CHEMICAL ENGINEERINGTRANSACTIONS
VOL. 43, 2015
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Chief Editors:SauroPierucci, JiříJ. Klemeš

ISBN 978-88-95608-34-1; **ISSN** 2283-9216
DOI: 10.3303/CET1543023

High–Pressure and Temperature Extraction of Phenolic Compounds from Corn Silage

Melita Kuzmanović[a], Marina Tišma[a], Ana Bucić-Kojić[a], Alessandro Alberto Casazza[b], Marco Paini[b], Bahar Aliakbarian[b], Patrizia Perego[b]

[a]Faculty of Food Technology Osijek, Josip Juraj Strossmayer University of Osijek, FranjeKuhača 20, 31001 Osijek, Croatia
[b]Department of Civil, Chemical and Environmental Engineering (DICCA), University of Genoa, Via Opera Pia 15, 16145 Genoa, Italy
bahar.aliakbarian@unige.it

Corn silage refers to stalks, leaves and cobs of maize plants that remain in fields after the corn harvest. Commonly it is used as a cattle feed, but recently it has been more often used together with cattle manure in biofuel production in anaerobic co-digestion.
In this work high-pressure and temperature extraction of phenolic compounds from corn silage was performed. Process parameters (temperature, T = 90 – 180 °C; extraction time, t = 40 – 120 min; liquid – solid ratio, 10 – 20 mL/g; and solvent concentration (10 – 90 % aqueous ethanol, v/v) were studied. Box-Bhenken design was used in order to obtain the maximal extractability of phenolic compounds (namely total phenolic compounds-TPC and total extractible proanthocyanidins - TPA) and the maximal antiradical power (ARP) of obtained extracts.
Experimental results of TPC, TPA, and ARP of corn silage extracts were in the range from 10.01 – 72.43 $mg_{GallicAcid\ Equivalent}/g_{drybiomass}$, 0.27 – 3.21 $mg/g_{drybiomass}$ and 1.25 – 16.76 $mg_{DPPH}/mL_{extract}$, respectively.
Statistical results confirmed that temperature was the most significant factor affecting the observed responses ($p < 0.05$). Optimal extraction conditions for TPA and ARP were achieved at 180 °C, 120 min using 65 % ethanol and L/S ratio 10 while optimal conditions for TPC were achieved at 180 °C, 120 min using 10 % ethanol and L/S ratio 20.
The results of this study evidenced that extracts from corn silage can be a good source of antioxidant compounds which can be used for production of food, cosmetic and pharmaceutical products.

1. Introduction

Corn silage is agricultural residue comprised of cellulose (31.2 %), hemicellulose (23.9 %) and lignin (20.1 %) (Jung et al., 2015). It has an excellent nutritional quality and good ensiling properties (Weinberg et al., 2010) and is mainly used as a cattle feed (Han and Zhou, 2013). Nowadays it is often used for biogas production in combination with cattle manure in anaerobic co - digestion process. The most recalcitrant part of the corn silage is lignin which is composed of variously linked phenyilpropane units, such as hydroxyphenyl, guaiacyl and syringyl (Kleinert and Barth, 2008).
Different methods can be used for degradation of lignin from corn silage as well as from the other lignocellulose materials for its further application in biofuel production. Some of the lignin degradation products are phenolic compounds that can act as inhibitors in biogas production (Zhu et al., 2014), but some of the products can have benefits to human health. Phenolic compounds are known to be good antioxidants and neuro - sedative, anti - inflammatory, anti - viral and anti - cancer agents (Aliakbarian et al., 2011).
There are many different extraction techniques that can be used for polyphenol isolation from different plant materials, such as microwave - assisted extraction (Costa et al., 2013), ultrasonic extraction (Dahmounea et al., 2014), supercritical fluid extraction (Dos Santos et al., 2013) and pressurized liquid extraction (Fernández-Ponce et al., 2013).

Please cite this article as: Kuzmanovic M., Tisma M., Bucic-Kojic A., Casazza A.A., Paini M., Aliakbarian B., Perego P., 2015, High–pressure and temperature extraction of phenolic compounds from corn silage, Chemical Engineering Transactions, 43, 133-138
DOI: 10.3303/CET1543023

In this study non - conventional extraction technology is used by employing high - temperature and pressure stirred reactor. Advantage of high temperature is enhancement of extraction process due to decreasing the viscosity of liquid solvent and resulting in better penetration of matrix particles. Furthermore, strong solute - matrix interactions caused by van der Waals forces, hydrogen bonding and dipole attractions of the solute molecules and the active sites on the matrix can also be disrupted by increased temperatures. The use of pressure enables solvent reaching into areas of the matrices that would not be reached by solvent under atmospheric conditions. Extraction cell pressuring is obtained to prevent the solvent from boiling at the extraction temperature and to ensure contact between solvent and sample during extraction time (Casazza et al., 2012).
The aim of this study was to investigate the influence of high - temperature and pressure extraction of corn silage on phenolic compounds extraction efficiency. Total phenolic compounds (TPC), total extractible proanthocyanidins (TPA) and antiradical power (ARP) of corn silage extracts were analysed. The effects of extraction time (t), temperature (T), solid/liquid ratio and solvent concentration were studied.

2. Material and methods

2.1 Material

Corn silage samples were collected from local biogas company (Bovis d.o.o., Ivankovo, Croatia). Before extraction corn silage was dried at 45 °C during 24 h and milled in a blender (HR 2860, Philips). Dry matter content of milled sample determined by fast moisture analyser (HR - 73, Mettler Toledo) was 91.48 %.

2.2 Extraction procedure

Extraction process was performed in high - temperature and pressure reactor model 4560 (PARR Instrument Company, Moline, IL, USA). Appropriate valves on the reactor allow changes of extraction atmosphere inside the reaction chamber. Corn silage was submitted to extraction using different concentration ethanol (10 – 90 % aqueous ethanol, v/v) with solid - liquid ratio 10 – 20 mL/g. The effects of extraction time (t = 40 – 120 min) and temperature (T = 90 – 180 °C) also were studied. Prior to extraction nitrogen was flushed through the reactor for 2 minutes. Obtained alcoholic extract were centrifuged by PK 131 centrifuge (ALC, Milan, Italy) at 7500 x g for 10 minutes. The supernatants were subjected to quantitative analyses.

2.3 Determination of total phenolic content

Using Folin - Ciocalteu colorimetric method total phenolic content (TPC) in extracts was determined (Swain and Hills, 1959). Measures were carried out using UV - Vis spectrophotometer (Lambda 25; Perkin Elmer, Wellesley; MA, USA). Calibration curve was made using standard solution of gallic acid (GA) ranging from 0.01 to 1.00 mg/mL and results were expressed as milligram of GA equivalent per gram of corn silage dried biomass. The method response was described by the linear equation Eq(1) (R^2 = 0.9940):

$$ABS_{725} = 0.0017TPC \quad (1)$$

Where ABS_{725} is the absorbance measured at 725 nm. All samples were performed in triplicate.

2.4 Determination of extractable proanthocyanidins content

Determination of total extractable proanthocyanidins is based on acid butanol assay where treatment in butanol leads to depolymerization of the proanthocyanidins to anthocyanidins and due to formation of red color they could be detected by spectrophotometer.
2 mL of diluted extract was mixed with 20 mL of Ferric sulphate solution (77 mg of $FeSO_4 \cdot H_2O$ in 500 mL of HCl: n-butanol = 2:3). After 15 min of incubation on 95 °C, reaction mixture were cooled and absorbance is measured at 540 nm on spectrophotometer (Lambda 25; Perkin Elmer, Wellesley; MA, USA). The blank was prepared with distilled water instead of extract (Bucić - Kojić et al., 2009). Samples were performed in duplicate. Total extractable proanthocyanidins content (TPA) was calculated according to molar extinction coefficient [ε = 34700 L/mol cm] and molar weight (MW = 287 g/mol) of cyanidin (Eq. 2) and expressed as g/L:

$$\mathrm{TPA} = \frac{A \cdot \mathrm{MW} \cdot \mathrm{DF}}{\varepsilon \cdot l} \quad (2)$$

where A - absorbance of sample, l - pathlength (cm), DF – dilution factor [ratio of total volume of reaction mixture for determination TPA (22 mL) and volume of extract (2 mL)].
In the final TPA calculation dilution of extracts were included. The final result of TPA content was expresses on dry basis of corn silage ($mg/g_{dry\ biomass}$).

2.5 Evaluation of antiradical power

According to corn silage extracts capacity to neutralize the free - radical 2,2 - diphenylpicryhydrazyl (DPPH) antioxidant activity was evaluated. Colorimetric method based on the reduction of DPPH in alcoholic solution

was used (Casazza et al., 2012). For each extract, different ethanolic dilutions were prepared. 100 µL of dilution was mixed with 3.9 mL of DPPH ethanolic solution. ARP was defined as $1/EC_{50}$ (µg $_{DPPH}$/µL$_{extract}$). Measuring of every sample was carried out in duplicate.

2.6 Experimental design

Box - Behnken design was applied to identify optimum levels of extraction conditions, including liquid - solid ratio, extraction temperature, extraction time and solvent composition regarding of response - content of total phenolic compounds (TPC), total extractible proanthocyanidins (TPA) and antiradical power (ARP) of corn silage extracts. The range and the level of the variables investigated in this study are given in Table 1. Box - Behnken design consisting of 29 experimental runs with three replicates at the central points was used for fitting a second - order polynomial model Eq(3):

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k} \sum_{j<1}^{k} \beta_{ij} X_i X_j \quad (3)$$

where Y is the predicted response (TPC, TPA and ARP); β_0, β_i, β_{ii}, β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction term, respectively; X_i, X_j are the independent variables; k is the number of variables.

Design Expert software version 7.1.6. (Stat Ease Inc., USA) was used for designing experiments and statistical data analysis (ANOVA). The lack of fit testing and coefficient determination (R^2) were used to verify the adequacy of the model. Models and regression coefficients were considered significant when p-values were lower than 0.05 (Table 2).

The fitted second - order polynomial equation was expressed as the three - dimensional response surface plots in order to visualize the relationship between predicted responses and experimental levels of independent variables and to deduce the optimum extraction conditions. Plots were drawn as function of interaction of significant terms for responses by holding insignificant terms at a constant central level.

Table 1: Uncoded and coded levels of independent variables used in Box - Behnken design for extraction process

Independent variables (Factors)	Variable levels		
	-1	0	+1
Liquid – solid ratio (X_1, mL/g)	10	15	20
Extraction temperature (X_2, °C)	90	135	180
Extraction time (X_3, min)	40	80	120
Solvent composition*(X_4, %, v/v)	10	50	90

**solvent composition - aqueous ethanol solutions*

3. Results and discussion

Experimental results of TPC, TPA, and ARP of corn silage extracts obtained from 29 experiments of Box - Behnken design were in the range from 10.01 - 72.43 $_{mgGallic\ Acid\ Equivalent}$/g$_{dry\ biomass}$, 0.27 – 3.21 mg/g$_{dry\ biomass}$ and 1.25 – 16.76 mg$_{DPPH}$/mL$_{extract}$.

Analysis of variance (ANOVA) for experimental set up was done to evaluate fitness of response function. Statistical significant of regression model ($p < 0.05$) for all responses and high coefficient of determination (R^2 = 0.91 – 0.98) were observed, indicating the reasonable fit of the model Eq(1) to the experimental data (Table 2).

Table 2: Regression coefficients of significant terms (p-values < 0.05), coefficient of determination (R^2) and lack of fit values of the predicted second order polynomial models for the response variable, TPC, TPA, and ARP of corn silage

Constant coefficients	TPC	TPA	ARP
β_0 - intercept	21.20	0.65	3.19
β_1- liquid-solid ratio	-	-	-1.70
β_2 - temperature	19.26	1.13	4.19
β_3 - time	-	0.15	-
β_4 - solvent composition	-	0.28	-
$\beta_1\beta_2$	-	-	-2.14
$\beta_1\beta_3$	-	-	-
$\beta_1\beta_4$	-	-	-
$\beta_2\beta_3$	-	0.25	2.20
$\beta_2\beta_4$	-11.65	0.38	-
$\beta_3\beta_4$	-	-	-
β_1^2	-	-	-
β_2^2	12.65	0.89	2.86
β_3^2	-	-	-
β_4^2	-	-0.14	-
$\beta_3\beta_4$	-	-	-
R^2	0.93	0.98	0.91
p value of lack of fit	0.02	0.36	0.01

Furthermore, ANOVA showed that temperature was the most significant factor affecting the observed responses ($p < 0.05$) which was confirmed by significant linear and quadratic terms of the model. For TPC interaction effect of temperature and ethanol concentration also was observed (Fig.1). By increasing the temperature and decreasing ethanol concentration, TPC increased significantly and the highest extraction yield of investigated phenolic compounds were reached at 180 °C using 10 % ethanol.

Significant terms for TPA beside temperature were time, solvent composition (linear and quadratic terms) and interaction terms (temperature - time and temperature - solvent composition). From 3D surface (Fig. 2), it was observed that TPA increased with the increase in temperature and ethanol concentration up to 180 °C and 90 % ethanol.

The other influencing model terms beside temperature for the ARP were liquid-solid ratio, interaction of temperature with liquid - solid ratio and with time. Fig. 3 demonstrates the influence of interaction of liquid-solid ratio and temperature. It is evident that increasing of temperature and liquid - solid ratio lead to higher ARP of corn silage extracts with best results reached at 180 °C using liquid-solid ratio of 20 mL/g.

The optimal extraction conditions obtained using numerical optimization based on model Eq(1) within the experimental range for TPA and ARP were obtained at 180 °C and 120 min using 65 % ethanol and liquid-solid - ratio of 10 mL/g. At this condition, the predicted response values for TPA and ARP were 18.17 $mg/g_{dry\ biomass}$ and 3.36 $mg_{DPPH}/mL_{extract}$, respectively. Liquid-solid ratio of 20 mL/g, 10 % ethanol, 180 °C and extraction duration 120 min were found as optimal conditions for HPTE extraction of TPC with predicted content by second-order model of 70.86 $mg_{Gallic\ Acid\ Equivalent}/g_{dry\ biomass}$ (Table 3).

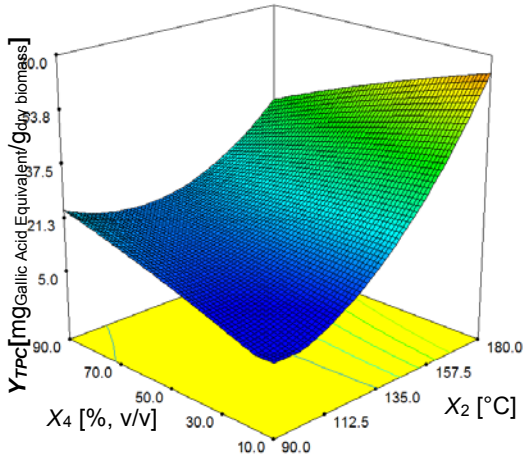

Figure 1: 3D graphics surface of TPC as a function of extraction temperature (X_2) and ethanol concentration (X_4) at fixed time (80 min) and liquid - solid ratio (15 mL/g) as for corn silage

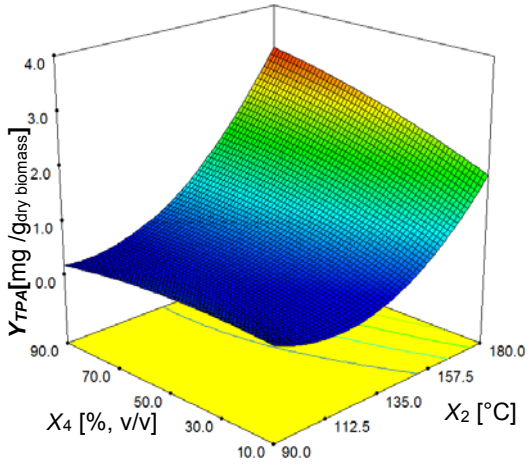

Figure 2: 3D graphics surface of TPA as a function of extraction temperature (X_2) and ethanol concentration (X_4) at fixed time (80 min) and liquid - solid ratio (15 mL/g) as for corn silage

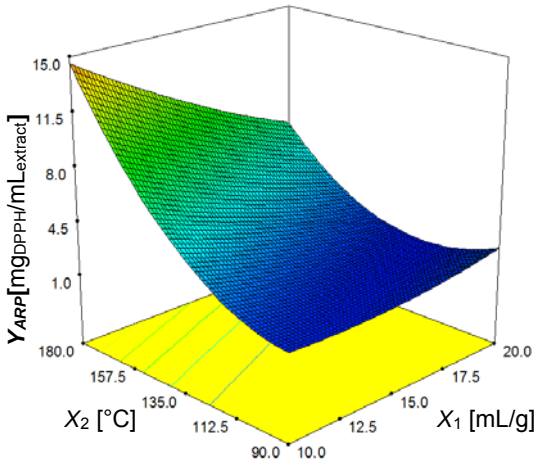

Figure 3: 3D graphics surface of ARP as a function of liquid-solid ratio (X_1) and temperature (X_2) at fixed time (80 min) and ethanol concentration (50 %, v/v) for corn silage

Table 3: Predicted and experimental values under optimum conditions for maximum total phenolic content (TPC), total extractible proanthocyanidins (TPA) and antiradical power (ARP)

Responses	Liquid - solid ratio (mL/g)	Extraction temperature (°C)	Extraction time (min)	Ethanol concentration (%, v/v)	Predicted value	Experimental value
TPC ($mg_{GallicAcid\ Equivalent}/g_{dry\ biomass}$)	20	180	120	10	70.86	56.29
TPA ($mg/g_{dry\ biomass}$)	10	180	120	65	3.36	3.68
ARP ($mg_{DPPH}/mL_{extract}$)	10	180	120	65	18.17	16.36

4. Conclusions

Second - order model was validated by performing the extraction of phenolic compounds under previously optimized process conditions (Table 3). According to the results it can be concluded that the extraction of TPA can be best described by the second - order model. The results of investigation of correlation between concentration of phenolic compounds and ARP showed that, high correlation was obtained between TPA and ARP (R = 0.85) while the lower one was calculated between TPC and ARP (R = 0.76). It can be concluded that total proanthocyanidins have significant influence on antioxidant activity of corn silage extracts obtained by high - temperature and high - pressure extraction.

Acknowledgements

This work was supported by Erasmus + Key activity 1 and by the EU-funded project ProBioTech (RC.2.2.08-0045).

References

Aliakbarian B., Casazza A.A., Perego P., 2011, Valorization of olive oil solid waste using high pressure – high temperature reactor, Food Chemistry, 128, 704-710.

Bucić-Kojić A., Planinić M., Tomas S., Jakobek L., Šeruga M., 2009, Influence of solvent and temperature on extraction of phenolic compounds from grape seed, antioxidant activity and colour of extract, International Journal of Food Technology, 44, 2394-2401.

Casazza A.A., Aliakbarian B., Sannita E., Perego P., 2012, High – pressure high – temperature extraction of phenolic compounds from grape skins, International Journal of Food Science and Technology, 47, 399-405.

Costa S.S., Arumugam D., Gariepy Y., Rocha S.C.S, Raghavan V., 2013, Spilanthol Extraction Using Microwave: Calibration Curve for Gas Chromatography, Chemical Engineering Transactions, 32, 1783-1788.

Dahmounea F., Moussia K., Reminia, H., Belbahia, A., Aouna, O., Spigno G., Madani K., 2014, Optimization of Ultrasound-Assisted Extraction of Phenolic Compounds from *Citrus sinensis* L. Peels using Response Surface Methodology, Chemical Engineering Transactions, 37, 889-894.

Dos Santos W.J., Silva E.A., Taranto O.P., 2013, Supercritical Fluid Extraction from Mango (*Mangifera indica* L.) Leaves: Experiments and Modeling, Chemical Engineering Transactions, 32, 2005-2010.

Fernández-Ponce M.T., Casas L., Mantell C., Martínez de la Ossa E.J., 2013, Potential Use of Mango Leaves Extracts Obtained by High Pressure Technologies in Cosmetic, Pharmaceutics and Food Industries Chemical Engineering Transactions, 32, 1783-1788.

Han L., Zhou H., 2013, Effects of ensiling processes and antioxidants on fatty acid concentrations and compositions in corn silages, Journal of Animal Science and Biotechnology, 4, 48.

Jung K.A., Woo S.H., Lim S., Park J.M., 2015, Pyrolitic production of phenolic compounds from the lignin residues of bioethanol processes, Chemical Engineering Journal, 259, 107-116.

Kleinert M. and Barth T., (2008). Phenols from lignin. Chemical Engineering and Technology, 31, 736 – 745.

Swain T., Hillis W.E., 1959, The phenolic constituents of *Prunus domestica*. The quantitative analysis of phenolic constituents, Journal of Science Food and Agriculture, 10, 63-68.

Winberg Z.G., Khanal P., Yildiz C., Chen Y., Arieli A., 2011, Ensiling fermentation products and aerobic stability of corn and sorghum silages, Japanese Society of Grassland Science, ISSN1744-6961.

Zhu J., Zhu Y., Zhang L., Yong Q., Xu Y., Li X., Lian Z., Yu S., 2014, Sodium hydroxide regeneration of trialkylamine extractant containing inhibitors from corn stover prehydrolyzate by liquid–liquid extraction, Separation and Purification Technology, 126, 39-43.