries. It was noted that coated fruits lost less weight and had a stable pH, indicating preservation of their juiciness, unlike uncoated hyaluronic acid analogues. In addition, the inclusion of hyaluronic acid significantly increased the antioxidant properties of the coating. This was confirmed by measurements of total phenol content, changes in ascorbic acid content and DPPH analysis (this test evaluates the antioxidant activity of substances by measuring their ability to neutralize free radicals through color change of the stable radical DPPH - 2,2-diphenyl-1-picrylhydrazyl).

A similar study was conducted by Zhou et al. (2024). A simple and effective method was developed for creating a multifunctional coating for fruit preservation by incorporating a complex of CIN and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) into hyaluronic acid, a natural polysaccharide with excellent film-forming properties. The finished HA/CIN@HP-β-CD coating demonstrated universal adhesion ability, excellent antimicrobial properties and good antioxidant activity without toxicity. Studies have shown that CIN from the coating is released gradually and constantly. Freshness tests on bananas and apples showed that this coating effectively preserves their color, reduces weight loss, prevents the development of microorganisms and significantly extends shelf life, making it promising for fruit preservation.

In addition, similar composite films of hyaluronic acid were created by adding curcumin and cellulose nanofibers (CNF) to the HA base, and their effectiveness was tested on egg preservation at 25 °C and 70% humidity. The addition of composite materials increased the thickness of the films. Curcumin at a concentration of 0.025% provided the best antimicrobial properties, while CNF significantly affected the viscosity, permeability and mechanical properties of the films. In a 56-day egg preservation test, a composite film with 0.5% HA, 0.025% curcumin and CNF, with a coating time of 2 minutes, showed the least weight loss - 13.88%. The final Haugh unit was 52.08, which exceeded control values by approximately the 35th day and extended the shelf life of eggs by at least 14 days (Fan et al., 2023).

Hyaluronic acid's role in modifying dairy and starch-based products

Due to its physicochemical properties, it is an excellent modifier and plasticizer for food products. Thus, Joshi et al., 2024 analyzed the effect of HA of different molecular weights on the properties of skimmed milk. Scientists studied the effects of HA in four sizes: 8, 320, 980 and 2550 kilodaltons (kDa), adding it to milk at a concentration of 0.25%. It was found that with increasing molecular weight of HA, the viscosity of milk and its pseudoplasticity increased, i.e. the ability to reduce viscosity when shaken. In milk samples, the formation of a weak gel-like structure was observed, caused by the interaction between polymeric particles of HA and the milk environment (Figure 2).

Hyaluronic acid with higher molecular weight (except for the smallest than 8 kDa) also improved milk's ability to retain water, its emulsifying properties and foaming ability. Interestingly, HA with the lowest molecular weight (8 kDa) did not significantly affect milk functionality, making it suitable for addition to dairy drinks to improve health without changing their properties.

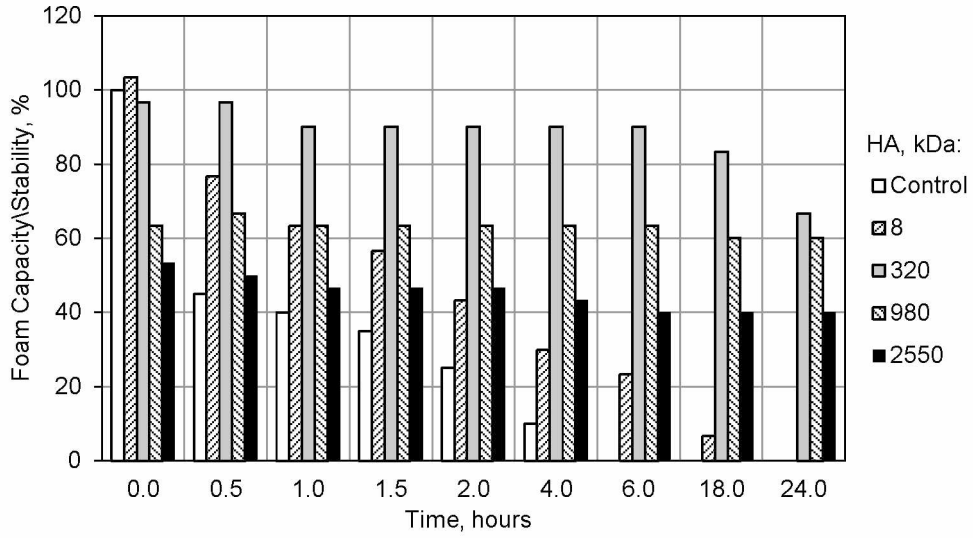

Figure 2. Foaming capacity and stability of milk samples treated with different molecular weights of hyaluronic acid at 0.25% (w/w) concentration

The study also showed that HA with a molecular weight of 980–2550 kDa did not cause significant differences in the effect on viscosity, protein separation and heat resistance of milk, which opens up possibilities for using HA in this range without additional fractionation, reducing production costs. The mechanism of HA action on milk is mostly related to the ability to bind water, while no changes were detected in the structure of milk protein fractions, and the hydrodynamic diameter of particles remained unchanged.

In another source (Wu et al., 2024), the property of HA to change the structure of cornstarch was considered. The results showed that the addition of HA significantly increased peak viscosity, solubility and water retention capacity in starch mixtures. At the same time, a decrease in pasting temperature, swelling power and amylose leaching was observed. Thus, hyaluronic acid can be used even as a conditional preservative for starch-containing products.

Hyaluronic acid as a natural flavor enhancer and salt reducer

In addition, it was noticed that HA contributes to changes in the taste properties of food (Huang et al., 2023). It discussed the effect of hyaluronic acid of different molecular weights on the perception of sweet taste in neutral and acidic solutions. HA with molecular weights of 100 kDa, 400 kDa and 1090 kDa increased the intensity and duration of sweet taste sensation at pH 4.0. This phenomenon is explained by the rupture of glycosidic bonds in HA in acidic environment, which changes its viscosity and interaction with mucin. In acidic solutions, the perception of sweet taste was slightly weaker, probably due to suppression by sour taste. Different molecular weights and concentrations of HA significantly improved the quality and thickness of the mucin layer, while the penetration of sucralose into the mucin layer decreased in acidic solutions. Hyaluronic acid with a molecular weight of 100 kDa showed the best results in enhancing sweet taste, probably due to its rigid rod-like structure, which allows more sucralose to penetrate the mucin layer. This study demonstrates the potential of using HA as a natural flavor enhancer to reduce sugar consumption without losing the desired level of sweetness.

Table 1

Applications of hyaluronic acid in food industry

Application	Description	Results/Effects	Source
Nanocapsules for antioxidants	Hyaluronic acid + curcumin for creating nanocapsules with antioxidants (curcumin, resveratrol)	Stability in gastric environment, gradual release of active substances, enhanced antioxidant activity	Guo et al., 2018
Multilayer coating for probiotics	Hyaluronic acid + gelatin for probiotic coating	Improved probiotic survival in high acidity and temperature, antioxidant properties	Wang et al., 2024
Edible coating for fruits	Hyaluronic acid + chitosan and gelatin for strawberry coating	Reduced weight loss, pH maintenance, and antioxidant properties	Al-Hilifi et al., 2024
Modification of dairy products	Impact of HA molecular weight on the properties of skimmed milk	Increased viscosity, foaming ability, and water retention with higher molecular weight	Joshi et al., 2024
Modification of starch	Hyaluronic acid as a modifier for corn starch	Increased peak viscosity, solubility, and water retention capacity	Wu et al., 2024
Enhancing flavor properties	Effect of different HA molecular weights on the intensity and duration of sweet taste	Enhanced sweet taste, reduced need for sugar	Huang et al., 2023
Salt reduction in products	Use of HA to reduce salt content in dishes (steaks, sauces)	10% salt reduction without loss of saltiness perception	Hu et al., 2023

Hu et al. (2023) noticed that adding hyaluronic acid to dishes such as steak, black pepper sauce, sour fish and packaged sour fish sauces allows reducing salt content by 10% while maintaining the sensation of saltiness. This discovery has significant potential for the food industry and health care, as excessive salt consumption is associated with an increased risk of cardiovascular diseases and hypertension. Using HA as a natural flavor enhancer can be an effective way to create low-sodium products that remain attractive to consumers.

Characteristics of modern cosmetic products containing hyaluronic acid

The beauty industry, particularly in the realm of cosmetic skincare, is undergoing a revolutionary transformation. The modern beauty paradigm has permeated deeply into human health and aesthetic consciousness, with contemporary pop culture acting as a powerful catalyst for this phenomenon. This symbiosis of demand and supply has catapulted the beauty sector into one of the most dynamic and lucrative industries globally (Lee et al., 2022). The digital age, characterized by the omnipresence of social media and the meteoric rise of e-commerce, has fundamentally altered consumer behavior, leading to increased expenditure on beauty and skincare products.

The landscape of skincare has undergone a seismic shift, transcending mere superficial enhancements to embrace a holistic approach to skin health. This paradigm shift is driven by an unprecedented level of consumer awareness and a collective aspiration to defy the aging process. Consequently, there is a surging demand for multifunctional products that not only enhance aesthetic appeal but also contribute significantly to skin health and longevity. In this context, hyaluronic acid has emerged as a revolutionary ingredient, heralded for its remarkable ability to retain moisture, boost skin elasticity, and exhibit potent antioxidant properties (Rathod et al., 2020).

One of the most popular cosmetic forms for GAGs is hydrogels. Hydrogels are gaining popularity as an innovative medium for the cosmetic application of GAGs, such as hyaluronic acid and alginate. These biocompatible polymers can form three-dimensional cross-linked networks that absorb and retain a large amount of water, making them ideal for moisturizing and regenerating skin care products. The hydrogel form provides prolonged action and optimal release of active ingredients like GAGs onto the skin over a long period. Additionally, it creates a pleasant cooling and refreshing sensation upon application, which appeals to consumers looking for effective and comfortable cosmetic products (Mitura et al., 2020).

Despite these promising outcomes, penetrating GAGs into the deeper layers of the skin remains a challenge for topical exogenous preparations. Consequently, new forms and delivery technologies are being actively developed to enhance the bioavailability of topical GAGs, including nano/microemulsions, liposomes, microneedles, and conjugation strategies. While these technologies are still in the experimental stage of development, there are already many scientific publications on this topic.

For instance, the authors point out that for the penetration of hyaluronic acid (the most popular GAG in cosmetics) into the deeper skin layers, auxiliary delivery methods are necessary (Wang et al., 2021). Most commonly used methods include microneedles with dermarollers, sliding or stamping microneedles, radiofrequency-based thermal needles, and fractional lasers.

A new method for producing microneedles from hydrolyzed hyaluronic acid could open new opportunities in the cosmetic and pharmaceutical industries. Research has shown that using a gel substance based on hyaluronic acid in combination with a vacuum imprinting method allows for the production of high-resolution microneedles with impressive precision,

a diameter of 13 micrometers, and a height of 24 micrometers, making them ideal for use in cosmetic and pharmaceutical products (Miura et al., 2022).

Microneedles have several advantages over conventional subcutaneous injections. Firstly, they are less painful; secondly, they do not require medical training, and many patients have a phobia of regular needles, making this method of HA delivery an excellent alternative.

Another alternative is nanoemulsions. These are transparent or semi-transparent substances with the following properties: low viscosity, effective penetration of active ingredients, increased interfacial surface area, high solubilization capacity, high kinetic stability, and the ability to carry both hydrophilic and hydrophobic substances. These characteristics make this type of nanocarrier an excellent candidate for delivering cosmetic ingredients to the skin. A study demonstrated that lipophilic hyaluronic acid could be encapsulated in nanoemulsions and effectively used as a transdermal delivery system for cosmetic applications. Nanoemulsions can also provide controlled release of hyaluronic acid over a long period, increasing the effectiveness and duration of its action (Oliveira et al. 2022).

Jegasothy et al. (2014) used nanotechnology to improve HA penetration. It was hypothesized that better HA penetration could be achieved by reducing its molecular weight and forming a polymer in the form of nanoparticles (nano-HA, 5 nm). Treatment based on several formulations (lotion, serum, and cream) containing nano-HA was tested on thirty-three women, and after 57 days, improvements in skin hydration and elasticity, along with reduced roughness, were noted. Although the effectiveness of products based on nano-HA was demonstrated, this study did not compare its results with treatment using regular-sized HA.

However, this was addressed in the next article (Tokudome et al., 2018). This study aimed to evaluate the passive delivery of HA nanoparticles, formed by polyionic complex formation, into the skin. HA nanoparticles were prepared by mixing and stirring anionic HA with the cationic polymer protamine at a charge ratio of 55:45. The penetration of fluorescent-labeled HA nanoparticles (HANPs) or free HA through the skin of hairless mice was characterized in vitro. HANPs or free HA were applied to the skin of UV-irradiated mice in vivo, and trans-epidermal water loss was measured after 4 days. The HA that penetrated the skin was separated and characterized using gel-permeation chromatography. The results showed that HANPs could deliver HA to the dermis both in vitro and in vivo, whereas free HA did not penetrate beyond the stratum corneum. After applying HANPs, HA in the skin was present as free HA, not nanoparticles. In vivo application of HANPs significantly reduced UV-induced trans-epidermal water loss.

Today, tissue filling procedures using collagen and HA fillers are becoming increasingly popular as they are affordable and produce noticeable long-lasting effects. They account for 9 out of 10 cosmetic procedures and 75% of the market value of cosmetic interventions, with the market size estimated at £2.27 billion.

There are many injectable HA-based products on the market for wrinkle correction, especially for nasolabial folds. Since the effect of these fillers is still temporary (usually less than 12 months for tissue fillers), many formulations have been proposed to increase the retention time, both for topical cosmetic creams and tissue fillers. For example, the preparation of partially depolymerized GAGs, gold or silver salts (Salgado et al., 2017).

This invention represents a revolutionary step in developing pharmaceutical and cosmetic products based on glycosaminoglycans. Partially depolymerized gold and silver salts of glycosaminoglycans obtained through a patented process have unique advantages over native high-molecular-weight forms of these compounds.

Firstly, the partial depolymerization process allows reducing the molecular weight of glycosaminoglycans, significantly increasing their ability to penetrate the skin barrier. This makes these compounds much more effective for topical use in pharmaceutical and cosmetic compositions for skin treatment and care.

Secondly, the presence of noble metal ions, such as gold and silver, adds additional therapeutic properties. These metals are known for their antimicrobial, anti-inflammatory, and regenerative effects on the skin, making these compounds ideal for treating wounds, burns, scars, and skin infections. Finally, these innovative compounds are promising for use in skin care cosmetics. Due to their moisturizing, regenerative, and anti-inflammatory properties, they can improve skin condition and appearance by reducing wrinkles and signs of aging.

Nevertheless, the risks associated with the use of dermal fillers include infections, lump formation, filler migration, the need for surgical intervention, scarring, blood vessel blockage, and even blindness (Pervez, 2021).

Thus, based on the above information, it can be concluded that one of the most optimal cosmetic forms for home use remains creams with hyaluronic acid. Unlike injectable fillers, cream is easy and safe to apply at home, without the risks of infection, lumps, surgical intervention, and so on. Moreover, high-molecular-weight hyaluronic acid is the most similar to the HA produced in the human body, and although it does not penetrate beyond the stratum corneum, it works quite effectively on the outer layers of the skin, acting as a barrier. The disadvantages of nanoemulsions, liposomes, and microneedles are that these technologies are still insufficiently studied, and using them on a large scale is risky, as we do not yet fully understand the possible negative effects. Compared to hydrogels, after application to the skin, creams feel more pleasant, particularly because they lack stickiness and viscosity.

Additional active components can be added to the HA cream formula, which in combination will provide better results. In the article (Juncan et al., 2021), it is noted that HA cream helps moisturize the skin and increase elasticity, thereby reducing wrinkle depth. It is assumed that when applied to the skin surface, HA solutions form an occlusive layer, absorbing moisture and thereby moisturizing the skin, filling in wrinkles. Additionally, the occlusive properties provided by HA may allow biologically active substances contained in the cosmetics to be retained in the skin layers, possibly facilitating their penetration into the epidermis.

Manufacturers of hyaluronic acid

As already mentioned, HA has quite a wide potential for use in the food and cosmetic industry, but its production must also meet standards so that this glycosaminoglycan can be safe for consumption. Since extraction from animal tissues is a somewhat inhumane method and more vulnerable to external contamination than microbial synthesis, it is obviously less common on an industrial scale now.

Since microbial synthesis has many more advantages, today many scientific sources are considering ways to improve the efficiency and reduce the cost of this process. Thus, in the article (Ferreira et al., 2021) they talk about improving the production process of this polysaccharide by *Streptococcus zooepidemicus*, because in the basic scenario HA is produced by batch fermentation, reaching 2.5 g/l after 24 hours. It is then centrifuged, diafiltered treated with activated carbon and precipitated with isopropanol. The product is suitable for local application, and its production cost is estimated at $1115/kg. Therefore, a similar production scenario was developed, based on fed-batch cultivation, which led to a higher product titer - 5.0 g/l and a lower cost of $946/kg.

In another study (Kumar et al., 2021), they optimized the production of hyaluronic acid by the *Streptococcus equisimilus* MK156140 strain. Using statistical optimization methods (Plackett-Burman design and central composite design), they determined the optimal cultivation conditions: pH 7.38, 12.15% meat extract, 7.64% yeast extract, 3% sucrose, temperature 35-38 °C, stirring speed 180 rpm, incubation time 28 hours. Under these conditions, a maximum HA yield of 7.16 g/l was achieved, which is close to the predicted value of 7.21 g/l. This is approximately 4 times higher than previous studies. The authors believe that the optimized process can be promising for industrial production of HA.

In another article (Jafari et al., 2022), the production of hyaluronic acid by a group G *Streptococcus* strain was optimized. Chemical mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine (NTG) was used in two rounds with concentrations of 120 and 200 μg/ml. Optimal cultivation conditions included pH 5.5 and a cultivation time of 4 hours. The original strain produced 1241±2.1 μg/ml of HA, while the best mutants Gm2-120-21-3 and Gm2-120-21-4 achieved 2470±8.1 μg/ml and 2856±4.2 μg/ml respectively, which is 16.1-45.5% higher. The molecular weight of the obtained HA was below 160 kDa. The mutants maintained stable production for 10 generations. This approach allowed obtaining strains with high yield of low molecular weight HA in a short cultivation time.

In the above-mentioned articles, representatives of the *Streptococcus* family are used as the target microorganism, as they are one of the most well known natural producers with a good yield of the target product. Despite this, this family of bacteria is quite pathogenic and produces exotoxins, and the HA obtained in this way must undergo additional processing stages, which increases the cost of production. Therefore, new producers are actively being sought, or they are being created using genetic engineering.

One such microorganism is *Lactococcus lactis*. The article (Sheng et al., 2015) describes the creation of an HA producer that has GRAS (Generally Recognized As Safe) status. The process of creating the HA producer involved cloning HA synthesis genes (hasA, hasB, hasC) from *Streptococcus zooepidemicus* into *L. lactis*. Several variants of recombinant strains were created with different combinations of genes, of which the best was strain NFHA03, which contained the hasA gene and enhanced expression of the endogenous ugd and glmU genes of *L. lactis*. Cultivation conditions included the use of LM17 medium with 1% lactose, cultivation at 30 °C for 12 hours, and induction of expression by adding nisin (10 ng/ml). The highest HA yield was 0.594 g/l for strain NFHA01, while strain NFHA03 produced 0.492 g/l of HA. The main advantages of the created system lie in the use of the food-grade *L. lactis* strain, the food-grade NICE expression system, and the food-grade selective marker lacF, making the process fully suitable for the production of food-grade hyaluronic acid.

In another study (Muthukrishnan et al., 2020), *Lactococcus lactis* was also used. The researchers used the non-pathogenic strain *L. lactis NZ9000*, widely used in the food industry, as a basis for genetic manipulations. A plasmid vector pNZ8148 containing the hasA and hasB genes from *Streptococcus zooepidemicus,* which encode key enzymes for HA synthesis, was introduced into this strain. Cultivation of the modified *L. lactis* was carried out in a specially adapted GM17 medium, enriched with glucose and chloramphenicol to maintain the plasmid. Optimal growth conditions included a temperature of 30°C and static conditions. Induction of HA synthesis genes was carried out using nisin at a concentration of 2 ng/ml, which was determined to be optimal for maximum expression. Bioreactors were used to scale up the process, where cells were grown at 30°C, with stirring at 200 rpm and pH maintained at seven. To ensure cell growth and HA synthesis, sterile glucose and chloramphenicol were periodically added to the medium.

In addition, this species was mentioned in the source (Jeeva et al., 2022). It studied the effect of respiratory metabolism on the production of hyaluronic acid by the modified strain *Lactococcus lactis* GJP5Δldh. Experiments were conducted at different levels of dissolved oxygen and with the addition of hemin. It was found that increasing oxygen levels and the presence of hemin positively affect HA production, reaching a maximum titer of 4.6 g/l. The researchers established a correlation between the concentrations of intracellular HA precursors and its final yield.

Recently, a study (Shaheen et al., 2023) for the first time revealed the ability of *Enterococcus durans* K11 and *Lactiplantibacillus plantarum* St3 strains to produce hyaluronic acid. These strains, isolated from natural sources - kefir and breast milk respectively, demonstrated high productivity: *E. durans* K11 synthesized HA at a concentration of 4.8 mg/ml, and *L. plantarum* St3 - 4.4 mg/ml. Strain identification was performed using 16S rRNA gene sequencing. HA obtained from both strains showed high antioxidant activity (about 66%) and significant protective effect against UV irradiation on human keratinocyte culture. It is important to note that these strains are natural isolates and have not undergone genetic modification to obtain the ability to synthesize HA. This study expands the range of known microorganisms capable of producing HA and opens new perspectives for the use of probiotic bacteria in the production of HA for the cosmetic and pharmaceutical industries.

In a study of Ahmed et al. (2023), *Klebsiella pneumoniae* H15 was identified as the best hyaluronic acid producer among 108 bacterial isolates analyzed. The strain was characterized morphologically, culturally, and biochemically, with its identity confirmed by 16S rDNA sequencing. Optimization of fermentation conditions showed the highest HA production at pH 8.0, 30 °C, 180 rpm, for 30 hours. The optimal medium contained 7% sucrose, 1.25% yeast extract, and 1.25% peptone, allowing a maximum HA concentration of 1.5 g/L. The bacterial HA demonstrated an inhibitory effect on MCF-7, HepG-2, and HCT cancer cells at concentrations of 0.98–3.91 μg/ml. On BHI agar, colonies were large, opaque, mucoid, and grayish-white, while on blood agar they were non-hemolytic, 3-4 mm in diameter. Alkaline medium favored higher HA production, with an optimal initial pH of 8.0, resulting in a final medium pH of 3.5-4.3. A temperature of 30 °C proved optimal, with satisfactory results at 25 °C and decreased production at 35-45 °C. The optimal fermentation time was 30 hours, after which a decrease in HA production was observed.

In addition, Du et al. (2021) optimized hyaluronic acid production in *Corynebacterium glutamicum* through metabolic engineering. Key strategies included improving glucose uptake by knocking out the iolR gene, intensifying cardiolipin synthesis by overexpressing pgsA1/pgsA2/cls genes, expressing Vitreoscilla hemoglobin (vgb) to enhance oxygenation, and supplementing the medium with glutamine. Cultivation conditions: medium with 40 g/L glucose, pH 7.2, temperature 28 °C, aeration 200 rpm, with periodic glucose addition and pH correction. These methods allowed achieving an HA yield of 32 g/L over 60 hours of fermentation, significantly exceeding previous indicators for *C. glutamicum*.

Radchenkova et al. (2020) optimized hyaluronic acid production by the halophilic bacterium *Chromohalobacter canadensis* 28, isolated from salt lakes of Pomorie. The strain was cultivated in a medium with 1% lactose, 15% NaCl, 1% tryptone, 0.5% yeast extract at pH 7.5. Optimal cultivation conditions included a temperature of 55 °C, pH 7.4, aeration 1.0 vvm, stirring 900 rpm. A continuous cultivation method was applied with an optimal dilution rate of 0.035 h^{-1}, ensuring stable production of high-purity exopolymer. This approach allowed avoiding the drawbacks of batch fermentation and optimizing HA production by a halophilic microorganism.

Kluyveromyces lactis as a safer alternative. The researchers inserted the xlhasB gene and created four strains with different hasA genes (three human, one bacterial). Transcript analysis confirmed hasA gene presence in three strains. The strain with the bacterial pmHAS gene produced HA, verified by electron microscopy. In bioreactor cultivation, this strain achieved maximum HA levels of 1.89 g/L with a molecular weight of 2.097 MDa. This represents the first report of HA production in *K. lactis* and the highest HA titers reported in yeast (Gomes et al., 2019).

Generalized data on hyaluronic acid producers are shown in Table 2.

Table 2

Hyaluronic acid production methods and yields across different bacterial strains

Producer	Modification method	HA yield	Cultivation conditions	Glucose source	Reference
Streptococcus zooepidemicus ATCC 39920	Fed-batch cultivation	5 g/l	pH 7, 37 °C, 12 h	Glucose	Ferreira et al., 2021
Streptococcus equisimilus MK156140	Statistical optimization	7.16 g/l	pH 7.38, 35-38 °C, 180 rpm, 28 h	Sucrose	Kumar et al., 2021
Streptococcus group G	Chemical mutagenesis (NTG)	2.856 g/l	pH 5.5, 4 h	Not specified	Jafari et al., 2022
Lactococcus lactis NFHA01	Cloning of hasA, hasB, hasC genes	0.594 g/l	30 °C, 12 h	Lactose	Sheng et al., 2015
Lactococcus lactis NZ9000	Introduction of pNZ8148 plasmid	2 g/l	30 °C, 200 rpm, pH 7	Glucose	Muthukrishnan et al., 2020
Lactococcus lactis GJP5Δldh	Introduction of pGJP5 plasmid	4.6 g/l	Various O_2 levels, hemin addition	Glucose	Jeeva et al., 2022
Enterococcus durans K11	Natural isolate	4.8 g/l	37 °C, 24 h	Skimmed milk	Shaheen et al., 2023
Lactiplantibacillus plantarum St3	Natural isolate	4.4 g/l	37 °C, 24 h	Skimmed milk	Shaheen et al., 2023
Klebsiella pneumoniae H15	Not specified	1.5 g/l	pH 8.0, 30 °C, 180 rpm, 30 h	Sucrose	Ahmed et al. 2023
Corynebacterium glutamicum	iolR gene knockout, overexpression of pgsA1/pgsA2/cls genes	32 g/l	pH 7.2, 28 °C, 200 rpm, 60 h	Glucose	Du et al., 2021
Kluyveromyces lactis.	pm *hasA*	1.89 g/l	24 hours, 30 °C and 200 rpm. 2 vvm, pH 6.0 maintained with 2 M NaOH.	Glucose	Gomes et al., 2019
Chromohalobacter canadensis 28	Not specified	2.1 g/l	55 °C, pH 7.4, 1.0 vvm, 900 rpm	Lactose	Du et al., 2021

Regulatory framework and standards

The use of HA in Ukraine is regulated by several key documents. In the cosmetics industry, the main one is the Technical Regulation on Cosmetic Products, approved by the Resolution of the Cabinet of Ministers of Ukraine on January 20, 2021, No. 65. This document establishes requirements for the composition, safety, and labeling of cosmetic products, including those containing HA.

In the pharmaceutical sector, DSTU 4765:2007 plays an important role, defining research methods, composition requirements, and quality standards for HA polymers used in pharmaceutical products. Additionally, the National List of Essential Medicines, approved by the Order of the Ministry of Health of Ukraine, includes information about medicinal products containing HA and their uses.

For medical devices containing HA, registration requirements and the Technical Regulation on Medical Devices (Resolution of the Cabinet of Ministers of Ukraine No. 753 of October 2, 2013) apply. These documents outline requirements for the safety, effectiveness, and labeling of medical devices, including those containing HA (Hryshchenko et al., 2024).

Chemical properties and quality standards

According to international standards, HA is described as a water-soluble substance available in the form of highly purified, lyophilized powder or aqueous solution. The molecular weight of HA can range from 5 to 1800 kDa, depending on the production procedures. Sodium hyaluronate, commonly used in cosmetic and medical preparations, has a molecular weight ranging from 80.2 to 4010 kDa, according to the Food Chemicals Codex (FCC) standards (Belsito et al., 2023)

Production and purification requirements

The production of HA can be carried out using various methods, including bacterial fermentation and extraction from natural sources such as rooster combs. Purification processes include solvent extraction (ethanol, acetone, etc.), ultrafiltration, ion-exchange chromatography, and other methods to remove impurities such as proteins, peptides, and nucleic acids.

It is important to note that for dermal fillers used in aesthetic medicine, the FDA requires that HA be cross-linked. This must be clearly stated in product descriptions and summaries to avoid misleading information.

Application in the food industry

In the food industry of Ukraine, the use of HA is regulated by several key documents. The main one is the Law of Ukraine "On the Basic Principles and Requirements for Food Safety and Quality," which sets out general safety requirements for food products, including additives and ingredients.

DSTU 4161-2003 "Food Additives. General Requirements" defines general requirements for food additives, their quality, labeling, and use in food products. This standard is important for manufacturers planning to use HA as a food additive.

The Order "On Approval of Requirements for Food Flavors, Food Additives, and Food Enzymes" (Resolution of the Cabinet of Ministers of Ukraine No. 133 of January 26, 2024)

defines the list of permitted food additives, including their acceptable levels and conditions of use in various food products. This document is key to determining the legality and conditions for the use of HA in the food industry in Ukraine.

Ukraine also adheres to international standards, including the Codex Alimentarius, which regulates the safety of food products and food additives at the international level. This means that the use of HA in the food industry in Ukraine must comply not only with national but also with international safety standards.

The List of Permitted Food Additives and Processing Aids, approved by the Order of the Ministry of Health of Ukraine, is another important document. It describes specific food additives, including HA, that are permitted for use in food products in Ukraine, as well as their allowable concentrations.

Safety considerations

The safety of HA use is a key aspect of its regulation. According to available data, various safety tests are conducted, including in vitro skin and eye irritation tests, human repeat insult patch tests (HRIPT), and comprehensive toxicity data.

It is important to note that clinical and safety studies on HA must clearly indicate and accurately describe the type of HA used (cross-linked or non-cross-linked) and the context of its use (e.g., cosmetic or medical applications). This is particularly important for ensuring transparency and accuracy of information for consumers and regulatory authorities.

The regulation of hyaluronic acid use in Ukraine is a complex process that covers various areas of application, from cosmetics to the food industry. The regulatory framework ensures quality control, safety, and effectiveness of HA in different products.

One of the key aspects of introducing hyaluronic acid into industrial production for cosmetic products is the compliance with regulatory requirements, including both international and national standards. In Ukraine, the production and circulation of hyaluronic acid for the cosmetic industry will be governed by the Technical Regulation on Cosmetic Products, which is harmonized with European legislation. This document will come into force on August 3, 2024. It is important to note that most national standards, which previously regulated the quality of cosmetic products, have already been repealed.

The primary regulator in the European Union that controls the use of hyaluronic acid in cosmetics is the European Parliament and Council Regulation (EC) No 1223/2009 of November 30, 2009, "On Cosmetic Products" (European Parliament & Council of the European Union, 2009). This regulation outlines a list of permitted ingredients, labeling requirements, packaging, storage, and procedures for evaluating the safety of cosmetic ingredients. Ukraine, as part of its legislative harmonization with European norms, is also implementing a Technical Regulation on cosmetic products, which will take effect on August 3, 2024. The document is based on EU Regulation No 1223/2009 and is aimed at ensuring the functioning of the domestic market while maintaining a high level of consumer health protection (Кабінет Міністрів України, 2021).

According to EU Regulation No 1223/2009, the inclusion of hyaluronic acid and its salts (sodium hyaluronate, potassium hyaluronate) is permitted in cosmetic formulations in concentrations of up to 100%. This gives manufacturers the flexibility to use HA in various cosmetic applications, such as moisturizers, serums, and anti-aging products, in concentrations optimized for specific skin types and purposes.

ISO Standards for GMP in Cosmetics

In addition to the EU regulatory framework, there are several standards within the ISO (International Organization for Standardization) series that regulate Good Manufacturing Practices (GMP) in cosmetic production. These include:

1. ISO 22716:2007 "Cosmetic Products. Good Manufacturing Practices (GMP). Guidelines" – This is the primary standard that defines the requirements for GMP in the production of cosmetics (International Organization for Standardization, 2007).
2. ISO 22717:2015 "Cosmetic Products. Microbiological Testing. Detection of *Pseudomonas aeruginosa*" – This standard outlines methods for detecting microbial contamination by *Pseudomonas aeruginosa*, a common bacterium that can contaminate cosmetic products (International Organization for Standardization, 2015a).
3. ISO 22718:2015 "Cosmetic Products. Microbiological Testing. Detection of *Staphylococcus aureus*" – This standard describes procedures for identifying *Staphylococcus aureus*, a bacterium responsible for skin infections, which can compromise product safety (International Organization for Standardization, 2015b).
4. ISO 29621:2017 "Cosmetic Products. Microbiological Testing. Guidelines for Risk Assessment and Identification of Products with Low Microbiological Risk" – This standard addresses methods for identifying pathogenic microorganisms in cosmetic products and provides guidance on risk assessment (International Organization for Standardization, 2017).

In Ukraine, two ISO standards have been harmonized for GMP and microbiological control in cosmetic production. These include DSTU EN ISO 22716:2015 "Cosmetics. Good Manufacturing Practice (GMP). Guidelines on Good Manufacturing Practice (EN ISO 22716:2007, IDT)", and DSTU EN ISO 29621:2016 "Cosmetics. Microbiology. Guidelines for risk assessment and identification of low microbiological risk products," which is harmonized with ISO 29621:2010 (although this version of the standard is no longer in effect in the EU).

Quality Standards for hyaluronic acid in cosmetics

There are also specific standards and pharmacopeial articles that establish quality requirements for hyaluronic acid. The European Pharmacopoeia includes a monograph on "Hyaluronic Acid," which outlines quality criteria for HA in both pharmaceutical and cosmetic applications. These criteria include appearance, solubility, identification, water content, heavy metals, and microbial limits (European Directorate for the Quality of Medicines & HealthCare, n.d.). In the United States, the USP-NF standard <1276> "Hyaluronan" provides similar requirements for hyaluronic acid quality, applicable to both pharmaceutical and cosmetic use (United States Pharmacopeial Convention, n.d.).

In Ukraine, the State Pharmacopoeia does not contain a separate monograph on hyaluronic acid. However, there is a general article on "Cosmetic Products" that includes requirements for microbiological and toxicological parameters (State enterprise "Ukrainian Scientific Pharmacopoeia Center for the Quality of Medicinal Products", 2015). Specific requirements in Ukraine for hyaluronic acid as an ingredient in cosmetics are defined in the upcoming Technical Regulation on cosmetic products, which will govern product safety, labeling, and consumer protection.

Regulation of cosmetic products in the United States

In the United States, the primary regulatory body overseeing the safety and marketing of cosmetic products, including those containing hyaluronic acid, is the U.S. Food and Drug Administration (FDA). Under the Federal Food, Drug, and Cosmetic Act, manufacturers are responsible for ensuring that their products are safe and that their labeling is truthful and not misleading. The FDA does not require premarket approval for cosmetic products, except for color additives, but it monitors adverse events and issues guidance on best practices for manufacturing and labeling.

The FDA has established specific quality standards for individual ingredients, including hyaluronic acid, when used in pharmaceutical and cosmetic formulations. In the case of hyaluronic acid, its production is expected to comply with GMP, which in the U.S. is regulated by standards such as ISO 22716, ensuring that products meet safety and quality requirements before reaching consumers.

Ukrainian regulatory authorities and harmonization with EU standards

In Ukraine, the regulation of cosmetic products is overseen by the State Service of Ukraine on Food Safety and Consumer Protection. Unlike in the United States, where the regulatory environment is somewhat less formalized, the regulation of hyaluronic acid in Ukraine is more structured, as it is based on the requirements of the Technical Regulation on cosmetic products. This regulation, harmonized with European standards, ensures that Ukrainian cosmetic products meet the safety and quality benchmarks of the EU.

Dermal fillers based on hyaluronic acid are becoming more and more popular in the world for cosmetic procedures. However, regulatory requirements for these medical devices still vary between jurisdictions, raising questions about their safety and efficacy.

For many years, there has been a problem of insufficient regulation of the safety of filers in the UK and the European Union (EU). Non-medical fillers were not subject to the same strict requirements as medical products and had less stringent safety standards than even toys or cosmetics (Bowes, 2014). This posed risks to consumers due to potentially unsafe products, unskilled filler insertion, lack of proper training and certification of providers, and unclear requirements for facilities and management of complications (Rowland-Warmann, 2020).

Approval for the sale of fillers in the US requires extensive clinical studies, while in the EU limited data was sufficient. The presence of CE marking for fillers did not guarantee a proper assessment of product safety. This has raised concerns about the lack of clear safety standards compared to the US.

However, the situation is changing with the release of the new EU Medical Device Regulation (MDR) 2017/745, which will replace the outdated Directive (MDD). The MDR imposes stricter requirements on Class III medical devices, particularly fillers (Kelso, 2020). This involves stricter requirements for pre-market clinical studies, post-market surveillance, product identification and transparency.

While the FDA approval process for fillers as prescription products may still be more rigorous at the pre-market stage, the new MDR brings EU regulation significantly closer to US standards. Both systems have their advantages and disadvantages, so the authors call for global harmonization of requirements (Kelso, 2020).

In summary, although previous EU filler regulation has been insufficient, the new MDR promises to raise safety and efficacy requirements to levels close to those of the US.

However, there is room for further improvement and harmonization of standards at the global level.

In Ukraine, regulation of dermal fillers is carried out in accordance with the legislation on medical products.

The main normative acts are Law of Ukraine "On technical regulations and conformity assessment"; Technical regulation on medical devices, approved by the resolution of the CMU No. 753 of October 2, 2013.

According to this Technical Regulation, dermal fillers are classified as class III medical products - products with a high degree of risk.

Basic requirements:

- Mandatory certification of the medical product in the authorized conformity assessment body before putting it into circulation on the territory of Ukraine.
- Clinical approval based on evaluation of clinical data obtained from relevant clinical studies.
- The manufacturer's quality management system must meet the requirements of technical regulations.
- Proper labeling of the product in accordance with the requirements of the regulation (name, manufacturer, instructions, etc.).

The sale and use of dermal fillers in Ukraine is possible only by those manufacturers/distributors who have passed the above-mentioned conformity assessment procedure. However, unlike the EU and the USA, Ukraine does not yet have specialized requirements specifically for dermal fillers. They are governed by the general rules for medical devices.

Post-fermentation stages in glycosaminoglycan production: an essential but complex process

In the biotechnological production of glycosaminoglycans, biosynthesis represents just the initial phase of a much longer and more complex process. Once the cultivation of microorganisms or cell cultures has been completed, glycosaminoglycans such as hyaluronic acid must be isolated from the supernatant and purified to remove impurities, including bacterial cell debris and proteins. This post-fermentation stage is crucial for producing a final product that is both high in quality and non-immunogenic, meaning it will not provoke an immune response when used in medical or cosmetic applications. However, this step is not only labor-intensive but also costly, especially at a large industrial scale. The purification and isolation process can be technically challenging due to the delicate nature of glycosaminoglycan molecules and the need to maintain their structure and biological activity (Sharma et al., 2022).

One of the most widely used methods for the extraction of hyaluronic acid from the supernatant involves precipitation techniques that closely resemble those used for protein precipitation. This approach typically requires the use of organic solvents. The principle behind this technique lies in the reduction of the dielectric constant of the aqueous phase by introducing an organic solvent. This modification in the system encourages macromolecular interactions, which, in turn, lead to an increase in the molecular weight of the hyaluronic acid molecules. As the molecular weight increases, the hyaluronic acid becomes less soluble in the solution, ultimately resulting in its precipitation.

According to recent research (Ucm et al., 2022) isopropanol is often the preferred organic solvent for hyaluronic acid precipitation. Isopropanol is particularly effective due to its properties that promote efficient precipitation while maintaining the structural integrity of

the hyaluronic acid. In laboratory settings, however, cold ethanol is sometimes used as a more accessible and cost-effective alternative. This precipitation method, while effective, requires a careful balance between the solvent concentration, temperature, and process duration to ensure that the hyaluronic acid does not degrade during the process.

Another study conducted by Abbas Mohammed and Niamah (2022) investigated similar precipitation methods using isopropanol. In this process, the authors employed 1% trichloroacetic acid (TCA) to remove protein contaminants, which is a common step in the refinement of biological molecules. Trichloroacetic acid denatures proteins, making them easier to separate from the target molecule—in this case, hyaluronic acid. Following protein removal, the hyaluronic acid was dialyzed against ultrapure water to further purification of the product. Dialysis is a method that utilizes a semi-permeable membrane to remove small impurities while retaining larger molecules like HA. The ultrapure water used in this stage is specifically treated to have extremely low mineral content, ensuring that no ions or particles interfere with the purification process. Finally, the dialyzed product was subjected to lyophilization, a freeze-drying technique that removes water from the sample by sublimation. This method allows the hyaluronic acid to be converted into a stable, dry form, which can then be processed into a commercial product.

An alternative approach to HA purification was described by Güngör et al. (2019). Their method began with the removal of cellular debris after fermentation using a 0.15% sodium dodecyl sulfate (SDS) solution, followed by centrifugation to separate the solid and liquid phases. SDS is a surfactant that helps to break down cell membranes and solubilize proteins, making it easier to remove unwanted cellular material from the mixture. After centrifugation, the supernatant containing hyaluronic acid was processed through a dialysis column equipped with a cellulose membrane. The column's dimensions were 25 mm × 16 mm, and the membrane had a molecular weight cut-off of 14,000, allowing only smaller molecules to pass through while retaining larger hyaluronic acid molecules. The column was immersed in a NaCl solution for five days to facilitate the gradual removal of impurities. Following the dialysis step, the dialysate was filtered through a series of increasingly fine filters, including cellulose acetate filters with pore sizes of 0.45 and 0.2 micrometers, and mixed cellulose ester filters with a pore size of 8 micrometers. This multi-step filtration process ensured the thorough removal of any remaining contaminants. The hyaluronic acid was then precipitated using 96% ethanol, resulting in an impressive yield of 12 g/L, which is considered a very high concentration for HA production.

A common theme across these studies is the reliance on organic solvents like isopropanol or ethanol to precipitate hyaluronic acid from the supernatant. While these solvents are highly effective, they are also expensive and may not be the most economical option for large-scale industrial production. Moreover, the use of organic solvents can pose environmental concerns and increase the complexity of waste management.

Recognizing these challenges, Gözke et al. (2017) explored the possibility of using electrodialysis as an alternative to organic solvent-based precipitation. Electrodialysis is a process in which ions are moved across a membrane using an electric field, allowing for the selective removal of charged impurities while retaining neutral molecules like hyaluronic acid. The study found that electrodialysis was able to preserve the molecular weight and structural integrity of the hyaluronic acid, which are critical for its biological function and commercial value. Additionally, the researchers reported an improvement in the overall yield of the product compared to traditional filtration methods. This technique offers a promising alternative for large-scale HA purification, potentially lowering costs and minimizing environmental impact.

Justification of methods for the extraction and purification of hyaluronic acid

Taking into account the processed literature, the most common methods of hyaluronic acid extraction at each purification stage were summarized and considered (Figure 3).

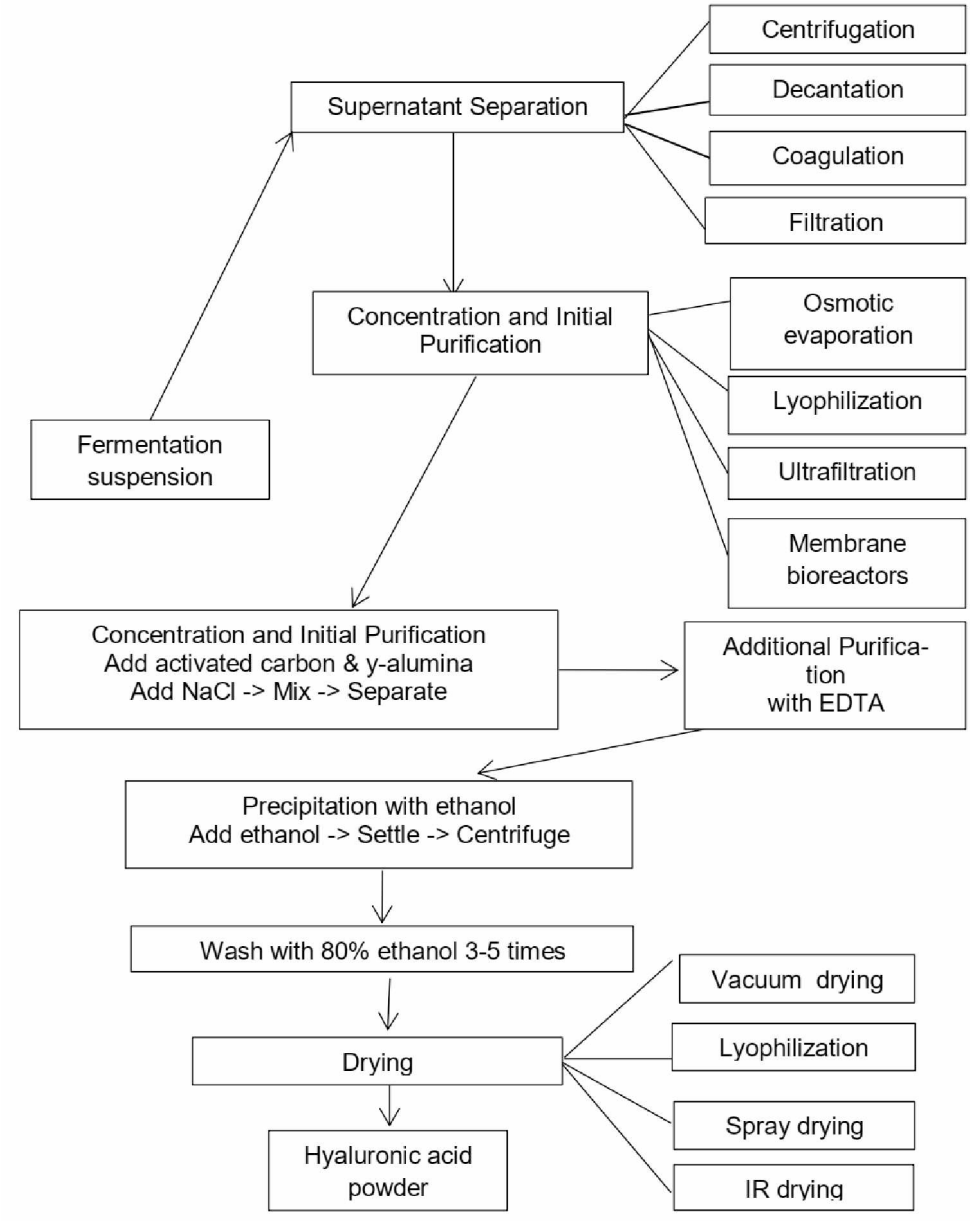

Figure 3. Common methods of hyaluronic acid extraction

Supernatant separation

The initial step in the hyaluronic acid extraction process is the separation of the supernatant from the bacterial culture. This step is critically important as it determines the quality and quantity of HA that can be obtained in subsequent stages. Several methods can be employed for this purpose, each with its own advantages and disadvantages (Choi et al., 2015):

Centrifugation:

- This is the most common and efficient method.
- Advantages include rapid separation, reproducibility of results, and scalability for industrial production.
- Typical conditions involve centrifuging at 5000-7000 g for 10-15 minutes.
- These parameters may vary depending on the specific bacterial strain and desired purity of the final product.
- It is crucial to optimize centrifugation speed and time to achieve maximum cell separation without causing cell lysis.
- Modern continuous-flow centrifuges can be used for large-scale operations, allowing for uninterrupted processing of large volumes of bacterial culture (Rodriguez-Marquez et al., 2022).

Filtration:

- While less common for bacterial cultures due to potential clogging issues, filtration can be useful for smaller-scale operations or specific bacterial strains.
- Diatomaceous earth can be added to improve filtration efficiency by creating a porous filter cake that enhances particle retention.
- Cross-flow filtration systems can be employed to minimize fouling and extend filter life in larger operations.
- The choice of filter pore size is critical: it must be small enough to retain bacterial cells but large enough to allow HA molecules to pass through (Cavalcanti et al., 2019).

Decantation:

- This method is simple but time-consuming and less efficient, making it unsuitable for large-scale production.
- It relies on gravity to separate the cells from the supernatant, which can take several hours to achieve adequate separation.
- While not practical for industrial processes, it might be used in laboratory settings for small-scale extractions or when specialized equipment is unavailable (Rossatto et al., 2023).

Coagulation:

- The addition of coagulants like aluminum or iron salts can aid in cell separation but may introduce unwanted impurities.
- This method can be particularly useful when dealing with difficult-to-separate bacterial strains.
- The choice and concentration of coagulant must be carefully optimized to ensure efficient separation without compromising HA quality.
- A subsequent purification step may be necessary to remove residual coagulants from the HA solution (Choi et al., 2014).

Centrifugation is generally preferred due to its efficiency, speed, and minimal risk of contamination. However, care must be taken to optimize centrifugation conditions to prevent cell lysis, which could release intracellular contaminants. The choice of separation method

may also depend on the specific characteristics of the bacterial strain used for HA production, as some strains may be more prone to cell lysis or may produce HA with different molecular weights that affect separation efficiency (Rodriguez-Marquez et al., 2022).

In industrial settings, a combination of methods may be employed. For instance, a preliminary filtration step might be used to remove large debris before centrifugation, enhancing the overall efficiency of the separation process.

Concentration and initial purification

Following supernatant separation, the HA solution undergoes concentration and initial purification. This step is crucial for removing water and low-molecular-weight impurities while retaining and concentrating the HA molecules. Several methods can be employed, each with its own advantages and considerations:

Ultrafiltration:

- This is the preferred method due to its efficiency in concentrating large molecules like HA while removing smaller impurities.
- A two-stage ultrafiltration process is often employed:
- First stage: Concentrates the solution to half its original volume using a 30,000 Da membrane.
- Second stage: Diafiltration with distilled water to reduce conductivity to around 2 mS/cm.
- The choice of membrane molecular weight cut-off is critical: it must be small enough to retain HA but large enough to allow impurities to pass through.
- Tangential flow filtration can be used to minimize membrane fouling and maintain high flux rates.
- Temperature control during ultrafiltration is important to prevent HA degradation.
- The process can be optimized by adjusting parameters such as transmembrane pressure, cross-flow velocity, and concentration factor (Cavalcanti et al., 2019).

Lyophilization:

- While effective in preserving HA structure, this method is time-consuming and energy-intensive, making it less suitable for large-scale production.
- It involves freezing the HA solution and then removing the ice by sublimation under vacuum.
- The freezing rate and temperature must be carefully controlled to minimize damage to the HA structure.
- Secondary drying may be necessary to remove residual bound water.
- While not ideal for initial concentration, lyophilization might be used as a final step to produce a dry, stable HA product (Aguilera-Bulla et al., 2022).
- Osmotic evaporation:
- This method uses osmotic pressure differences to remove water but can be slow and challenging to scale up.
- It involves using a concentrated salt solution separated from the HA solution by a semipermeable membrane.
- The osmotic gradient drives water from the HA solution into the salt solution, concentrating the HA.
- While gentle on the HA molecules, this method requires careful management of the salt solution and may introduce salt contamination if the membrane integrity is compromised (Lambe et al., 2021).

Membrane bioreactors:

- These combine fermentation and filtration but require significant initial investment.
- In this system, HA is produced continuously while being separated from the bacterial cells and concentrated in real-time.
- This approach can lead to higher productivity and reduced processing time but requires careful design and operation to maintain optimal conditions for both HA production and separation.

Ultrafiltration is typically chosen for its balance of efficiency, scalability, and preservation of HA quality. The process not only concentrates HA but also removes low-molecular-weight impurities, enhancing the purity of the product. The multi-stage approach allows for fine-tuning of the concentration and purification process, with the diafiltration stage being particularly effective at removing salts and other small molecules.

In industrial settings, the ultrafiltration process may be further optimized by using a cascade of membranes with different molecular weight cut-offs, allowing for more precise fractionation of the HA based on molecular weight. This can be particularly useful when producing HA for specific applications that require a narrow molecular weight distribution.

The choice of concentration and initial purification method will depend on factors such as the desired final HA concentration, the nature and quantity of impurities present, the molecular weight distribution of the HA, and the intended application of the final product. Careful optimization of this stage can significantly affect the efficiency of subsequent purification steps and the quality of the final HA product (Karami et al., 2021).

Adsorption of impurities

The concentrated HA solution is then subjected to an adsorption process to remove high-molecular-weight impurities. This step is crucial for achieving high-purity HA suitable for cosmetic and pharmaceutical applications. Common adsorbents include activated carbon granules (e.g., Norit C Gran), gamma-alumina, diatomaceous earth.

The process typically involves:

- Adding 2% activated carbon granules and 1% gamma-alumina to the HA solution.
- Adding sodium chloride to a concentration of 1 M to enhance impurity adsorption.
- Mixing the solution for about 5 hours to allow adsorption of proteins, nucleic acids, and endotoxins.
- Separating the adsorbents by centrifugation or filtration.
- The use of both activated carbon and gamma-alumina provides a synergistic effect: activated carbon effectively removes high-molecular-weight proteins and nucleic acids, while gamma-alumina is particularly effective at removing endotoxins.
- The presence of sodium chloride aids in detaching impurities from HA and increases the adsorption capacity of the adsorbents. After adsorption, the adsorbents can be regenerated for reuse, typically by heating to 900 °C in the absence of oxygen and in the presence of steam (Choi et al., 2015).

Additional purification

An additional purification step using EDTA (ethylenediaminetetraacetic acid) is often employed to further enhance the purity of the HA solution. This step serves multiple purposes:

- Removal of divalent cations: EDTA chelates divalent metal ions that may be present in the solution.
- Inhibition of protease activity: EDTA can inhibit metalloproteinases that might degrade HA.
- Removal of residual aluminum: EDTA helps in removing any remaining aluminum from the previous adsorption step.
- The process typically involves:
- Adding EDTA to the HA solution to a final concentration of 1 mM.
- Mixing the solution for about 10 minutes to allow chelation and impurity removal.

This step is particularly important for cosmetic-grade HA, where high purity is essential for product safety and efficacy (Mónico et al., 2017).

Precipitation

The purified HA is then precipitated using an organic solvent, most commonly ethanol. This step serves to isolate the HA from the aqueous solution and remove any remaining water-soluble impurities.

The precipitation process typically involves:

- Adding ethanol to the HA solution in a ratio of 2:1 (ethanol to HA solution).
- Allowing the mixture to stand for a period to ensure complete precipitation.
- Separating the precipitate by centrifugation (typically at 6000 g for 5 minutes).

Ethanol is preferred over other organic solvents due to its lower cost and reduced toxicity. The 2:1 ratio is chosen to balance efficient HA precipitation with minimizing HA losses.After precipitation, the ethanol can be recovered through distillation for reuse, improving the process's economic efficiency (Cavalcanti et al., 2020)

Washing

The HA precipitate is washed with 80% ethanol to remove any remaining impurities. This step is crucial for achieving a high-purity final product suitable for cosmetic applications.

- The washing process typically involves:
- Resuspending the HA precipitate in 80% ethanol.
- Mixing thoroughly to ensure all surfaces of the precipitate are exposed to the ethanol.
- Separating the washed precipitate by centrifugation.
- Repeating the process 3-5 times to ensure thorough purification.

The 80% ethanol concentration is chosen to effectively remove impurities while minimizing HA loss. The ethanol used in this step can also be recovered and reused after appropriate purification (Cavalcanti et al., 2020).

Drying

The final step in the process is drying the HA precipitate to obtain the final powder product. Several drying methods can be employed (Aguilera-Bulla et al., 2022):

Vacuum drying:

- This method is often preferred due to its balance of efficiency, cost-effectiveness, and preservation of HA quality. The process involves:
- Placing the HA precipitate in a vacuum chamber.

- Applying vacuum to lower the boiling point of residual water.
- Gently heating to facilitate water removal without degrading the HA (Collins et al., 2013).

Lyophilization:

- While effective in preserving HA structure, this method is time-consuming and energy-intensive. It involves:
- Freezing the HA precipitate.
- Applying vacuum to sublime the ice directly to vapor.
- Secondary drying to remove bound water.

Spray drying:

- This method is fast and suitable for large-scale production but may lead to some HA degradation due to high temperatures. The process involves:
- Atomizing the HA suspension into a hot air stream.
- Collecting the dried powder at the outlet of the drying chamber.

Infrared drying:

- This method provides rapid heating and drying but may result in uneven drying and potential HA degradation (Fallacara et al., 2019).
- Vacuum drying is often chosen for its ability to dry the HA at relatively low temperatures, preserving its quality while still being efficient and scalable for industrial production.
- The dried HA powder is then collected and packaged under sterile conditions to maintain its purity and quality.

Each of these steps in the extraction and purification process plays a crucial role in obtaining high quality HA suitable for cosmetic and pharmaceutical applications. The choice of methods at each stage depends on factors such as required purity, scale of production, available equipment, and economic considerations. Continuous optimization of these processes contributes to more efficient and cost-effective production of this valuable biopolymer.

The production of glycosaminoglycans, particularly hyaluronic acid, involves several post-fermentation steps that are essential for obtaining a high-purity, biologically active product. While traditional methods such as organic solvent precipitation, dialysis, and filtration are effective, they present challenges in terms of cost, scalability, and environmental sustainability. As the demand for hyaluronic acid continues to grow in fields such as medicine, cosmetics, and biotechnology, the need for more efficient and sustainable purification methods is becoming increasingly important. New technologies, like electrodialysis, show promise in addressing these challenges, offering a more cost-effective and environmentally friendly approach to glycosaminoglycan production.

This expanded version provides a more in-depth analysis of the various post-fermentation techniques and the innovations aimed at improving the efficiency and sustainability of glycosaminoglycan purification.

Conclusions

Based on the conducted review, it can be concluded that hyaluronic acid has broad commercial applications in the food industry, medical and cosmetic fields. In particular, the most promising strain for industrial production of HA is *Corynebacterium glutamicum* (32

g/l during 60 hours of cultivation), which has received GRAS status, confirming its safety for use in food products.

For the food industry, it is especially important to comply with the requirements established by the Law of Ukraine "On Basic Principles and Requirements for Food Safety and Quality", DSTU 4161-2003, and the Technical Regulation on Food Additives. These documents define the conditions for using HA as a food additive, its permissible concentrations, and labeling requirements.

Manufacturers and importers of products containing HA must carefully adhere to all regulatory requirements, ensure proper quality and safety of products, and provide consumers with accurate and complete information about the composition and properties of the products.

Given the constant development of technologies and research in the field of HA application, further improvement of the regulatory framework governing its use can be expected. This may include updating quality standards, expanding areas of application, and implementing new safety control methods.

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Cite:

UFJ Style

Hryshchenko M., Starovoitova S. (2024), From production to regulation: the comprehensive role of hyaluronic acid in the food and cosmetic industry, *Ukrainian Food Journal*, 13(3), pp. 476–506, https://doi.org/10.24263/2304-974X-2024-13-3-5

APA Style

Hryshchenko, M., & Starovoitova, S. (2024). From production to regulation: the comprehensive role of hyaluronic acid in the food and cosmetic industry. *Ukrainian Food Journal*, *13*(3), 476–506. https://doi.org/10.24263/2304-974X-2024-13-3-5