Современная наука и инновации. 2024. № 3. С. 84-91.
ТЕХНОЛОГИЯ ПРОДОВОЛЬСТВЕННЫХ ПРОДУКТОВ

Modern Science and Innovations. 2024;(3):84-91.
TECHNOLOGY OF FOOD PRODUCTS

Научная статья
УДК 637.344 637.146.2
https://doi.org/10.37493/2307-910X.2024.3.8

Перспективы использования дрожжей для переработки вторичного молочного сырья

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Аннотация. *Обоснована актуальность применения различных видов дрожжей для синтеза коммерческих продуктов. Проанализированы направления переработки вторичного молочного сырья на основе биоконверсии лактозы. Показана возможность применять дрожжи, которые не способны к гидролизу лактозы в целях утилизации вторичного молочного сырья.*

Ключевые слова: вторичное молочное сырье, молочная сыворотка, дрожжи, Kluyveromyces marxianus, напитки, биоэтанол

Для цитирования: *Лодыгин А. Д., Нерсесян Т. В., Куликова И. К. Перспективы использования дрожжей для переработки вторичного молочного сырья // Современная наука и инновации. 2024. № 3. С. 84-91.* https://doi.org/10.37493/2307-910X.2024.3.8

Research article

Prospects for yeast using in secondary dairy raw materials processing

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Abstract. *The relevance of different types of yeasts application for synthesis of various types of commercially available products is substantiated. Directions of secondary dairy raw materials processing based on lactose biotransformation is analyzed. The possibility of using yeast, which is not capable of hydrolyzing lactose, for the purpose of recycling secondary milk raw materials has been shown.*

Keywords: secondary dairy raw materials, whey, yeasts, Kluyveromyces marxianus, beverages, bioethanol

For citation: *Lodygin AD, Nersesyan TV, Kulikova IK. Prospects for yeast using in secondary dairy raw materials processing. Modern Science and Innovations. 2024;(3):84-91.* https://doi.org/10.37493/2307-910X.2024.3.8

Introduction. The development of whey processing in Russia, despite numerous studies in this area, is held back by several reasons. Among them are insignificant investments in the dairy industry, lack of funds for the implementation of modern technologies and the purchase of equipment, insufficient consumer awareness of the benefits of whey products and the promotion of a healthy lifestyle, the absence of mass production of multifunctional products based on milk whey, and the liberalism of the environmental service regarding the discharge of whey into wastewater.

Different whey processing technologies should be used depending on its type and daily volume. According to MMF, the annual volume of whey in the world is more than 130 million tons, and the problem of its processing in many countries remains relevant.

There are three main directions of industrial processing of whey: full use of all whey components (fresh and fermented drinks, condensed and dry products, etc.), separate use of raw material components (extraction of fat, proteins, lactose, etc.), obtaining derivative components of whey (hydrolysates of whey proteins, glucose-galactose syrups, lactulose, etc.) [2, 6, 7].

To process whey, thickening, drying, electromembrane (electrodialysis, electroactivation) and baromembrane processes (ultrafiltration, nanofiltration, reverse osmosis) are used [3, 4, 6].

The use of the latter is relevant for the production of whey protein concentrates. Most of the dry matter in cheese whey is lactose (45–50 g/l). In addition, it contains proteins (6–8 g/l), lipids (4–5 g/l) and minerals, primarily calcium, potassium and phosphorus. Among the whey ingredients, whey protein concentrates (WPC) are of greatest interest to global consumers. Their production, however, does not fully solve the problem of recycling secondary dairy raw materials, as it is associated with the production of a significant amount of whey permeate, which is a by-product of the technological process.

Currently, new applications have been found for whey permeate in the food industry, for example as a source of unsweetened sugars [6]. Also, due to the fact that β-galactosidase is synthesized by a fairly large number of microorganisms, cheese whey and whey permeate can also be used as sources of nutrients for fermentation processes [7–10].

Materials and research methods. The purpose of this article is to analyze the possibility of enzymatic utilization of various types of secondary dairy raw materials, which can result in the production of various products suitable for sale with added value. One such option may be the production of the so-called single-cell oil (SCO), accumulated by oily yeasts [9, 30, 34].

Research results and their discussion. SCO yeast has a fatty acid profile similar to vegetable oils [14], which makes this microbial product attractive for biodiesel production. According to [17], the growing demand for biofuels has increased the cost of vegetable oils, from which 90% of biodiesel is obtained. Thus, microbial oil may represent a promising alternative to solve the ethical issues associated with the “food or fuel” dilemma. On the other hand, SCO may be a suitable additive for animal and human nutrition due to its high content of unsaturated fatty acids [30]. Oleaginous yeasts exhibit the ability to accumulate intracellular lipids up to 70% of dry weight and are considered “easy” for industrial production, fast-growing and land-efficient [12, 25, 26]. However, the cost of microbial oil production is still high, which does not allow the implementation of the bioprocess on a large scale. Kutinas et al. [23] reported that the cost of oil using glucose as a carbon source and oleaginous yeast Rhodosporidium toruloides would be US$5.5/kg at a glucose price of US$0.4/kg. However, a reduction in this cost could be achieved by using secondary dairy raw materials as a substrate. [33] Furthermore, the use of liquid waste such as whey permeate can save fresh water required for fermentation, reducing the impact on water resources throughout the production cycle [20].

It is also known about the technology of obtaining a beer-like drink based on whey permeate [1]. As is known, when using different types of whey (curd, cheese) for further processing, clarification is necessary in order to remove casein dust and whey proteins. When using permeate in the technological chain, this operation is absent, which reduces the costs of producing a beer-like drink. The beer-like drink based on curd whey permeate differed little in composition from ordinary beer, contained 2-3 times less alcohol (1.5-2.0%); was rich in extractive substances (about 8%), their content was 2 times more than in ordinary beer. This technology is interesting in that it allows for almost waste-free utilization of curd whey permeate, the processing of which for the purpose of obtaining lactose is difficult due to the large number of impurities.

Bioconversion of secondary milk raw material by Kluyveromyces marxianus yeast has been proposed as a method for producing various types of alcohols, such as phenylethyl alcohol [18]. 2-Phenylethanol (2-PE) is an alcohol with rose scent and antimicrobial activity, so it is widely used in food and cosmetic industries as a flavoring agent and preservative. The results showed that the production yield of 2-PE was increased by 60% in continuous cultivation compared with batch fermentation. Along with a remarkable decrease in chemical oxygen demand for whey permeate, the present study reports a complete, efficient and environmentally friendly method for the production of 2-PE with a space-time yield of 57.5 mg/L/h.

Another popular alcohol that has been obtained from the fermentation of secondary dairy raw materials with Kluyveromyces marxianus yeast is ethanol. [16] The production of ethanol using K. marxianus from various types of whey obtained from the production of organic cheeses was studied. Cultivation was carried out in batch and continuous modes. The results showed that pasteurization before the process is not required, which is a great advantage from an industrial point of view, since pasteurization or sterilization of whey leads to an increase in the financial costs of the process. Batch fermentation of unsterilized whey showed high ethanol yields (0.50 g ethanol/g lactose) at both 30 °C and 40 °C using low pH (4.5) or without pH control. Continuous fermentation of unsterilized whey was carried out using K. marxianus yeast immobilized in calcium alginate capsules. High ethanol production (4.5 g/L/h) was achieved at a dilution rate of 0.2/h, and K. marxianus was able to maintain high production at low pH in unsterilized whey. K. marxianus was able to compete with lactic acid bacteria present in whey and was found to be a very robust microorganism capable of producing ethanol at high temperature and low pH in whey.

The production of bioethanol from secondary dairy raw materials was also demonstrated using immobilized E. coli microorganisms expressing Vitreoscilla hemoglobin [31].

According to data [29], ethanol can be obtained not only from native samples of secondary dairy raw materials, but also from reconstituted ones. This method of bioconversion has a certain advantage, since it makes it possible to control the yield of a useful product by increasing the dry substances of the nutrient substrate by changing the reconstitution recipe.

Bioconversion of lactose into bioethanol during fermentation of secondary milk raw materials is possible not only with the help of lactose-fermenting yeast. It has been shown that in a combined culture consisting of lactobacilli and Saccharomyces cerevisiae yeast, which are not capable of fermenting lactose, the possibility of producing ethyl alcohol is preserved [5]. The mechanism is based on the ability of lactobacilli to hydrolyze lactose to oligosaccharides, which are suitable as a nutrient medium for Saccharomyces cerevisiae. An interesting fact is that in this environment there is no competition for nutrition, since yeast can feed on both oligosaccharides and the products of lactobacilli. Bioconversion of lactose with the help of homofermentative lactic acid bacteria allows cultivating many types of yeast on milk whey, which themselves are not capable of assimilating lactose, but absorb lactic acid well.

Among the organic substances that can be obtained by processing secondary dairy raw materials, the proposed method for obtaining ethyl acetate seems interesting. Ethyl acetate is a valuable organic solvent and is currently produced from fossil hydrocarbons. An interesting alternative could be microbial synthesis of this ester from carbohydrate-rich waste. Synthesis of ethyl acetate [21] using Kluyveromyces marxinanus DSM 5422 from lactose-free whey permeate (DWP) was tested in an aerated stirred bioreactor at 40 °C.

In addition to obtaining the sought-after organic substances through the bioconversion of secondary milk raw materials, there is the possibility of obtaining valuable products from the biomass of lactose-fermenting yeasts themselves, cultivated on lactose-rich nutrient media, which can be whey or permeate obtained during its baromembrane treatment. The enzyme β-galactosidase, which allows these types of yeast to ferment secondary milk raw materials, can be isolated from them, as was shown in one of the proposed methods [8].

Another component of yeast biomass that was obtained after culturing Kluyveromyces marxinanus on secondary dairy raw materials is mannoprotein, which has pronounced emulsifying properties [27]. It was extracted from the cell walls of Kluyveromyces marxianus grown on a lactose-based medium by autoclaving the cells in citrate buffer at pH 7. The purified product was evaluated for chemical and physical stability to determine the possibility of its use as a natural emulsifier in processed foods. The yield of purified bioemulsifier from this K. marxianus strain was 4–7% of the initial dry cell weight. The purified product at a concentration of 12 g/L formed emulsions stable for 3 months when exposed to a range of pH (3–11) and NaCl concentration (2–50 g/L). The composition of this mannoprotein was 90% carbohydrate (mannan) and 4-6% protein. These values are similar to the mannoprotein extracted from Saccharomyces cerevisiae cells, which is a traditional source. Therefore, K. marxianus grown in inexpensive lactose-based media such as whey or pure lactose-rich dairy waste could synthesize a bioemulsifier for use in food industry. The emulsifying properties of the bioemulsifier from K. marxianus were similar to that of the same product obtained from S. cerevisiae biomass reported by Cameron [13] and Torabizadeh et al. [32]. As an emulsifier, the mannoprotein of K. marxianus grown in lactose-rich media may have certain advantages over other yeast species. Firstly, the yeast can be grown on whey, a widely available by-product of the dairy industry that contains sufficient lactose. Secondly, the difficulty in removing residual hydrocarbons from alkane-grown yeast bioemulsifiers will hinder their use in some applications, such as food. Since K. marxianus is classified as having "generally recognized as safe" (GRAS) status, its mannoprotein bioemulsifier can be expected to be non-toxic. Thirdly, it is stable over a wide pH range from 3 to 11. Finally, it can be used in formulations containing a wide range of NaCl concentrations from 2 to 50 g/L). The new bioemulsifier from K. marxianus was successfully isolated and evaluated for its emulsifying properties and potential use in the food industry, where emulsification plays an important role in consistency and texture formation, as well as in phase dispersion.

Conclusion. Despite the wide possibilities of processing secondary dairy raw materials, a significant amount of it is still not used to obtain valuable commercial products. It can be assumed that this is due to the high cost of re-equipping enterprises for these purposes, limited access to technology, logistical and economic obstacles.

On the other hand, as the conducted analysis of literary data shows, an interesting alternative to processing can be microbial conversion of secondary milk raw materials, which does not require large financial investments and allows obtaining biotechnological products that are in demand and popular on the market.

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Вклад авторов: все авторы внесли равный вклад в подготовку публикации.

Конфликт интересов: один из авторов – доктор технических наук, доцент А. Д. Лодыгин является членом редакционной коллегии журнала «Современная наука и инновации». Авторам неизвестно о каком-либо другом потенциальном конфликте интересов, связанном с этой рукописью.

Статья поступила в редакцию: 10.09.2024;
одобрена после рецензирования: 14.10.2024;
принята к публикации: 27.10.2024.

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Contribution of the authors: the authors contributed equally to this article.

Conflict of interest: one of the authors AD Lodygin, Dr. Sci. (Techn.), Associate Professor, is a member of the Editorial Board of the journal "Modern Science and Innovations". The author is unaware of any other potential conflict of interest related to this manuscript.

The article was submitted: 10.09.2024;
approved after reviewing: 14.10.2024;
accepted for publication: 10.10.2024.