MYROSINASE ACTIVITY IN ARMORACIA RUSTICANA

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Abstracts. This work is part of a study, which is more complex regarding the activity of sinigrin-myrosinase complex. With the help of this study we are following the optimization and the characterization of myrosinase activity with the help of HPLC chromatography and the comparison of the obtained results by spectrophotometric analysis.

At different temperatures conditions, (25°C ÷ 75°C), and reaction time (30 ÷ 390 minutes) were determined sinigrin concentration in extract (CSinExtr) and sinigrin concentration consumed in enzymatic reaction (CSinCons) by HPLC analysis.

The appreciation of sinigrin-myrosinase system activity was done by transforming CSinExtr and CSinCons in µg/glucose/g sample*hour.

The best parameters, adequate to myrosinase maximum activity, in Armoracia rusticana extracts were the following: pH = 7, temperature of 55°C, and reaction time was of 210 minutes for rubbed out horseradish samples and of 240 minutes for unrubbed horseradish samples.

INTRODUCTION

Glucosinolates are un group of plant secondary metabolites found exclusively in dicotyledonous plants. The highest concentrations are found in the Brassicaceae family. The Brassicaceae family comprises many commonly consumed vegetables, condiments, forages and oil containing plants, such as cabbage, broccoli, horseradish, mustard, cauliflower, Brussels sprouts and rape. Over 120 different glucosinolates have been identified to this date (Oerlemans et al., 2006).

These sulfur-containing compounds seems to play an important role in resistance to fungi, nematodes and other plant pathogens (Bonnes and Rossiter, 1996). On the other hand glucosinolates and/or their breakdown products have recently attracted intense research interest because of their possible cancer chemoprotective attributes (Fahey and Stephenson, 1999; Fahey and Talalay, 1999).

Glucosinolates have a well defined structure with a side chain (R-group) and D-glucopyranose as β-thioglucoside attached to carbon atom no. 0 in (Z)-N-hydroxime sulfate esters (Hansen et. al, 1995; Sorensen, 1990).

According to their structure they can be classified as aliphatic, aromatic, ω-methylthioalkyl and heterocyclic (e.g., indole) glucosinolates (Fahey et. al., 2001).

Because of their high bioactivity and because of the variety of compounds that can be obtained from them, GLS exhibit a great potential for their use in chemistry, food processing...
and food applications. In spite of being considered antinutritional compounds at the beginning, after wards their efficiency in preventing sickness and in preparing and storage at some foods, was proved (Hemingway, 1995; Li and Kushad, 2004).

Glucosinolates and their degradation products are responsible for the characteristic taste and odor of crops such as horseradish, cabbage, mustard and broccoli. The GLS content is higher in black mustard seeds and horseradish roots (over 10% by dry weight), than in the other constituent parts of the Cruciferae (Rosa et al., 1997).

The glucosinolate known as sinigrin (thio-β-glucopyranosyl-1-N-sulphate-2-propenylimidate) are in significant content in horseradish (Armoracia rusticana) roots (Thies, 1988).

Myrosinase, or thioglucoside glucohydrolase EC 3.2.3.1., is the trivial name for the enzyme (or group of enzyme) responsible for the hydrolysis of glucosinolates (Fenwick and Heaney, 1983). Autolysis or tissue damage brings myrosinase in contact with glucosinolates and hydrolysis occurs. Myrosinase activity results in the release of the glucose moiety to leave an unstable intermediate (Mithen at al, 2000), which spontaneously rearranges to produce several products. Which product is formed depends on several factors, such as pH, substrate or availability of ferrous ions (Bones and Rossiter, 1996).

The products of glucosinolate hydrolysis include isothiocyanates, nitriles, thiocyanates, indoles and oxazolidinethiones; from which isothiocyanates and indoles in particular have an impact on levels and thus intake of phytochemicals (Dekker, et al, 2000; Wilkinson et. al., 1984).

![Figure 1. Hydrolysis of glucosinolates in Armoracia rusticana, under different conditions](image-url)
MATERIAL AND METHODS

Vegetable material

The horseradish roots were harvested from Recas locality (in June 2005), were cleaned of impurities, and then were dried at room temperature.

Apparatus

A shaking machine, JT30 B, THERMO ANALOGIQ (Jouan SA, France) was used to accommodate at different temperature.

A HPLC chromatograph with the help of which were determined the adequate areas of sinigrin content.

Reagents

All reagents used were analytical reagent grade.

Acetonitrile HPLC grade and phosphate buffer dry powder blend, pH 7 at 25°C were purchased from Sigma Aldrich Chemie GmbH, Taufkirchen Germany. Deionized water was used for the preparation of all solutions.

Sinigrin (C_{10}H_{16}NO_{9}S_{2}K·H_{2}O) from horseradish was obtained from Sigma Aldrich Chemie GmbH, Taufkirchen Germany.

AgNO_{3}, from which was prepared the solution of 0,01M concentration, was acquisitioned from Merck Biocar Diagnostic, Germany.

The horseradish roots extracts were obtained as we described below: each of 1 g sample (rubbed out and unrubbed horseradish) was mixed with 10 ml phosphate buffer solution (pH = 7).

To appreciate more exactly the myrosinase activity in glucosinolates hydrolysis reaction, were done kinetically and thermodynamically studies (Al-Turki and Dick, 2003).

A. The influence of temperature on myrosinase activity from Armoracia rusticana

The temperature effect on myrosinase activity was studied by variation of this parameter in the interval of 25°C ÷ 85°C, maintaining a constant time of 30 minutes for each sample.

B. The kinetically studies on sinigrin enzymatic hydrolysis from Armoracia rusticana

The kinetic of sinigrin enzymatic reaction was studied in the following conditions:

- phosphate buffer pH was of 7;
- reaction time was of 30 ÷ 390 minutes;
- temperature of 25°C-75°C.

The samples were shaken and heated at adequate temperatures, with the help of a shaking machine. To each temperature, in the interval of 30 ÷ 390 minutes, was taken a sample, after every 30 minutes, which was cooled and then treated with AgNO_{3} solution, 0,01 M, for the inhibition of enzymatic reaction.

The myrosinase activity appreciation was done through the measurement of growth or decrease of sinigrin concentrations (CSinCons and CSinExtr) using HPLC technique.

HPLC-conditions: system HPLC: system 1100 (Hewlett Packard with DAD), column: ZORBAX XDB C18 150 x 2.1 mm; 20μl, flow: 0.55 ml/min, gradient: A (H2O) / B
Sinigrin-myrosinase system activity was appreciated by transforming \( CSinCons \) and \( CSinExtr \) obtained, by HPLC technique in glucose (mg/L).

The reaction is the following:

\[
\text{MYROSINASE} \quad \text{SINIGRIN} \rightarrow \text{GLUCOSE} + \text{ISOTHIOCYANATES}
\]

\[\begin{align*}
\text{(S)} & \quad \text{(E)} & \quad \text{(P)} \\
\text{MYROSINASE} & \quad \text{SINIGRIN} & \quad \text{GLUCOSE} + \text{ISOTHIOCYANATES}
\end{align*}\]

\(A. \) The temperature effect

The temperature effect on myrosinase activity from horseradish roots (rubbed out and unrubbed horseradish) may be observed in figure number 2. As we can see from the figures myrosinase activity grew once with the temperature growth to 55°C where it reached maximum values and at the other temperatures the activity diminished. We can say that temperature of 55°C is the optimal for maximum activity.

Also it was found that at temperatures more than 65°C, concomitantly with enzymatic hydrolysis of sinigrin, begins to show, at first a lower, then more intensely (at 75°C and 85°C), another enzymatic reaction, having as a substrate, sinigrin, namely desulphatase reaction, in the presence of sulphatase enzyme (van Doorn et al., 1999).

\(B. \) Reaction time effect

Reaction time effect on myrosinase activity from horseradish roots (rubbed out and unrubbed horseradish) is presented in figures number 3 and 4. Myrosinase activity evolution in horseradish roots was followed at different temperatures (25°C ÷ 75°C) in the interval of 30 ÷ 390 minutes.

As a result of the effected studies we can also say that:

- the optimal temperature for myrosinase maximum activity is of 55°C;
- the optimal reaction time for rubbed out horseradish extracts was of 210 minutes, and for the unrubbed horseradish was of 240 minutes.

The activation energy of the catalyzed reaction by myrosinase was of 928673.80 J/mol for rubbed out horseradish, and of 928008.68 J/mol for unrubbed horseradish.

On the basis of regression we can see that the enzymatic hydrolysis of sinigrin, is a reaction of first order.

![Figure 3. Effect of time reaction over myrosinase activity (unrubbed horseradish)](image)

![Figure 4. Effect of time reaction over myrosinase activity (rubbed out horseradish)](image)

**CONCLUSIONS**

The results obtained as a part of this study may be correlated with those obtained using another method analytical, namely, colorimetical determination of D-glucose.
The optimal parameters corresponding at maximum activity of sinigrin-myrosinase complex are the following: temperature of 55°C (enzymatic activity: 22.689 µg glucose/g sample*h, for unrubbed horseradish samples, and 42.907 µg glucose/g sample*h for rubbed out horseradish samples), and reaction time of 210 minutes for rubbed out horseradish samples (enzymatic activity: 64.906 µg glucose/g sample*h), respectively of 240 minutes for unrubbed horseradish samples (enzymatic activity: 61.513 µg glucose/g sample*h).

The method, which we presented here, is exactly, rapidly, and may be used in the majority of the laboratories.

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