61st International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA)

VARIATION OF CHEMICAL COMPOSITION OF *EPILOBIUM ANGUSTIFOLIUM* DURING FERMENTATION

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Introduction

Infusion from fermented *Epilobium angustifolium* L. herb (Ivan tee) is traditionally consumed in Russia for the treatment of stomach ulceration, gastritis, and sleeping disorders. It has been used in folk medicine to treat a variety of ailments such as benign prostate hyperplasia and the associated problems of micturition. Flavonoids and tannins, particularly oenothein B, dimeric hydrolyzable ellagitannin seemed to be responsible for these activities.

Aim of study

In continuation of our studies on fermentation procedures evaluation of chemical composition of *E. angustifolium* during fermentation was done.

Materials and methods

Material.

Aerial parts of *E. angustifolium* were harvested from plantation in Mikkeli (Finland) (61°44' N, 27°18' E) in different vegetation phases and fermented as described [1].

Methods.

The total tannins were calculated as tannin equivalents [mg tannin/g dry weight] as described in the State Pharmacopoeia USSR [2]. Briefly, 2.0 g of botanical material was macerated with 250 ml of hot water during 30 min by refluxing. 25 ml aliquot was mixed with 500 ml of water and 25 ml of indigo sulphuric acid solution, then titrated using a 0.02 M $KMnO_4$ solution.

The total flavonoids content was calculated as rutin equivalents (mg rutin / g dry weight of material) according to the method described in a USSR State Pharmacopoeia [3]. About 0.5 g of the botanical material was macerated with 50 ml EtOH (50% v/v) for 30 min on water bath and filtered. A 1.0 ml aliquot of the extract was mixed with 5 ml of $AlCl_3$ (10% (w/v) in EtOH) and 1 drop of Acac, and then diluted to 25 ml with EtOH. The absorption at 410 nm was recorded on PharmaSpec UV-1700 (Shimadzu, Japan). *Flavonoids* were analyzed by HPLC. EtOH extract was injected into Shimadzu HPLC-system (Japan) with SPD-M20A DAD detector. Separation was done on Luna C18 (4.6x150 mm, 5 µm) (Phenomenex, USA) column. The linear gradient from 10% of acetonitrile in 0.03% water solution of TFA with increasing of acetonitrile concentration 1%/min was used; flow rate 1 ml/min. Detection was done at 360 nm. Hyperoside (Extrasynthese, France) was used as a reference compound.

Oenothein B was analyzed by HPTLC [4]. The content of oenothein B in the samples was calculated on basis of content of free gallic acid and total gallic acid (after HCl hydrolysis). HPTLC was done on silicagel pre-coated plates (Merck, Germany) in solvent system toluene/ethylacetate/methanol/formic acid (3/3/0.2/0.8 v/v) with following densitometric quantification at 280 nm.

Statistical analysis

Data were analyzed using Statistica 6.0 version (Statsoft, Moscow, Russia). The results are expressed as the M±m.

References: 1. Shikov A.N. et al. J Agric Food Chem 2006; 54: 3617-3624; 2. European Pharmacopoeia. 5 ed.; 3. Russian State Pharmacopoeia, XI ed. Vol. 1 and 2; 4. Shikov A.N. et al. J. Planar Chromatography 2010; 23(1), 70-74.

Results

The concentration of total tannins during fermentation was decreased in 36-42% (Fig. 1). It is associated with hydrolysis of tannins.

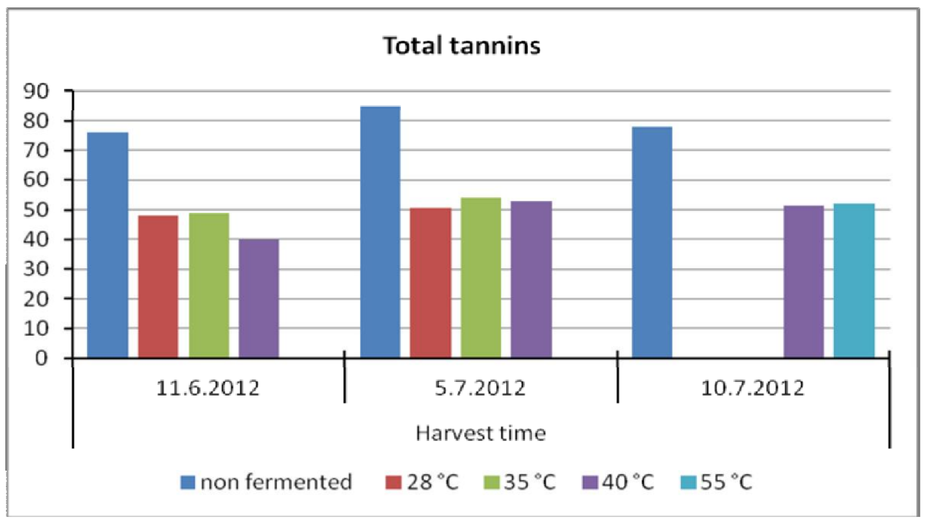

Fig. 1. Concentration of tannins, mg/g dry mass

The variation in total flavonoids content was depended from vegetation phase. In the beginning of flowering it decreased linearly according to increase of temperature, while in flowering time it was statistically not significant (Fig.2). However, concentration of hyperoside was decreased in result of fermentation in double.

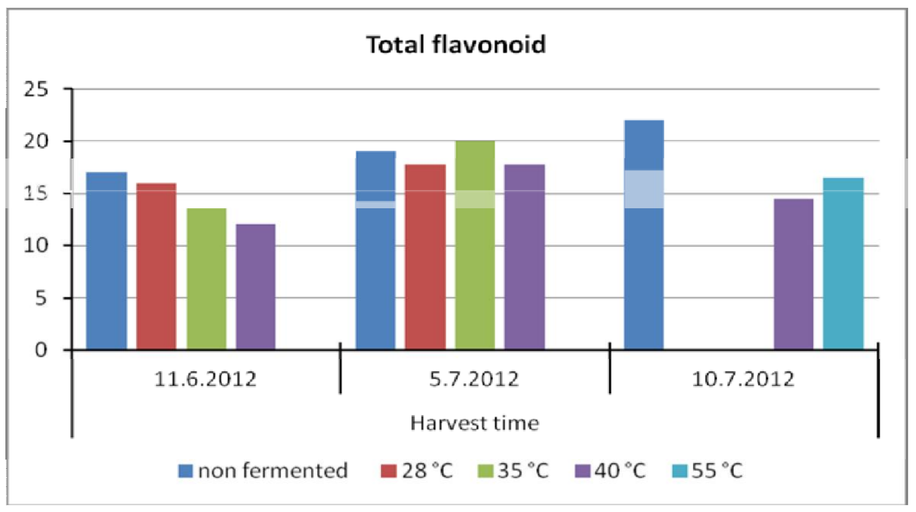

Fig. 2. Concentration of total flavonoids, mg/g dry mass

The concentration of Oenothein B was varied during fermentation not linearly. Depending fron vegetation phase minimal decrease was observed at 40ºC (by 60 %), while at the 28ºC and 35ºC the decreases were by 90 and 75 %.

Conclusion

Maximal concentration of tannins and oenothein B was observed at the beginning of flowering, while flavonoids and hyperoside at the flowering phase. The fermentation process decrease significantly the contents of total tannins, hyperoside and Oenothein B, and less effected on the total flavonoids. The minimal decreases were observed at 35-40 ºC fermentations (especially for Oentohein B, as a key compound).

The study was supported by Specicrops ENPI CBC2007-2013 project, co-funded by the European Union, the Russian Federation and the Republic of Finland