IDENTIFICATION AND QUANTITATIVE EVALUATION OF AMYGDALIN FROM APRICOT, PLUM AND PEACH OILS AND KERNELS

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Key words: plum, apricot, peach kernels, vegetable oil, glycoside, amygdalin.
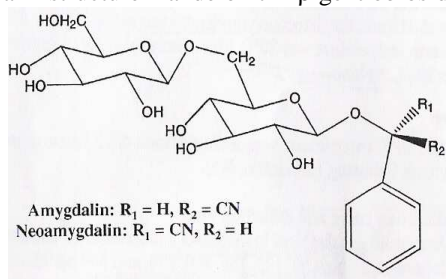
Abstract. Some kernels of different fruits, as plums,apricots, peaches, have considerable quantity of amygdalin with is a toxic substance for human organism. The paper prezents experimental data having in view the identification and quantitative evaluation of amygdalin from apricot, plum or peach oils and kernels. The amygdalin quantity was establish following the quantitative analyses using the reaction for nitrile group (-CN) with hidroxylamine as colorimetric method and using the identification and quantitative analyses of amygdalin as HPLC method. Comparing the results with some standards we conclude that the amygdalin concentration was 7-24 mg/Kg in kernels, while in oils the amygdalin was absent.

INTRODUCTION

In the industry of different cans and juices the kernels from some fruits as plums, apricots and peaches remain uncapitalized [1,2]. But these kernels can become raw material for obtaining semnificative quantities of good quality oils. Thus, the researchers from all over the world and our country, try to find various ways to put in the market these potential raw materials for the technology of obtaining vegetal edible oils with good quality and high nutritional functions [3,4].

In bitter almond and plum, apricot, peach or cherry kernels there is a glycosylated cyanogen compound, named D-amygdalin.

Fig.1 Amygdalin structure mandelonitril-β-gentiobiosid ($C_{20}H_{27}NO_{11}$)

Amygdalin: R_1 = H, R_2 = CN
Neoamygdalin: R_1 = CN, R_2 = H

The amygdalin gives the characteristic taste of "almond", agreated by the consumers, but it also has toxic

potential at doses over 500 ppm.

This compound with molecular formula $C_{20}H_{27}NO_{11}$, is found generally in kernels and other parts of the plant from various species of the *Rosaceae* family, as some fruit kernels. Glycosides that contain hydrocyanic acid are toxic substances and are named cyanogenic glycosides [5].

D-amygdalin in hydrolysis reaction, under action of emulsine, is emiperased at neoamygdalin [7,8].

Our researches follow the identification and HPLC quantitative elavuation of amygdalin in some kernel, and

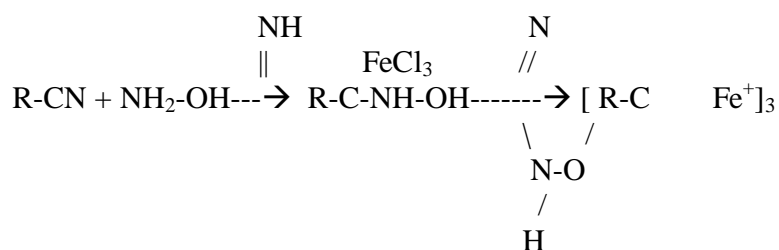also the oils obtained in extraction with organic solvents.

MATERIALS AND METHODS

We used plum, apricot and peach kernels (5 samples: 1 – peach kernel, 2 – plum boiled kernel, 3 – apricot grind kernel with wood cover, 4 – apricot kernel, 5 – plum raw kernel), harvested from west geographical part of the country. After the breaking kernels, the kernels were ground and homogenated, and then the oil was extracted with Soxthlet aparatus, using petroleum ether as solvent. The solvent was then distilated and was dried with anhydrous $CaCl_2$.

I. Amygalin identification with coluor reaction

At 1 g sample we added 10 ml warm water, then we made the extraction during 1 hour and after, 1 ml of

filtated obtained solution was used for the analyse.

The identify reaction for nitrile group (-CN) was made using hidroxylamina NH_2-OH as colouring agent [6], (red-violet colour), concordant to the next reaction:

$$
\begin{array}{ccc}
 & NH & N \\
 & \| \quad FeCl_3 & // \\
R\text{-}CN + NH_2\text{-}OH \text{---}\rightarrow & R\text{-}C\text{-}NH\text{-}OH \text{-------}\rightarrow & [\, R\text{-}C \qquad Fe^+]_3 \\
 & & \backslash \quad / \\
 & & N\text{-}O \\
 & & / \\
 & & H
\end{array}
$$

II. Identification and quantitative analyse of amygdamin using HPLC method.

II.1. Extraction

For the kernel extraction we used Soxhlet method which is based on repeated and continuous extraction of analysed material (5 g), for 3 hours at the 90°C, with a determinated methanol 99% volum (100ml).

The obtained extract was washed for 4-5 times with petroleum ether into a separation funnel to the complete cleaning. Samples were then concentrated to the rotative evaporator until we obtained a 80-100 ml of volume.

The amygdalin extraction from oil samples was made directly, as a result of repartition in separation funnel using methanol 99%. A sample containing 5 ml oil was introduced in separation funnel together with methanol 99%. The separation process was repeated for 4-5 times, when the methanolic phase was collected. After that, we washed the methanolic phase with petroleum ether for the complete purification, obtaining 10-20ml extract.

II.2. HPLC analyse of amygdalin using an amygdalin standard

In case of amygdalin separation and identification from our samples we used HPLC Chromatograph Waters type with Supelcosil column LC-18 (25cm x 4.6mm, 5μm) with PDA detector Waters 990 type. The mobile phase that we used was methanol **:** water 15 **:** 85 (v/v), at a 1ml/min debit for 22 minute. The detection was made at wave length of 215nm. The injector was washed with the mobile phase between succesive injections and also after experiment.

We prepared a stock solution contained amygdalin 1mg/ml in methanol 99%. Using the stock solutions we made succesive dilutions which were injected in HPLC Waters 900 Chromatograph with photodiod detector (PDA).

To obtain the etalonation curve, the pick´s areas were graphicaly represented in corelation with concentration of standards.

Etalonation curve (fig. 2) represents the HPLC – area of the signal corelated with standard amygdalin concentration (0-1mg/ml). Retention time speciffic for amygdalin is $t_R = 16.6min$.
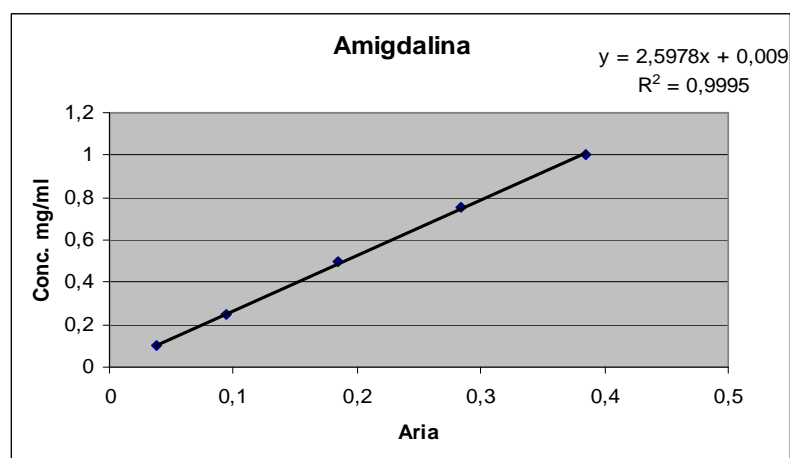
For identification of examined samples components the retention time of samples and standard have been compared. Thus, the concentration of amygdalin in samples was dwtermined using pick´s area from absorbtion spectrum UV-VIS based on etalonation curve [7,8].

RESULTS AND DISCUSSION

I. Etalonation curve and HPLC chromatograme for D-amygdalin

Figure 2 represents the etalonation curve with a correlation factor $R^2 = 0.999$, that demonstrate a significant linearity of area–concentration corelation for amygdalin standard solution.

Fig. 2 Etalonation curve (with the mention of the calibration straight line equation)

$$y = 2{,}5978x + 0{,}009$$
$$R^2 = 0{,}9995$$

Amigdalina

II. HPLC analyse of samples containing apricote and plum kernels and analyse of oil samples

Figure 3 represent HPLC chromatograme of standard solution 1mg/ml. The etalonation curve was obtained from the chromatogrames of the 5 standards with different concentrations.

Figures 4A and 4B represent chromatogrames obtained from the extracts of the samples 1 (apricots raw kernel) and 2 (plums boiled kernel). We can observe the presence of amygdalin to $t_R = 16.6$ min. and the presence of a "shoulder" signal to $t_R = 18.0$ min.

Generally, all the samples which contained kernel presented the same type of chromatogrames.

Figures 5A and 5B represents the plum oil chromatogrames (sample 7). In this case the major signal appeared to $t_R = 18.5$ min, and at the $t_R = 16.6$ min. appeared a minor signal, but characteristic for amygdalin. To demonstrate the absence of amygdalin in oil, the chromatograme for this sample was made with standard solution of amygdalin, and we observed the increasing of the signal compared to the initial value $t_R = 16.6$ min. (fig. 5B).

Table 1. Area of HPLC semnals, retention times t_R and the amygdalin contend of 1-8 analysed samples (details in Meterials and Methods)

No. of samples	Pic area	Retention time* t_R	Amygdalin mg/kg sample
1.	0.251454	16.33	15.9
2.	0.265205	16.44	13.5
3.	0.055603	16.89	3
4.	0.380123	16.24	24
5.	0.162787	16.66	7.2
6.	0	-	0
7.	< 0.05	16.66	urme
8.	0	-	0

The position of the signal at the t_R characteristic for amygdalin was confirmed trough co-chromatography using standard solution of amygdalin. It can be observed the appearance of an amygdalin degradation compound, present at the $t_R = 18+18.8$ min. (fig. 5A şi 5B).

Figure 6 shoned the absence of the signal to $t_R = 16.6$ min. and a intense signal to $t_R = 18.0$ min., demonstrate than the oil doesn't contain amygdalin.

Otherwise, the amygdalin concentration in oil was really decreased, which can be explained trough a low solubility in organic solvents (Table 1).

Using the spectroscopic UV analyse of the HPLC signals (Fig. 7A-C) we can compare the characteristic spectrum for amygdalin standard (Fig. 7A, maximum at 193 and 208 nm), amygdalin presence in raw kernel (Fig. 7B) and of degraded forme(fig. 7C).

Fig. 3 The HPLC chromatograme in case of amygdalin standard solution (1 mg/ml)

Fig. 4A The HPLC chromatograme in case of apricot kernel extract
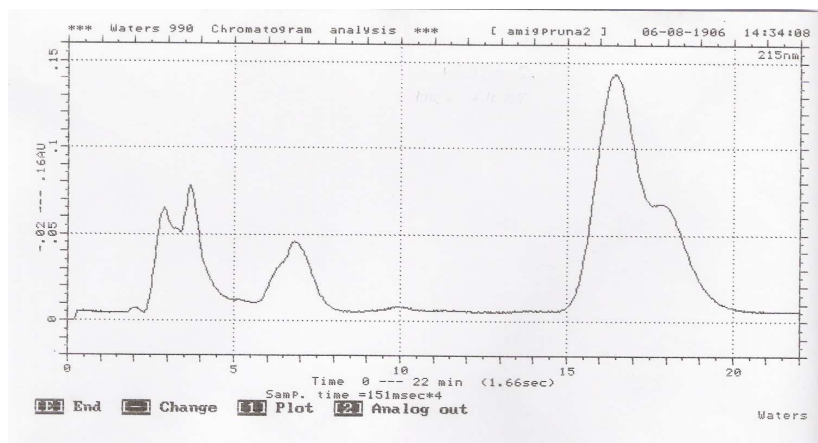
Fig. 4B The HPLC chromatograme in case of plum kernel extract
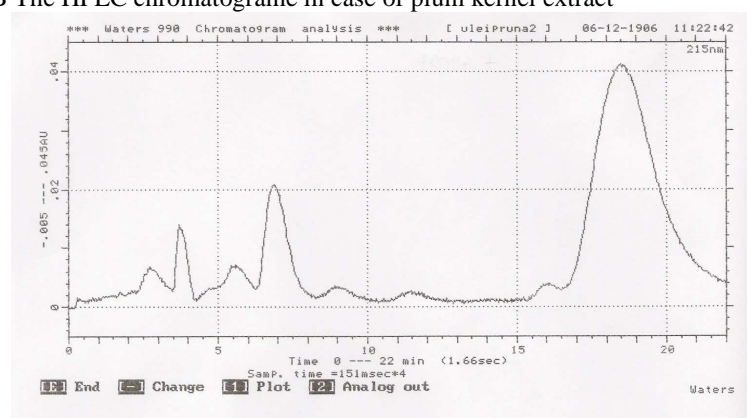

Fig. 5A The HPLC chromatograme in case of plum oily extract
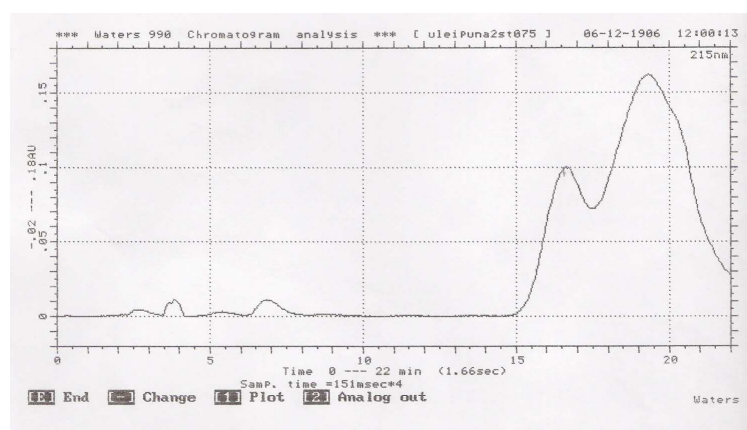

Fig. 5B The HPLC co-chromatograme in case of plum oily
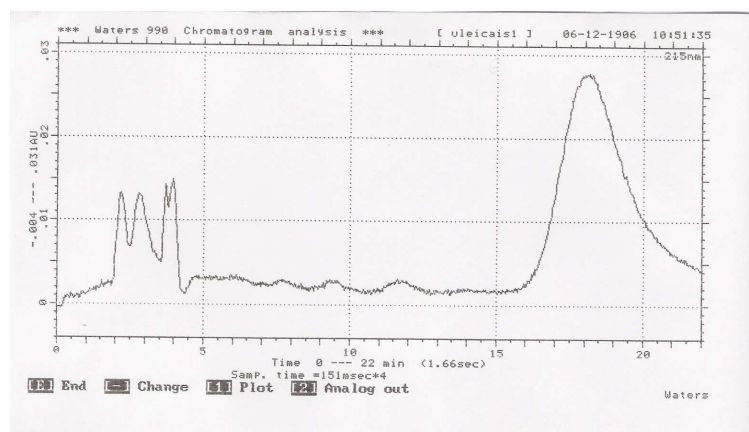extract with addition of amygdalin standard

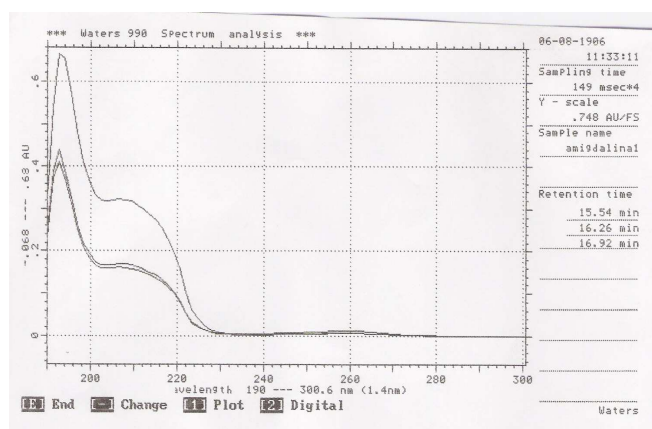Fig. 6 The HPLC chromatograme in case of apricot oily extract

Fig. 7A Amygdalin characteristic UV spectrum ($\lambda_{max.}$= 193.208 nm)
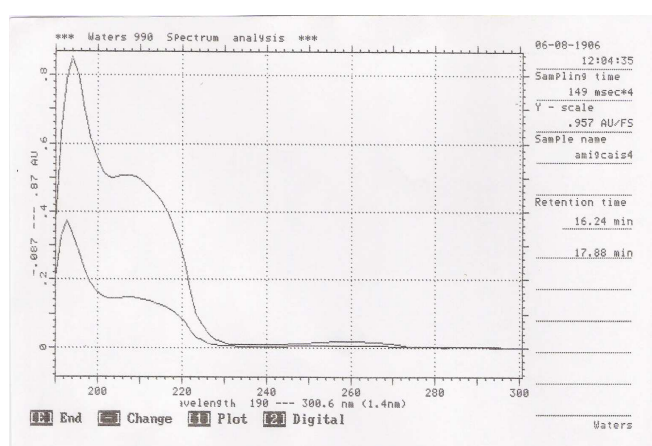

Fig. 7B Amygdalin characteristic UV spectrum in case of apricot oily extract
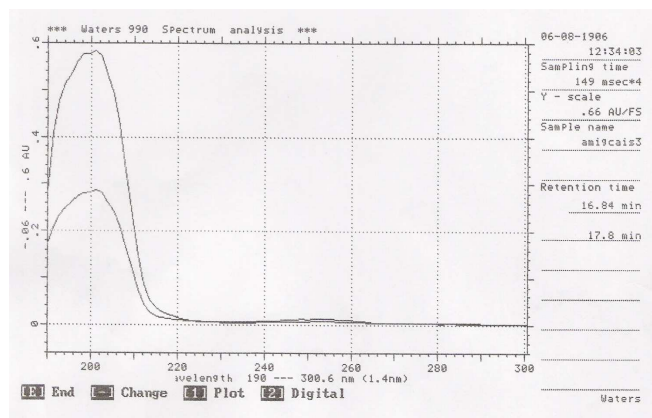
Fig. 7C Amygdalin characteristic UV spectrum
of degraded forme in case of termic processing products

CONCLUSIONS

We appreciate that the amygdalin concentration in samples which contained kernel from apricots and plums is between 3-24 mg/kg, and a high value is found in apricots. In oil samples there is no amygdalin, and this aspect have positive implications over the quality and use of oils in foods industry.

BIBLIOGRAPHY

1. Amitabh, C.; Nair, M.G., 1993, - *"Characterization of pit oil from Montmorency cherry (Prunus cerasus L.)"*, J. Agric. Food Chem. 41 (6), 879

2. Belitz, H.D., Grosch, W., 1987, -Food Chemistry, Springer-Verlag, New York-Berlin-Heidelberg-London-Paris-Tokyo-Hong Kong-Barcelona-Budapest

3. Carpenter D.L., Lehmann J., Mason B.S., Slover H.T., 1976, – *„Lipid composition of selected vegetable oils"*, J. Am. Oil Chem. Soc.

4. Gunstone, F., 1994, - *"Speciality oils of vegetable origin"*, Ind. Food Ingred., 3, 51-54.

5. Neamţu G., 1997, -"Biochimie alimentară",Editura Ceres, Bucureşti

6. Neamţu G., Carmen Socaciu, Sanda Crăpătureanu, Roşca E.G., Muntean E.,1998, Lucrări practice de chimie, Ed. a-II-a, Tipografia AGRONOMIA Cluj-Napoca, USAMV Cluj-Napoca

7.Wei-Feng Lv, D. Ming-Yu, Z. Rui, 2005, -*"Isolation and Quantitation of Amygdalin in Apricot-kernel and Prunus Tomentosa Thunb. By HPLC with Solid-Phase extraction"*, Journal of Chromatographic Science, Vol. 43, Department of Chemistry, Tsinghua University, Beijing 100084, P.R. China

8. Ja-Yong K., H. Eun-Young, C. Sonhae, L. Je-Hyun, L. Yong-Moon, H. Seon-Pyo, 2004, -*"Quantitative determination of amygdalin epimers from armeniacae semen by liquid chromatography"*, Journal of Chromatographic B, 814 (2005) 69-73, College of Pharmacy, Chungbuk national University, Chongju 361-763, Korea