

Free amino acids in glycerol macerates of *Ribes nigrum* L.

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Introduction

In gemmotherapy, the buds of the black currant is used successfully. In the past, the macerates from *Ribes nigrum* (black currant) have attracted attention due to their health promoting benefits. Until now, only a few studies of the ingredients of the black currants have been made. The aim of this study was to identify the free amino acids of *Ribes nigrum*. Macerates from different batches had to be checked with HPTLC and HPLC. The essential ingredients had to be quantitatively determined. Other aims of this study were to gain more informations about the homogeneity of different batches, the stability of retain samples and the difference between the macerates of buds and leaves. The results were compared with the literature.

Test solutions

Different batches of macerates from *Ribes nigrum* were used. All macerates were prepared according Ph.Eur. 7.0/2371 V.2.1.3. 10 batches of the macerates of the buds were used, whereof the following were presented: 17110305, 17120307, 17130701*. All batches except the marked one were supplied by Spagyros, the other is commercial. The batches 22.06.2012 and 29.07.2013 are macerates of the leaves.

Reference substances

12 amino acids from different suppliers.

Determination of free amino acids made with HPTLC

Equipment:
HPTLC plates: silica gel 60 F254, 20*10 cm, Merck, lot No. HX389048; ADC 2, CAMAG; ATS 4, CAMAG; TLC Plate Heater III, CAMAG; TLC Visualizer, CAMAG; Desaga DS 20, Sysmex Digitana; visionCATS version 1.3.0.0, CAMAG.
Test solutions:
Filter macerates.

Material and Methods

Reference substances:

Dissolve 0.003 mol/l of each amino acid in methanol:water (7:3 V/V). Filter.

Application:

Test solution: 6-8 µl, reference solution: 2 µl as a band of 8 mm.

Mobile phase:

Extraction: 1-butanol : diethyl ether (3:2 V/V)
1_mobile: 1-propanol : ethanol abs. : diethyl ether (2:1:1 V/V)

2_mobile: citric acid buffer: citric acid (0.075 mol/l) : disodiumhydrogen phosphate (0.150 mol/l) (37:63 V/V), ethyl acetate : methanol : citric acid buffer (3:3:4 V/V/V), developing distance: 70 mm.

Process:

Extract glycerol for 45min (bands below phase!). Dry. Develop 2 times. Dry.

Drying:

1. 118°C for 3 minutes.
2. 105°C for 3 minutes.

Derivatisation:

Spray the plate with 0.016 mol/l ninhydrin in ethanol 94% (m/m) : acetic acid 98% (m/m) (97:3 V/V), dry by 105°C for 3 minutes.

Detection:

Examine in white light.

Determination of free amino acids made with HPTLC (extern)

Equipment:

Column: Hibar Purospher 150-4.6 RP18e, 3.0µm N1.50470.0001, VWR; Precolumn Purospher 30-4 RP18e, 3.0µm N1.50225.0001, VWR; Fluorescence Detector, Shimadzu; HPLC, Shimadzu

Test solutions:

Macerat : water R (1:1 V/V). Filter.

Reference substances:

Dissolve 0.2 mg/l of each amino acid in water. Filter.

Solvent:

A: methanol : water (90:10 V/V); B: sodium acetate (50 mmol/l), pH 6.9 : methanol (90:10 V/V).

Gradient:

Pre column derivatisation with OPA/FMOC.

time (min)	mobile phase B (% V/V)
0-11	78
11-15	78→65
15-31	65→25
31-32	25
32-36	5

Flow: 0-32 min 1.35 ml/min; after 1.8 ml/min

Injection volume: 30 µl (derivative)

Detection: At 330 nm / 450 nm.

Results

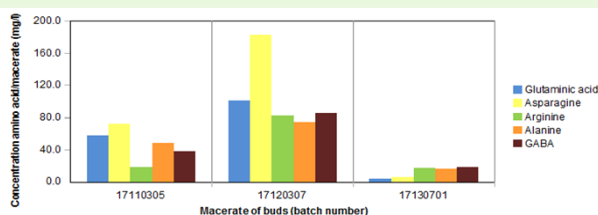


Fig. 1: Concentrations of main free amino acids in glycerol macerates of buds.

Identification and homogeneity of glycerol macerates of *Ribes nigrum*

Figure 1 demonstrates the content of main free amino acids (Alanine, Arginine, Asparagine, γ -Aminobutyric acid (GABA) and Glutamic acid) in different batches of bud glycerol macerates. The main free amino acids appear in all batches but the concentrations differ. (17110305 and 17120307 Spagyros macerates, 17130701 commercial macerate.)

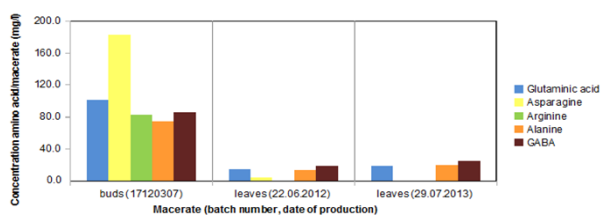


Fig. 2: Comparison of the concentrations of main free amino acids in one macerate of buds and two macerates of leaves.

Comparison of the macerates of buds and leaves of *Ribes nigrum*

The glycerol macerate be made of buds (17120307) contains considerably higher concentrations of all main free amino acids (see figure 2) compared to two macerates be made of leaves.

Tab. 1: Comparison of the measured concentrations of main free amino acids in glycerol macerates of buds and leaves with literature data (Tetau, 1997), nd = not detectable.

Amino acid	Bud macerate		Leave macerate	
	Value measured (mg/g buds)	Value literature (mg/g buds)	Value measured (mg/g leaves)	Value literature (mg/g leaves)
Alanine	15.0	0.654	4.0	0.405
Arginine	16.5	5.699	nd	0.771
Asparagine	36.7	12.457 (+Glutamine)	0.2	10.062
Aspartic acid	2.2	0.787	0.5	0.522
γ -Aminobutyric acid	17.3	1.038	5.1	1.444
Glutamic acid	20.2	0.926	3.8	1.436
Glycine	nd	0.121	0.1	0.076
Lysine	1.3	0.815	0.1	0.579
Proline	5.7	1.408	0.8	1.011
Serine	1.8	1.993	nd	1.552
Threonine	6.4	0.787	0.7	0.522
Valine	6.9	0.991	0.4	1.117

Comparison of free amino acids concentrations with literature data

Table 1 compares the detected concentrations of free amino acids in glycerol bud and leave macerates of *Ribes nigrum* with literature data (Tetau, 1997). In the bud macerate the measured concentrations of most free amino acids are higher compared to literature data.

Discussion

The literature data of free amino acids in buds and leaves of *Ribes nigrum* could be confirmed for *Ribes nigrum* glycerol macerates of Spagyros AG. The influence of conditions as harvest time or origin of the plants should be evaluated in further investigations.

The higher concentrations of free amino acids in bud extracts could be a hint that buds and leaves differ in their primary metabolism. The result encourages to investigate buds as an independent part of a plant. Therefore gemmotherapy using glycerol macerates of different buds could be a real contribution to utilize the whole potential of healing plants.

References:

Lapke, C. et al (2002). Detection of amino acids via TLC as a rapid method for the screening of hop and valerian. Revista de Fitoterapia 2 (S1).

Tetau, M. et al. (1997) Un anti-inflammatoire d'origine végétale: *Ribes nigrum* bourgeons. Cahiers des Biothérapie n63:69-72

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