



LYOFILISATION PROCES FOR THE ISOLATION OF NATURAL COMPOUNDS - ANTHOCYANINS

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Abstract:

Isolation of natural substances, like anthocyanins, from the plant material is carried out by steam distillation and extraction in various extraction reagents. The methods of separation with their specific conditions (high temperature, aggressive solvents, etc.) means, in many cases, changes in the structures of secondary metabolites, thereby reducing their therapeutic effect. In connection with these facts insulation of components by the method of lyophilisation appears to be a real method, in which the structure of qualitative and quantitative properties are not changed.

*The aim of the research project had been to study the isolation of natural compounds - anthocyanis – from the fruits of selected plants species by the method of drying freezing. Blueberry (*Vaccinium corymbosum* L.) and blackcurrant (*Ribes nigrum* L.) was selected for the initiation lyophilisation proces and was done with cooperation by the company GEA Lyophil SMART® SL 2. Fruits (or extracts) were cleaned and mixed in + 10 °C. Mixture was stored in stainless steel tape and the lyophilization was made.*

The behavior of the product was studied while it was being cooled down to the temperature lower than -80 °C and subsequently reheated in an alcohol bath. During the cooling proces most of the components crystalized at the temperature between -5 and -45 °C. Some few components were frozen at a temperature ranged from -25°C until -75 °C. The degree range for beginning start melting during reheating process was between -20 °C - -45°C.

Keywords: freeze-drying, extracts, *Vaccinium corymbosum* L., *Ribes nigrum* L.

INTRODUCTION. Anthocyanins are naturally occurring compounds that impart colour of fruits, vegetables and plants. They are probably the most important group of visible plant pigments besides chlorophyll visible to the human eye. They belong to the widespread class of phenolic compounds collectively named flavonoids. The differences between individual anthocyanins related to the number of hydroxyl groups, the nature and number of sugars attached to the molecule, the position of this attachment and the nature and number of aliphatic or aromatic aids attached to sugars in molecule. Apart from imparting colour to plants, anthocyanins also have an array of health-promoting benefits, as they can protect against a variety of oxidants through a various number of mechanisms. However, anthocyanins have received less attention than other flavonoids, despite this [1]. More than 400 anthocyanins have been found in nature [2].

Lyophilization is a process which extracts the water from foods and other products so that the foods or products remain stable and are easier to store at room temperature (ambient air temperature). Lyophilization is carried out using a simple principle of physics called sublimation. Sublimation is the transition of a substance from the solid to the vapour state, without first passing through an intermediate liquid phase. To extract water from samples, the process of lyophilization consists of freezing the sample so that the water in it become ice, under a vacuum, sublimating the ice directly into water vapour, drawing off the water vapour and once the ice is sublimated, the samples are freeze-dried and can be removed from the machine. Lyophilization has many advantages compared to other drying and preserving techniques. Lyophilization maintains samples quality because it remains at a

temperature that is below the freezing-point during the process of sublimation. The use of lyophilization is particularly important when processing lactic bacteria, because these products are easily affected by heat. Foods which are lyophilized can usually be stored without refrigeration, which results in a significant reduction of storage and transportation costs. Lyophilization greatly reduces weight, and this makes the products easier to transport. For example, many foods contain as much as 90% water. These foods are 10 times lighter after lyophilization. Because they are porous, most freeze-dried samples can be easily rehydrated. Lyophilization does not significantly reduce volume, therefore water quickly regains its place in the molecular structure of the food [3].

The aim of our project was to develop the optimal technique for anthocyanins extraction and to preservation it by lyophilisation. Final product of anthocyanins could be used as food supplement. Anthocyanins are instable in higher temperature and lower pH. Pharmaceutical companies often use freeze-drying to increase the shelf life of products, such as vaccines and other injectables. By removing the water from the material and sealing the material in a vial, the material can be easily stored, shipped, and later reconstituted to its original form for injection.

MATERIAL AND METHODS. The tests were carried out at the GEA Lyophil laboratory (Germany) with greatest care in the apparatus and machines available in the laboratory. The freezing behavior (crystallization after sub-cooling) of the product was determined by the cooling speed, the sensor used and the transfer of heat. The condition at the GEA Lyophil laboratory were not sterile.

For a process of lyophilisation were used six samples. There were used two plant material – blueberry (*Vaccinium corymbosum* L.) from the locality Krivá, Orava region, Slovakia and blackcurrant (*Ribes nigrum* L.) from the locality Uzhorod, Ukraine. The fruits of this two species were used for preparation of three different extracts. The first was plain juice of fresh fruits purified from all particules. The second and the third were water and ethanol extract. The aim of the research was to study the isolation of natural compounds - anthocyanis – from the fruits of selected plants species by the method of drying freezing with adjusted various parameters, to optimize the process of isolation and lyophilization and to stabilize them.

Extracts were studied by the resistance measurements. The behavior of the products were studied while it was being cooled down to a temperature lower than – 80 °C and subsequently reheated in an alcohol bath. The resistance and temperature of the extracts were measured during cooling and reheating. With this method it is possible to study the behavior of products while it is being frozen and heated. It is also possible to determine the temperature that the product must reach during freezing, as well as the product temperature that must be maintained during primary drying (sublimation temperature T_{ice}).

The measuring system used was AW 2 Eutectic monitor with a programmable TS 2 Cooling and Heating Unit. The values measured were registered by the the DES software of the AW 2 Eutectic Monitor. The measurements can be avaluated using the curves thus obtained.

RESULTS AND DISCUSSION. We have been evaluated the process of lyophilisation for each extract and compared properties and possibilities of anthocyanins isolation from two different species – blueberry and blackcurrant.

There were used 3 g net weight of the blueberry and blackcurrant juice. Cooling rate was 1.0 °C/min (pre-set value) and heating rate was 3.0 °C/min (pre-set value). During the cooling analysis most components of blueberry crystallized at temperature between 0°C and -31°C and some few components were also frozen at a temperature of – 75 °C. For the juice of blackcurrant temperatures were shifted as follows. During the cooling most of components crystallized between 0°C and -45 °C and few components were frozen at a temperature of -65 °C. During the heating some of blueberry components began to melt at a temperature -75°C and most components melted at temperature of -31 °C. Black currant components began to melt at temperature of -65 °C and most components melted at temperature of -45°C (Figure 1 and 2).

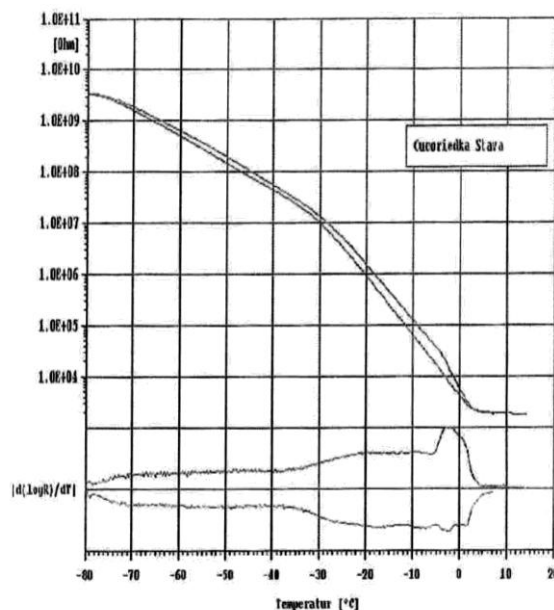


Figure 1 Results of the resistance measurements of blueberry juice.

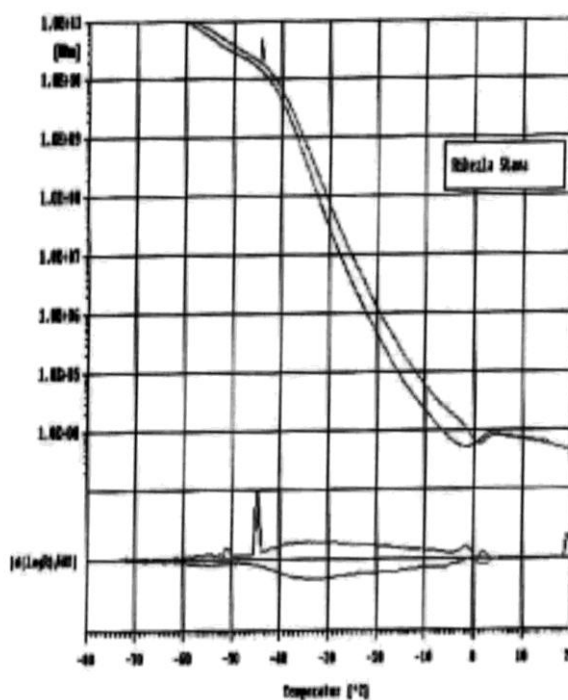


Figure 2 Results of the resistance measurements of blackcurrant juice.

Analysis of water extracts of the fruits of both species required 3 g net weight. Cooling rate was 1.0 °C/min (pre-set value) and heating rate 3.0 °C/min (pre-set value). Most of the components of blueberry extract crystallized at temperature between 0 °C and - 25 °C. Some few components were frozen at temperature - 65 °C. Components at blackcurrant water extract crystallized earlier than components at blueberry water extract – between 0°C - - 5°C. Some components were frozen at temperature - 45 °C.



During reheating some frozen components began to melt at temperature of - 65 °C but most of components melted at -30 °C (Figure 3). Melting components from black currant water extract started at -45 °C but most components melted at temperature of - 20 °C (Figure 4).

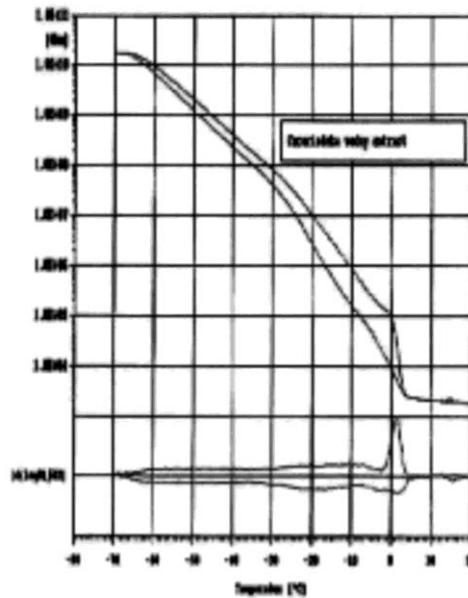


Figure 3 Results of the resistance measurements of blueberry water extract.

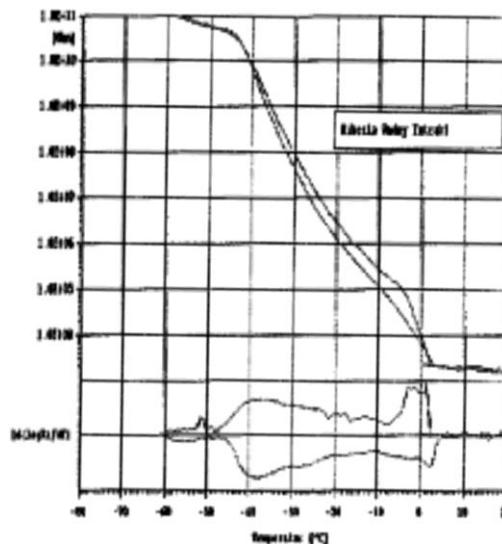


Figure 4 Results of the resistance measurements of black currant water extract

Last analysis were carried out with the ethanol extracts of mentioned samples. Cooling rate was 1.0°C/min and heating rate was 3.0 °C/min. We evaluated differences between the temperature of crystallization proces. Most of the components of blueberry crystallized between 0°C and -39°C. Some few components were frozen at temperature -55°C and -72 °C. The components of blackcurrant start to crystallize later at -5°C and finished at -40 °C, but few components were frozen between -40 °C and -50 °C and also at a temperature of -65°C.



Reheating proces almost copied the line of cooling. Frozen components began to melt at temperature of -72°C and -55°C , but most o the components of blueberry melt at a temperature of -39°C . The components of blac currant began to melt during the reheating at a temperature of -65°C and -55°C . Most of the components melt at a temperature od -40°C .

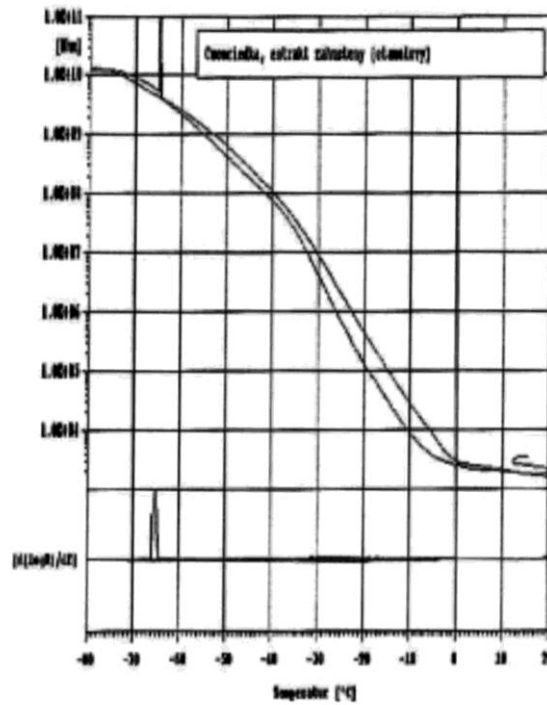


Figure 5 Results of the resistant measurements of blueberry ethanol extract

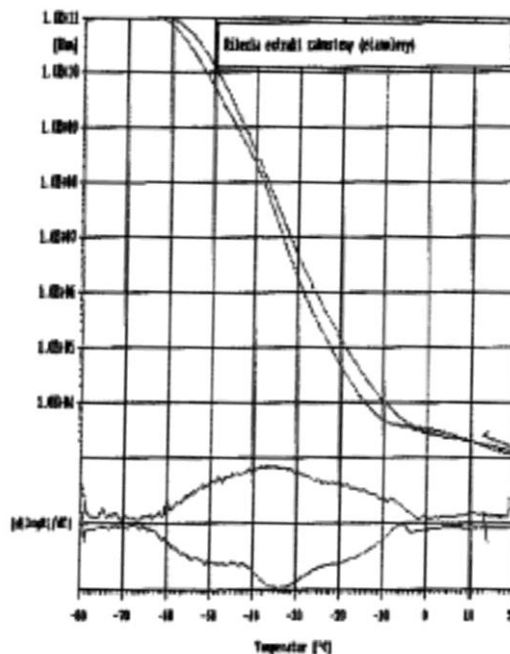


Figure 6 Results of the resistance measurement of black currant ethanol extract

CONCLUSION. Freeze-drying science and technology continues to evolve and increase in importance because of the emergence of biotechnology drugs that are too unstable to be commercially available as ready-to-use solutions [3]. The freezing method used during lyophilization can substantially affect the structure of the ice formed, the watervapor flow during primary drying, and the quality of the final dried product. Controlling how a solution freezes can shorten lyophilization cycles and produce more stable formulations [4].

According to results of lyophilization proces we can conduct the most suited parameters for the maintaining quality of the samples (*Vaccinium corymbosum* L. and *Ribes nigrum* L.). Comparing the freezing of juices of two species, the product of blueberry must be frozen at a temperature below -40 °C and sublimation occurred at the temperature lower than -31°C while blackcurrant at -45°C and at the same temperature begun sublimation.

Water extract of blueberry had almost the same properties for freezing (-40 °C) and sublimation (-30°C) as it was in its juice. Sublimation process changed in blackcurrant water extract and begun earlier at -20°C while temperature of freezing did not changed compared to its juice.

Presence of alcohol in ethanol extracts shifted lines. Freezing of the ethanol extract of blueberry must be frozen at a temperature below -55°C and sublimation occurred during primary drying, the ice – temperature lower than -39°C. Ethanol extracts of blackcurrant samples require for the freezing temperature below -45/-50 °C and for sublimation temperature lower than -40 °C.

On a larger scale, freezing is usually done using a freeze-drying machine. In this step, it is important to cool the material below its triple point, the lowest temperature at which the solid and liquid phases of the material can coexist. This ensures that sublimation rather than melting will occur in the following steps. Usually, the freezing temperatures are between -50 °C and -80 °C. The freezing phase is the most critical in the whole freeze-drying process, because the product can be spoiled if badly done.

If a freeze-dried substance is sealed to prevent the reabsorption of moisture, the substance may be stored at room temperature without refrigeration, and be protected against spoilage for many years. Preservation is possible because the greatly reduced water content inhibits the action of microorganisms and enzymes that would normally spoil or degrade the substance.



Freeze-drying also causes less damage to the substance than other dehydration methods using higher temperatures. Freeze-drying does not usually cause shrinkage or toughening of the material being dried. In addition, flavours, smells and nutritional content generally remain unchanged, making the process popular for preserving food. However, water is not the only chemical capable of sublimation, and the loss of other volatile compounds such as acetic acid (vinegar) and alcohols can yield undesirable results.

Freeze-dried products can be rehydrated (reconstituted) much more quickly and easily because the process leaves microscopic pores. The pores are created by the ice crystals that sublime, leaving gaps or pores in their place. This is especially important when it comes to pharmaceutical uses. Freeze-drying can also be used to increase the shelf life of some pharmaceuticals for many years.

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