TECHNICAL TRANSACTIONS | CZASOPISMO TECHNICZNE
ENVIRONMENT ENGINEERING | ŚRODOWISKO
1-Ś/2016
DOI: 10.4467/2353737XCT.16.197.5946

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UTILIZATION OF ANIMAL BLOOD PLASMA AS EXAMPLE OF USING CLEANER TECHNOLOGIES METHODOLOGY

UTYLIZACJA PLAZMY KRWI ZWIERZĘCEJ JAKO PRZYKŁAD STOSOWANIA METOD CZYSTSZEJ PRODUKCJI

Abstract

Dried animal blood plasma, which is offered to the market as a feed product, should meet appropriate standards of quality and epidemiological safety. That is why blood drawing, its transportation, storage and processing must be performed with absolute observance of the principles of hygiene and sanitation. The paper discusses the possibilities of application of techniques used in the food industry for the pretreatment of animal blood plasma to ensure a high quality of the final product. Both the proper treatment of this by-product from the animal's production as well as its recycling and re-use as a feed or food additives are examples of practical use of cleaner production methods.

Keywords: *blood plasma, pre-treatment, bacteria amount reduction, anticoagulation, blood stabilization, cleaner production*

Streszczenie

Suszona plazma krwi zwierzęcej, aby mogła być oferowana na rynku jako produkt paszowy, powinna spełniać odpowiednie standardy jakości oraz bezpieczeństwa epidemiologicznego. Dlatego ważne jest właściwe pobranie surowca, jego transport i magazynowanie oraz obróbka przy bezwzględnym przestrzeganiu zasad higieniczno-sanitarnych. W artykule omówiono możliwości aplikacyjne technik stosowanych w przemyśle spożywczym do obróbki wstępnej plazmy krwi zwierzęcej w celu zapewnienia wysokiej jakości produktu finalnego. Zarówno odpowiednie przetwarzanie tego produktu ubocznego z produkcji zwierzęcej jego recykling, jak i wtórne zużycie do produkcji pasz czy dodatków do żywności jest przykładem zastosowania w praktyce metod czystszej produkcji.

Słowa kluczowe: *plazma krwi, obróbka wstępna, redukcja flory bakteryjnej, antykoagulacja, stabilizacja krwi, czystsze produkcje*

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1. Introduction

Cleaner production includes pollution production, reduction at source, recovery of materials end energy, with or without proper processing of waste or byproducts and their recycling [1]. The proposed methods of management and processing of blood plasma are examples of recycling and reuse of this valuable product. Recycling and re-use of blood plasma is possible only in this case if sampling and treatment of animal blood and its processing into blood plasma [2].

Spray-dried blood plasma is a rich source of nutrients. It may therefore be used as a feed component in feeding monogastric animals. The dried pork plasma is a powder ranging in color from cream to pale yellow; it is dry, odorless, with specific density of 0.60–0.65 kg/dm^3 and a moisture content of 6–9%. It is readily dissolved in water (88%) [3–5]. A commercial product should contain min. 70% of protein and other components should be present in the following maximum amounts: fat – 2.0%, fibers – 0.3%, Na – 6.0% K – 0.6%, Ca – 0.15% P – 0.15% Cl – 3.0% Fe – 50 ppm, while the amount of ash obtained from the sample material should not exceed 14.0%. In addition, the plasma should have very low content of heavy metals and bacteria, especially from the group of the *E. coli* and *Salmonella*. Pb concentration limit is 0.3 mg/kg, for Cd – 0.05 mg/kg, Hg – 0.02 mg/kg, As – 0.2 mg/kg) [6–8]. The major protein fractions of the blood fraction are: albumin (approx. 50%), α-globulins (15%), β-globulins (15%) and γ-globulin, which include valuable immunoglobulins (15%) naturally stimulating the functioning of the immune system of animals. Typical composition of plasma proteins is as follows [%]: alanine – 3.8; arginine – 4.2; aspartic acid – 7.1; cysteine – 2.5; glutamic acid – 10.6; glycine – 2.7; histidine – 2.5; isoleucine – 2.6; leucine – 7.0; lysine – 6.1; methionine – 0.6; phenylalanine – 4.1; proline – 11.5; serine 4.2; threonine – 4.3; Tryptophan – 1.2; tyrosine – 3.3; valine – 4.8 [5, 9].

Drying of the animal plasma solution requires specific, properly selected process parameters to maintain its functional and biological values. Too aggressive treatment could in fact harm the quality of the finished product. Thus, the process is carried out by spray drying of plasma solution in a drying chamber through which hot air flows at the same time, causing a rapid evaporation of water droplets (mist), which then turn into powder particles falling to the bottom. Drying under such conditions allows using of high temperatures at a short contact time to minimize protein denaturation and to preserve the biological function of the plasma [10, 11].

Modification of the above-described method is spraying drying using a fluidized bed. In this case, drying is carried out as follows: the plasma solution is sprayed on a bed of small balls made of polycarbonate or dextrose, suspended in a stream of warm air. The balls were coated with a thin film of plasma solution; the applied layer immediately evaporates water. The balls rub against each other's surfaces, causing the abrasion of the applied material and increase in its bulk density. The beads are supported by a wire mesh through which dried powder is poured. Using of this method is preferred for economic reasons [5].

In order to commercial the product – dried porcine plasma, blood collected at slaughter must be subjected to stabilization with anticoagulants to prevent the clotting process, and then separated into plasma and material morphogenesis (blood cells). The resulting solution of plasma is treated further using processes of initial concentration, sterilization, and reduction of the mineral's content and discoloration. The use of all these operations should

ensure compliance with the requirements of the quality parameters of the product and meet the microbiological criteria to guarantee the safety of animal health, food products of animal origin and the environment [12, 13].

In the paper, we discussed the possibilities of application of techniques used in the food industry, for the pretreatment of animal blood plasma to ensure the high quality of the final product. Both the proper treatment of this by-product from the animal's production as well as its recycling and re-use as a feed or food additives is are examples of practical use of cleaner production methods.

2. Initial concentration

Blood plasma is an animal protein aqueous solution (8%) and mineral component (1%). The dry weight is about 9%. Thus, the main component of the plasma forming unwanted ballast in the production of dried plasma is water that must be removed. Its quantity is approx. 91%. Due to the protein denaturation occurring at an elevated temperature with the use of the simplest methods, concentration of the solution by heating and evaporation of water is not possible. Therefore, two leading techniques of initial plasma concentration are usually used: ultra-filtration and evaporation processes carried out under vacuum.

3. Minimization of the bacterial flora in the raw material and centrifugation products

Animal blood and blood products are highly microbiologically sensitive materials and can be easily contaminated. Therefore, both during slaughter and further processing of blood, the highest standards of hygiene must be maintained. However, when the raw material does not meet the microbiological purity, it becomes necessary to use special techniques to reduce microbial growth. The most common methods of sterilization are thermal processes, which, in this case, due to the specific properties of the blood and its derivatives (high temperature sensitivity), are practically not used. It is therefore necessary to use more technologically advanced processes, such as e.g. bactofugation or membrane techniques [14] and radiation [15]. Other proposals include sterilization of a porcine plasma solution using a bactericidal preparation based on nanosilver and dry plasma sterilization by irradiation in an electron accelerator.

Radiation techniques

For the sterilization, ionizing radiation, i.e. gamma radiation (γ), accelerated electrons (e-), and sometimes X-rays, causing the electrically neutral atoms and molecules, changes in electric charges or ionization, are used. Depending on the dose of radiation and the conditions in which this process takes place, inter alia temperature and oxygen, radiation in food preservation technology can be used:

- sterilization of food; large doses from 10 kGy to 50 kGy;
- extension of the storage stability of food products; middle dose, from 1 to 10 kGy;
- increase the stability of certain agricultural products, the prevention of parasitic diseases and food poisoning; small doses to 1 kGy.

Global standards for the use of techniques of radiation for food irradiation are defined by the Food Codex Commission FAO and WHO [15].

Radiation techniques allowing for the preservation of various kinds of food products are mainly used in the United States [16]. In the European Union and in other European countries, legal restrictions and a lack of experience in the application of these techniques mean that they are used to a lesser extent. In the EU, there are two Directives on the use of radiation techniques. The first unifies the law on the use of irradiation in the Member States [17], while the second establishes a positive list of foods that may be irradiated [18]. In the above-mentioned European directives, as foods authorized for treatment with radiation, these only include dried aromatic herbs, spices and vegetable seasonings. The maximum overall average absorbed radiation dose for these products is defined as 10 kGy. In Poland, all issues related to radiation preservation of foods are regulated by the Regulation of the Minister of Health of 15 January 2003. Under this regulation, the country can irradiate: onions, garlic, fresh and dried mushrooms, potatoes, dry spices, dried vegetables and pork plasma.

In addition to the formal and legal conditions, wider application of radiation techniques for sterilization of food is difficult, due to the high costs of preparing such an installation and problems associated with the necessity of cooperation with the specialized agencies of Atomic Energy and the difficulties of disposing spent radioactive element. In addition, in order for the development of such technology to be profitable, sterilization should be subjected to approximately 200–300 t of product per day, which in Polish conditions /rather low production capacity of poultry plants/ is not easy [16].

Tests of powdered plasma sterilization by irradiation with high-energy electrons at a dose of 2 × 10 kGy were conducted on the accelerator Electronics 10/10 at the Institute of Nuclear Chemistry and Technology at the Department of Chemistry and Radiation Technology in Warsaw. Their results confirmed the usefulness of methods for removing bacterial flora from the product, regardless of the size of the batch of exposed material. The material used in the tests was powdered plasma obtained in the experimental installation of Meat Plant Duda-Bis, and it did not meet the microbiological standards. Total number of bacteria exceeded 108, and the content of bacteria *E. Coli* group was at 5×10^3. Plasma sterilization under these conditions resulted in the total removal of the bacterial flora plasma.

The processes of the application importance used for the preservation of food, water sterilization, disinfection of production rooms, apparatus or technical equipment may also include ultraviolet light, ultrasound or microwaves. However, none of these techniques used in the laboratory tests – the irradiation of UV light in the so-called ultrasonic washer and microwave reactor Plazmatronika – gave positive results. On the contrary, in many cases, in the samples, elevated bacterial count was not observed. The time of exposure to radiation of sterilized material was limited by rising temperatures and the possibility of denaturation of proteins. It appears that the used exposure times are insufficient to achieve the desired effects, and the increased temperature favors the development of microflora.

Bactofugation

Bactofugation is the process of separating microbial cells from the liquid by centrifugation. The principle of the process is using the difference in the density of bacterial cells and the liquid itself. The distribution is made on disc drum centrifuges with s special design. Since the density of microbial cells is increased compared to the density of liquid plasma phase, they behave in the spaces between the discs analogously to the behavior of particulate matter in the sedimentation centrifuges. Bacterial concentrate flows toward the outer circumference of the drum, whereas the stream of liquid free of microorganisms is directed to the axis of rotation. The effectiveness of the separation of microbial cells from the stream dosed to the centrifuge plasma depends on the continuous discharge of the concentrate fraction bacterial outside. The increase in cell concentration in the outer part of drum can block the inflow of new particles, which can lead to contamination of the new batch of plasma [14, 19].

Membrane techniques

Membrane techniques [20] allow separating with different sizes of particles on the specially prepared filtration membranes. It is necessary to select such a membrane to separate undesirable substances, while at the same time not reducing the content of the essential components of the medium, thereby changing its physic-chemical properties. Removal of bacteria from liquid products is carried out using microfiltration membranes.

In work [12], the use of membranes TAMI Industries with the separation limits 1.4 μm; 0.45 μm; 0.07 μm; and 300 kD to eliminate the bacterial flora from pig's blood plasma was described in detail. The research results indicate that the target micro-plasma unit should work on the membranes with the separation limit 1.4 μm, enabling to obtain a satisfactory level of bacteria reduction in the raw material. The membrane retains almost the entire bacterial microflora and the filtrate does not indicate the presence of bacteria from group *E. Coli* and is fully permeable to proteins and mineral salts, the component responsible for the quality of plasma. Other membranes have a much greater efficiency of removal of bacteria, but the process of their use is accompanied by significant losses of proteins, increasing with decreasing separation limits of membranes. In the case of a 0.45 μm membrane, these losses are ~20%, while for membranes of 0.07 μm and 300 kD, they are close to 90%.

Bactericidal preparations

The use of bactericidal preparations to reduce the level of microorganisms in pig plasma is determined mainly by their composition. The addition of any substance is not such as to cause changes in the properties of the raw material, or limit its use.

The results of tests available on the market with an innovative formulation containing silver particles with bactericidal, fungicidal and deodorant properties for sterilization porcine plasma are presented below. The chemical composition of the preparation is as follows [%]: silver – 0.2; polyvinyl alcohol – 2.5; water – 97.3. The formulation is a colorless liquid with a slight odor and acid pH range of 6–9. The test were conducted with concentrate containing 2000 mg/L Ag, which, before the introduction into the plasma samples, was diluted with water to a concentration of about 100 mg/L Ag. Nanosilver solution was dispensed into the sterilized samples so as to obtain any suitable concentration of silver ions, and then left for

48 hours in sterile, sealed containers. After this time, microbiological analyzes specifying the total number of bacteria (OLB) and the number of bacteria *E. Coli* group in preparations were carried out. The results are presented in Table 1.

Table 1

Bactericidal effect of formulation obtained on the basis of silver nanoparticles, the level of bacteria in animal blood drawn

Test	Ag content [ppm]	Number of bacteria from the group *E. Coli*	Total number of bacteria
1	0	1×10^6	$> 10^8$
2	2	3×10^5	$> 10^8$
3	5	3×10^5	$> 10^8$
4	10	2×10^2	$> 10^8$
Limit	–	0	$< 1 \cdot 10^4$

The presented results demonstrate the unsuitability of this type of formulation for sterilization. It was observed that with an increase of silver ions, the number of *E. Coli* bacteria in the samples decreases; however, in each case, the standard requirements are not met. There were no positive results for the reduction in the level of total bacteria. Operation of preparations based on silver in the pig plasma sterilization process is not possible most likely due to interaction of silver ions with sulfur compounds contained in the feed and the unsuitable pH for formulation, causing its deactivation.

4. Decrease of mineral substances in the blood

An important role in the blood-collecting is played by its stability made in order to prevent coagulation. The process is realized by dosing a coagulant solution into the blood drawn from the aorta of a slaughtered animal, whose function is:

– decrease of the possibility of rapid development of bacterial flora,
– decrease of the possibility of the occurrence of the process of blood hemolysis,
– elimination of the blood clotting process.

The most commonly used as coagulants are sodium citrate citric acid, a mixture of phosphates (22% Na_2HPO_4, 22% $Na_4P_2O_7$, 16% $Na_2H_2P_2O_7$ and 40% NaCl), aqueous solutions of heparin and oxalate, oxalate, sodium or potassium hydroxide, the disodium salt of ethylene-diamine-tetra-acetic acid, proteolytic enzymes. The addition of most of these formulations in the blood increases the concentration of minerals in the liquid part of plasma blood. To maintain the quality standards of dried plasma, these substances must be removed. Most often, ultrafiltration is used for this purpose [14].

5. Discoloring of blood plasma

Blood pork plasma may contain impurities resulting from the improper treatment of both blood and plasma itself. Due to the improper treatment of blood, its hemolysis is possible. This means that plasma obtained by centrifugation will contain too much the particles of hemoglobin, causing its color to differ in comparison to the market standards. If the contamination of plasma with hemoglobin occurred during the blood fractionation, the selection of process parameters and operating parameters of the centrifugation is significant. They must take into account both the product quality and the process efficiency. From the practical point of view, there is no possibility of a complete separation of the blood. The increase in the yield of plasma results in the penetration of hemoglobin into liquid plasma, causing the deterioration of its color or transparency, and vice versa – decrease of the plasma outflow is associated with the improvement of its quality, but it drastically reduces the quantity of the obtained product. This has negative influence on the economics of the process. Theoretically, it is possible to further purify the obtained plasma in the second, additional centrifuge; however, incurred costs of operation increased the cost of the overall process, making it unprofitable.

The available techniques of plasma discoloring can be divided into two main groups. The first is the mechanical separation of the particles, causing coloration or turbidity of the plasma fraction. These are plasma centrifugation and filtration, including membrane filtration [20, 21]. The second group used the chemical methods [22], changing the characteristics of the particles so as not to affect the quality of the product [23, 24]. The usefulness of all these methods for the discoloration of plasma is described below.

Membrane techniques

Membrane filtration [19] is one of the most common methods for the purification of liquids and it usually helps to seperate fine impurities that are in them. Therefore, it seemed promising to use membrane to discoloration of plasma solutions.

Below are described the results of tests carried out with:

- the microfiltration membranes made by TAMI Industries with 0,14 μm separation limit;
- ceramic membranes of UNIPEKTIN (TiO_2 membrane in a cover made from Al_2O_3) with separation limits of Section 0.1;
- 0.075 and 0,05μm and membrane disc type M37GR40PP, M37FS40PP, M37FSM045PP offering the possibility of the discoloration of plasma and to obtain clear solutions, but at the expense of almost a total loss of the protein, which disqualifies membrane processes for the discoloration plasma.

Filtration

In addition, the application of filtration techniques, using a candle and disc, turns out to be useless. Filtration of raw plasma using them does not bring satisfactory results because the color of the solution does not change. Furthermore, the process complicated by drastically due to reducing of the process flow through the filtration partition, even to its complete stop.

The use of ion exchangers was pointless. Preliminary tests carried out using various types of ion exchange resins available on the market have not given the desired results; the degree of discoloration of the plasma was negligible.

Chemical methods

To reduce the dark characteristic color of hemoglobin, hydrogen peroxide bleaching can be used. Tests carried out with the use of H_2O_2 confirmed the usefulness of this solution for discoloring liquid plasma. Optimization of the process on a laboratory scale allowed to choose process parameters, i.e. temperature, pH and process time. The transfer of the process into a quarter-scale did not cause deterioration of the quality of the obtained solution.

The developed plasma discoloration procedure is as follows: plasma solution (pH 7.5–8.5) is alkalized slowly with a 4M solution of NaOH to obtain pH 13. Next, at the bottom of the reactor, hydrogen peroxide is introduced with concentration of 10% H_2O_2 in the amount of 1 vol. of H_2O_2 solution into 50 vol. of plasma solution. The system is left for 6 hours at 3°C and mixed extemporaneously so as not to cause foaming of the solution. After completion of the plasma, discoloring is made also with 2.5% HCl adjustment of pH to 7.

Comparison of the characteristics of crude and bleached plasma is reported in Table 2. Discolored plasma had a 1.36% lower protein content and 0.87 higher salts content. These results are not satisfactory.

Table 2

Analyses of blood plasma before and after discoloring process (quarter-technique scale)

Raw material	Color	pH	Content [%]		
			protein	water	salts
Raw plasma	Dark pink	6.90	5.97	91.53	0.66
Discolored plasma	Tea color	7.04	4.61	92.64	1.53

6. Summary

Proper treatment of blood, by-product from the animal's production, its processing into high quality of blood plasma, recycling and re-use of dried blood plasma as a feed or food additives, is an example of the practical use of cleaner production methods.

Production of dried pig's blood plasma with quality corresponding to the proper standards requires using (before the drying process) additional operations to improve its quality. In many cases, these are the methods of physical separation, whose task is to eliminate undesirable components of plasma; therefore, they are rarely chemical methods. This pre-treatment may often be used in techniques known and used in the food industry.

Due to the characteristic for proteins, the possibility of their denaturation under the influence of physical (temperature, pressure, ultrasonic) and chemical factors (heavy metal salts, acids, bases, urea, certain organic solvents, detergent) resulting in loss of their biological activities as well as the usefulness of each of the used methods, have to be verified. In the case of developing the techniques already used in other technologies, their procedures require modification and optimization of parameters.

To maintain quality standards of dried plasma, mineral substances must be removed. Most often, ultrafiltration is used for this purpose. Tests carried out with hydrogen peroxide bleaching confirmed the usefulness of this solution for discoloring of liquid plasma. Membrane filtration seemed promising to use membrane to discoloration of plasma solutions.

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