

Economical Analysis of a Pressurized Fluid-Based Process Applied for Phytochemicals Recovery in a Sustainable Biorefinery Concept for Brazilian Ginseng Roots

Juliana Albarelli^{*a}, Renata Vardanega^a, Nino Wilkins^b, Diego T. Santos^a, François Maréchal^b, M. Angela A. Meireles^a

^aLASEFI/DEA/FEA (School of Food Engineering) /UNICAMP (University of Campinas) Cidade Universitária "Zeferino Vaz", Rua Monteiro Lobato, 80; 13083-862, Campinas, SP, Brazil.

^bIndustrial Process and Energy Systems Engineering (IPESE), École Polytechnique Fédérale de Lausanne (EPFL), Station 9, 1015, Lausanne, Switzerland.

jualbarelli@gmail.com

A growing number of technologies are being developed in order to produce different high added value products, such as phytochemicals, ethanol and other chemicals from biomass, representing an important trend that needs to be evaluated. The use of pressurized-fluid technologies to obtain phytochemicals from certain biomasses has shown encouraging results in terms of selectivity, enabling its use prior bioenergy generation since generally only a small part of the solid material is extracted. The phytochemical compound which is most financially interesting to extract from the Brazilian Ginseng (*Pfaffia glomerata*) roots is ecdysterone (β -ecdysone), an ecdysteroid. Ecdysteroids are a class of natural steroids acting as hormones and β -ecdysone is the most important steroid employed in cosmetic formulations. It is also used in other industries such as the food and pharmaceutical industry. Due to its health properties, large amounts of *Pfaffia glomerata* roots are being exported from Brazil to countries that use phytochemical therapies widely. This work is part of a project that aims to present a solution to perform phytochemicals recovery step in Brazil envisioning decentralized local-scale production. In this study, first the influence of the extraction temperature (353–413 K) and static extraction time (5–15 min) using pressurized ethyl acetate as extracting solvent on the β -ecdysone recovery was experimentally evaluated and compared with literature results employing other pressurized fluids. Afterwards, an economical analysis was done for comparison using ASPEN PLUS software and OSMOSE platform. It was observed that the relationship of the β -ecdysone recovery, extraction temperature and static extraction time was linear. An increase in temperature and static time resulted in enhancement of the β -ecdysone recovery from the biomass. On the other hand, the increase of the extraction temperature beyond 373 K possibly might enhance the degradation of the β -ecdysone extracted decreasing its relative amount in the extract. Since for the economical analysis performed the former response variable (β -ecdysone recovery) is most important than the latter, it was calculated the net present value of this process considering high extraction temperatures. Considering an installation time of two years, a lifetime of 25 years and an interest rate of 15 % the obtained net present value for the different pressurized extracting solvents were in the following order: ethyl acetate > ethanol > CO₂-ethanol mixture. The results demonstrate that the inclusion of a pressurized fluid-based process using ethyl acetate or ethanol for β -ecdysone recovery prior further bioenergy production is very promising.

1. Introduction

Biomass valorization is an important topic for the energetical future of our planet. As the fossil fuels are getting more scarce, it is necessary to find alternative solutions to meet the energetic needs of the growing population. Many different processes for many different kinds of biomass have been proposed and it is very likely that in the future a significant part of our energy will be produced by efficiently valorizing biomass (Tuck et al., 2011).

In this specific work different ways of biomass valorization were analyzed but they all follow the same scheme. In a biomass there are many different compounds of different values. Very interesting phytochemicals, the most financially interesting class of compounds to be extracted, can be found in various biomasses. This brings up the interesting idea of optimizing the biomass valorization process by finding a way to extract these phytochemicals and then sell them. After the extraction of these high value compounds, the lower value waste can be used to produce energy or electricity, for example through combustion. In this work the focus was on the valorization of *Pfaffia glomerata* roots, also known as Brazilian Ginseng.

The bioactive compound which is most financially interesting to extract from the Brazilian Ginseng is ecdysterone (β -ecdysone), an ecdysteroid. Ecdysteroids are a class of natural steroids acting as hormones and β -ecdysone is the most important steroid employed in cosmetic formulations and is also used in other industries such as the food or pharmaceutical industry (Zimmer et al., 2006). Other bioactive compounds which can be extracted are the aforementioned saponins, which can be used as emulsifiers in various industries. The saponins are natural surfactants present in over 500 different plant species. Due to its amphiphilic structure with hydrophilic sugar chains and lipophilic aglycone they are surface active compounds with emulsifying, foaming, detergent or wetting properties. It has been shown that saponins extracted from plants can be a good replacement of synthetic emulsifiers (Santos et al., 2013a; Rosa et al., 2016).

Therefore the option to first extract β -ecdysone and then saponins in a next step is an interesting possibility. Different solvents for different types of extractions were taken into account and after a screening it was decided to focus on a limited number of potential processes. One of the emerging technologies which was analyzed is pressurized fluid-based processes, such as, pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE). The advantage of pressurized fluid-based extractions is that it allows fast extraction and a reduced consumption of the used solvent (Santos et al., 2013b). The feasibility of β -ecdysone recovery from *Pfaffia glomerata* roots has been proven on a laboratory scale with different solvents, including the use of supercritical CO₂ with ethanol as co-solvent and pressurized ethanol (Santos et al., 2014). For the so-called supercritical fluid extraction (SFE), the CO₂, the most common solvent employed in SFE, is used in a supercritical state as an extractant either on its own or by mixing it with another organic solvent. The main advantages of SFE include an even further reduced consumption of solvent, very little solvent residue in the product and therefore an increased environment-friendliness of the process.

Therefore, this work aims to present a solution to perform phytochemicals recovery step in Brazil envisioning decentralized local-scale production. In this study, first the use of a different solvent, i.e. pressurized ethyl acetate, as extracting solvent on the β -ecdysone recovery was experimentally evaluated, being the influence of the process parameters extraction temperature (353–413 K) and static extraction time (and 5–15 min) further analyzed. Then, the obtained results were compared with literature results employing other pressurized fluids, such as supercritical CO₂ with ethanol as co-solvent and pressurized ethanol (Santos et al., 2013b; Santos et al., 2014) in terms of economical parameters using ASPEN PLUS software.

2. Materials and methods

2.1 Materials

Brazilian ginseng roots (*Pfaffia glomerata*) were washed and dried in a forced air circulation dryer at 313 K for 5 days. The dried roots (8.9% moisture) were then comminuted in a pulse mill (Marconi, model MA 340, Piracicaba, Brazil) for few seconds. Next, the particles of higher size were milled again, this time using a knife mill (Tecnal, model TE 631, Piracicaba, Brazil) for 2 s at 18,000rpm and finally, they were separated according to their size using sieves (Series Tyler, W.S. Tyler, Wheeling, IL). The milled roots were stored in freezer (Metalfrío, model DA 420, São Paulo, Brazil) at 263 K.

Ethyl acetate (analytical grade) purchased from Merck (Darmstadt, Germany) was used as extracting solvent.

2.2 Pressurized Liquid Extraction (PLE)

Dried and milled pieces of Brazilian ginseng roots (4.5 g with a moisture content of 8.9 %) were placed in a 6.57-cm³ extraction cell (Thar Designs, Pittsburg, USA) containing a sintered metal filter at the bottom and upper parts. The cell containing the sample was heated, filled with extraction solvent (ethyl acetate) and then pressurized. The sample was placed in the heating system for 6.5 min to ensure that the extraction cell would be at the desired temperature during the filling and pressurization procedure. After pressurization, the sample with pressurized solvent was kept statically at the desired pressure for the desired time (static extraction time). The pressure of the extraction was set in all experiments to 12 MPa. Thereafter, the back pressure regulator (BPR) valve (Model #26–1761-24–161, Tesco, Elk River, USA) was carefully opened, keeping the pressure at an appropriate level for the desired flow (1.0 cm³/min), to rinse the extraction cell with fresh extracting solvent for 20 min (dynamic extraction time). After pressurized liquid extraction (PLE), the extracts were rapidly cooled to 268 K in ice water using glass flasks to prevent extract degradation. After extraction, the solvent was

evaporated using a rotary evaporator (Laborota, model 4001, Vertrieb, Germany) with vacuum control (Heidolph Instruments GmbH, Vertrieb, Germany) and a thermostatic bath held at 313 K.

2.3 Beta-ecdysone quantification

Beta-ecdysone quantification was performed by HPLC according to the methodology described by Rostagno et al. (2014).

2.4 Process simulation

The mass and energy balances were done with ASPEN PLUS software version 7.3 and the energy integration with the OSMOSE platform version 2.7.8. OSMOSE (Optimisation Multi-Objectifs de Systemes Energetiques integres, which means "Multi-Objective Optimization of Integrated Energy Systems") is a computation platform that was built in Matlab, developed and continuously improved at École Polytechnique Fédérale de Lausanne in Switzerland for the design and analysis of integrated energy systems. The platform allows one to link several software such as Belsim Vali and ASPEN PLUS for a complete suite of computation and result analysis tools (optimization, sensitivity analysis, Pareto curve analysis, etc.).

The amount of feed was calculated according to the overall amount of Brazilian Ginseng which is exported to Japan each year. 700 tons of *Pfaffia glomerata* roots are shipped away each year and ideally, this biomass could be treated in Brazil, providing economical, social and environmental benefits (Junior, 2005). With a flowrate of 97.22 kg/h of wet biomass feed, the desired 700 t are achieved considering work at 300 days of the year for 24h each day. It is important to check the time planning to see if the process has enough residence time in the extractor for each batch and if the t_{cer} , the ideal time of extraction (duration of the constant extraction rate period) can be reached. According to Santos et al. (2012a) the minimum cycle time of the described PLE process is 36 minutes, due to 4 minutes of static extraction followed by 14 minutes of dynamic extraction. Since there are two extractors working at the same time, while one of them is cleaned, in the other one the extraction takes place. This is why it is possible to simulate a steady state process in ASPEN PLUS software, buying two batch reactors, which together work just like a continuous process.

Using 300 days with 24 hours each, the process is running during 7200 h, which with 36 minutes per batch allows a maximal number of 12000 batches. This means that the minimal flow per batch must be 58.33 kg/batch of wet ginseng, which is achieved. In fact, even considering 54 minutes per batch, for example by doing two extractions in sequence, there is enough time. With 54 minutes per batch, the maximal batch number is 8000, leading to a minimal flow of 87.5 kg/batch, which is also less than the 97.22 kg/h with which the simulations are done.

Other inputs were dependent on the lab results which were available to run the simulations. The solvent to feed ratio used is 4.44 for PLE and 31.667 for SFE (Santos et al., 2013b; Santos et al., 2014).

The biomass pretreatment starts with cleaning the roots and washing away a significant amount of soil (60% according to Santos et al., 2012a). This step was not simulated by ASPEN PLUS but it is taken into account with the OSMOSE simulations. The first step simulated with ASPEN PLUS was the drying of the raw material, since it is necessary to limit the moisture content before cutting and milling. The drying process was done by adding a fixed amount of hot air at 373 K. The amount of the air necessary was defined with a design specification, setting the desired moisture content of the outgoing material to 8.9%. The reactor used was a reservoir without heat duty and at the pressure of 0.1 MPa. The air coming in is pre-heated with a standard heater and wet air going out is cooled down to 298 K in order to recover the energy. After the drying, the material is cut and milled. Both the cutting and milling were left as a black box in ASPEN PLUS and were simulated in OSMOSE platform.

For the extraction a black-box process was used both for PLE and for SFE. Whereas in real life, the extraction takes place in a semi-batch process, it can be simulated as a continuous process under the assumption that there are multiple reactors, which run alternately, therefore assuring the approximated continuous production.

The composition of the biomass after the extraction was given from lab results obtained experimentally and this information was used to determine the different outgoing streams. The solvent's influence on extraction yield and leftover material composition was taken into account. The different biomass leftovers have different thermal properties, which was reflected in the calculations.

The solvent was pre-heated and pressurized with a standard pump and then mixed with the biomass input. In the case of SFE, it is important to take into account the cooling of the CO₂ before the pressurization, as CO₂ at ambient temperature would not be able to be pumped as a liquid at 6.5 MPa, the pressure in which the CO₂ is bought. Therefore, it is cooled down to 273 K before it is pressurized. Once both the ethanol and CO₂ are at the predefined pressure, the mixture is heated to the desired solvent temperature.

Since the organic solvent should be recovered to obtain an economically feasible process, an evaporation step is placed after the extraction, in which the organic solvent is evaporated and then recollected. The organic solvent is evaporated and the dry extract is obtained. The organic solvent subsequently is cooled

down to ambient temperature and recycled into the system. A loss of 10 % to the residue and the condenser was considered, which is represented in ASPEN PLUS by a split-off stream. This quantity which is lost has to be added to the process continuously and is used for the economic evaluation.

In the SFE the extract is placed in a flash tank at ambient temperature, where the CO₂ is vaporized. This CO₂ is then compressed to 6.5 MPa and cooled down to be liquified and recycled as fresh solvent. A loss of 2 % of the CO₂ is considered during the depressurization and it has to be therefore added to the process and taken into account for the economical evaluation.

The non-extracted material can be used as source of energy for the process, which is why after depressurization, it is envisioned that the waste of the extraction would be burned.

2.5 Economical evaluation

The economical evaluation was done with OSMOSE platform, using approximations for the cost of the extractors based on the books of Ulrich (1984) and Turton et al. (1998). This method uses approximations to determine the so-called grass root cost, which is a cost approximation including labor, installation and maintenance cost. Adding all the different elements bought, a total estimated investment cost is obtained. This cost, while of course not extremely precise, is a good indication of how expensive the process is and can give indications about the feasibility.

The waste treatment costs were not taken into consideration due to the ecofriendliness of the waste products, since the dry solid biomass matrix can be incorporated in the soil and does not contain any toxins. In small quantities, the CO₂ and the organic solvent which are lost do not present harm to human health or the environment.

For the variable costs, estimations for the price of the different raw materials and products had to be done. The exact approximations can be found in the Table 1. For different parameters a sensitivity analysis was performed, in order to see their respective impact.

Table 1: Economical analysis assumptions.

Material	Price (USD)
Brazilian Ginseng Roots (kg)	1.5
Ethyl acetate (kg)	2.5
Ethanol (kg)	0.65
CO ₂ (kg)	0.15
Electricity (kWh)	0.07
Steam (kg)	0.02
β-ecdysone (kg)	8000

3. Results and discussion

In this study, first the influence of the extraction temperature (353–413 K) and static extraction time (and 5–15 min) using pressurized ethyl acetate as extracting solvent on the β-ecdysone recovery was experimentally evaluated and compared with literature results employing other pressurized fluids. It should be pointed out that we have previously presented the results regarding the influence of these process parameters on extraction yield (Santos et al., 2012b) and here we plotted these results together with the new finding for a better visualization. It was observed that the relationship of the β-ecdysone recovery, extraction temperature and static extraction time was linear (Figure 1). An increase in temperature and static extraction time resulted in enhancement of the β-ecdysone recovery from the biomass. On the other hand, the increase of the extraction temperature beyond 373 K possibly might enhance the degradation of the β-ecdysone extracted decreasing its relative amount in the extract. Since for the economical analysis performed the former response variable (β-ecdysone recovery) is most important than the latter, it was calculated the net present value of this process considering high extraction temperatures.

In order to efficiently analyze if the obtained results are promising or not we compared our results using ethyl acetate as extraction solvent with those of when using supercritical CO₂ with ethanol as co-solvent and pressurized ethanol (Santos et al., 2013b; Santos et al., 2014). The different solvents and extraction conditions were evaluated for their energetical and economical aspects with the goal of focusing on the most promising settings for the process. After getting the energetical values from ASPEN PLUS software, the parameters were analyzed with OSMOSE platform, where an economical evaluation was done. First the solvents were analyzed separately and the parameter under which they were compared was the net present value (NPV) of the process. An installation time of two years, a lifetime of 25 years and an interest rate of 15 % were considered. This interest rate is rather high since the plant should be built in Brazil.

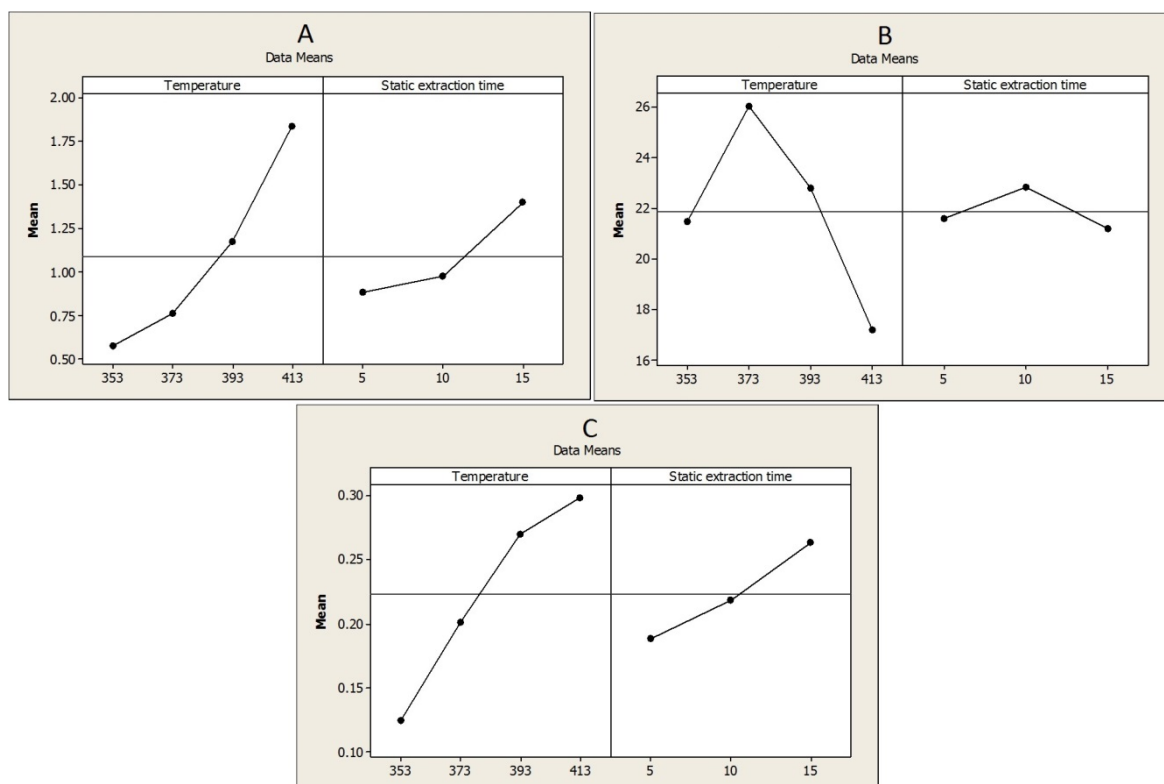


Figure 1: Influence of the extraction temperature (353–413 K) and static extraction time (5–15 min) using pressurized ethyl acetate as extracting solvent on: A) extraction yield, B) β -ecdysone content regarding the extract (%), C) β -ecdysone content regarding the initial biomass (%).

Table 2 shows that ethyl acetate extracts are more selective for β -ecdysone recovery. On the other hand, the extraction yield is larger for pure ethanol. The main advantage of ethanol as opposed to ethyl acetate is the lower price (0.65 USD/kg vs. 2.5 USD/kg). Compared to pure ethanol, the addition of CO₂ increases the selectivity of the β -ecdysone extraction, on the other hand, the amount of extract stays fairly low, which leads to a low overall β -ecdysone yield regarding the initial biomass. In the analysis with OSMOSE only four of the processes showed a positive NPV when considering a selling price of β -ecdysone of 8000 USD/kg.

Table 2: Summary of the process conditions and results. Data from: ^aSantos et al. (2012b); ^bSantos et al. (2013b); ^cSantos et al. (2014).

Extracting solvent	Temperature (K)	Pressure (MPa)	Extraction yield (%)	β -ecdysone content regarding the extract (%)	β -ecdysone content regarding the initial biomass (%)	Net present value (NPV) sign
Ethyl acetate	353	12	0.74 ^a	21.61	0.160	-
Ethyl acetate	373	12	0.93 ^a	27.46	0.255	+
Ethyl acetate	393	12	1.45 ^a	24.14	0.350	+
Ethyl acetate	413	12	1.54 ^a	20.67	0.318	+
Ethanol	323	10	1.95 ^b	2.54 ^c	0.049	-
Ethanol	363	10	4.9 ^b	4.5 ^c	0.221	+
50EtOH:50CO ₂	323	10	1.32 ^b	6.47 ^c	0.085	-
50EtOH:50CO ₂	363	10	2.12 ^b	7.50 ^c	0.159	-
10EtOH:90CO ₂	323	10	0.26 ^b	7.99 ^c	0.021	-
10EtOH:90CO ₂	363	10	0.43 ^b	8.04 ^c	0.042	-

If ethanol is chosen, the conditions of 363 K and 10 MPa are clearly the most favorable whereas for ethyl acetate the best NPV is achieved at 393 K and 12 MPa. The obtained NPV for the different pressurized extracting solvents were in the following order: ethyl acetate > ethanol > CO₂-ethanol mixture.

Unfortunately, not enough values were available to compare at the same pressures and temperatures but it can clearly be said that both organic solvents are interesting and can be economically feasible to extract β -ecdysone, meanwhile the use of supercritical CO₂ should be avoided, since the β -ecdysone content in their extracts in terms regarding the initial biomass (%) are very low, thus losing a considerable amount of nonextracted β -ecdysone in the biomass. In a general aspect it should be concluded that a minimum amount around 0.160 - 0.221 g of β -ecdysone per 100 g of Brazilian ginseng roots (dry basis) should be obtained for a pressurized fluid-based process to become the process economical attractive.

4. Conclusions

The obtained net present value for the different pressurized extracting solvents were in the following order: ethyl acetate > ethanol > CO₂-ethanol mixture. The results demonstrate that the inclusion of a pressurized fluid-based process using ethyl acetate or ethanol for β -ecdysone recovery prior further bioenergy production is very promising. In addition, it could be indicated that the energy generated from the leftover material after the extraction process could be used to fulfill its own energetic requirements and the surplus energy might be sold to a local concessionary or to the local community, being also this option interesting in terms of sustainability aspects.

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Reference

- ASPEN PLUS software <<https://www.aspentech.com/products/engineering/aspem-plus>>.
- Junior I.M., 2005, Avaliação de genótipos de *Pfaffia glomerata* (spreng.) pedersen visando seu cultivo comercial, Master Thesis, Instituto Agrônomo de Campinas (in Portuguese).
- OSMOSE Platform <<http://leni.epfl.ch/osmose>>.
- Rosa M.T.M.G., Silva E.K., Santos D.T., Petenate A.J., Meireles M.A.A., 2016, Obtaining annatto seed oil miniemulsions by ultrasonication using aqueous extract from Brazilian ginseng roots as a biosurfactant, *Journal of Food Engineering*, 168, 68–78.
- Rostagno M.A., Debien I.C.N., Vardanega R., Nogueira G.C., Barbero G.F., Meireles M.A.A., 2014, Fast analysis of β -ecdysone in Brazilian ginseng (*Pfaffia glomerata*) extracts by high-performance liquid chromatography using a fused-core column, *Analytical Methods*, 6, 2452-2459.
- Santos D.T., Albarelli J.Q., Meireles M.A.A., 2012a, Simulation of an integrated sustainable production of extract from brazilian ginseng roots with a cogeneration plant, *Chemical Engineering Transactions*, 29, 91–96.
- Santos D.T., Albarelli J.Q., Rostagno, M.A., Ensinas A.V., Marechal F., Meireles M.A.A., 2014, New proposal for production of bioactive compounds by supercritical technology integrated to a sugarcane biorefinery, *Clean Technologies and Environmental Policy*, 16,1455–1468.
- Santos D.T., Barbosa D.F., Broccoli K., M. S. Gomes M.T.M.S., Vardanega R., Meireles M.A.A., 2012b, Pressurized organic solvent extraction with on-line particle formation by supercritical anti solvent processes, *Food and Public Health*, 2, 231–240.
- Santos D.T., Barbosa D.F., Gomes M.T.M.S., Vardanega R., Meireles M.A.A., 2013a, Novel method to produce emulsions containing essential oils from saponin-rich pressurized aqueous plant extracts, *Journal of Colloidal Science and Biotechnology*, 2, 1–7.
- Santos D.T., Vardanega R., Albarelli J.Q., Ensinas A.V., Marechal F., Meireles M.A.A., 2013b, Energy consumption versus antioxidant activity of pressurized fluid extracts from *Pfaffia glomerata* roots, *Chemical Engineering Transactions*, 35, 1099–1104.
- Tuck C., Pérez E., Hörvath I., Sheldon R., Poliakov M., 2011, Valorization of biomass: Deriving more value from waste, *Science* 10, 337, 695–699.
- Turton R., Bailie R.C., Whiting W.B., Shaiwitz J.A. 1998, *Analysis, Synthesis and Design of chemical processes*. Prentice Hall, New Jersey, USA.
- Ulrich G., 1984, *A Guide to Chemical Engineering Process Design and Economics*. Wiley, New Jersey, USA.
- Zimmer A., Bruxel F., Bassani V., Gosmann G., 2006, HPLC method for the determination of ecdysterone in extractive solution from *Pfaffia glomerata*, *Journal of Pharmaceutical and Biomedical Analysis*, 40, 450–453.