

Determination of Amygdalin and Cyanide in Industrial Food Samples using Enzymic Methods *

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The use of enzymes in analysis promotes specific determination of a compound in complex mixtures. Especially in monitoring of biotechnical processes, for instance in food and pharmaceutical industry as well as in waste water treating plants, such methods appear to be of particular value.

Here are reported two such enzyme-based analytical procedures, tailor-made for the analysis of samples from a plant manufacturing almond pastes (from almond and apricot kernels) used in pastry and confectionery. Apricot and almond kernels contain the flavouring substance almond oil. Amygdalin, the major constituent of almond oil, is decomposed to glucose, benzaldehyde and hydrogen cyanide by the enzyme β -glucosidase which naturally occurs in the kernels,¹ Scheme 1.

In the manufacturing of almond paste the kernels are debittered by extraction first in 40–50 °C water and then in cold water during 2–4 days. This procedure is highly water and energy consuming and furthermore it delivers a rather toxic waste water that requires detoxification before discharge. Since the feedstocks contain varying amounts of amygdalin, the need for monitoring and control is apparent and should preferably be performed in a continuous and automatic fashion.

For the analysis of amygdalin we have chosen to measure the heat evolved by the enzymic reaction shown above with an enzyme thermistor,² which is a semi-adiabatic flow micro-calorimeter. The instrument contains a small column (0.8 ml) filled with β -glucosidase (E.C. 3.2.1.23, from sweet almond, 50 U/mg; salicine, 25 °C; Boehringer-Mannheim, FRG.) immobilised on controlled pore glass (CPG).

To 1 ml of CPG 10–700 80/120 mesh (Corning Glasswork, USA) was added 40 mg of β -glucosi-

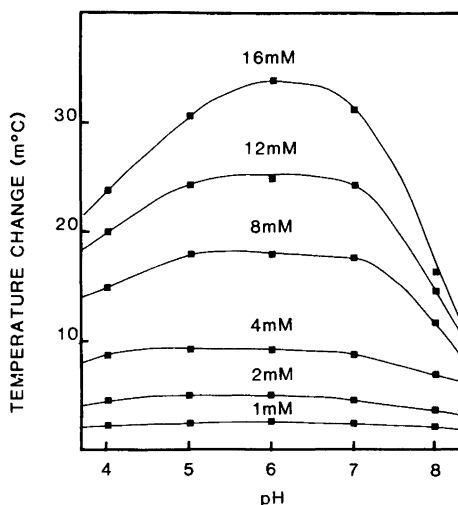


Fig. 1. Temperature responses of the enzyme thermistor versus pH at different concentrations of calibration solutions of amygdalin.

dase. Coupling to the alkyl-amino-derivatised glass beads was performed using glutaraldehyde.³ The β -glucosidase completely converts the amygdalin present in intermittently injected sample pulses, resulting in a corresponding temperature pulse that is recorded with a thermistor placed at the outlet of the column. Fig. 1 shows the heat released, expressed as a temperature change, as a result of injecting calibration solutions made up in 0.1 M sodium phosphate and 0.1 M sodium citrate buffers with pH ranging from 4 to 8. The optimum pH for the assay was found to lie between 5.0 and 7.0. The calibration curve then was linear up to 15 mM amygdalin using 0.5 ml sample at a flow rate of 0.8 ml/min. These samples were injected manually. In some experiments, however, we also used an automatic sample injection system with a sampling interval of 5 min.⁴

Table 1. Determination of amygdalin.

Sample of kernels	Enzyme thermistor (mg amygdalin per gram of kernels)	Spectrophotometer
Bitter almond	39.3	44.5
Apricot	11.8	12.2
Sweet almond	0	0

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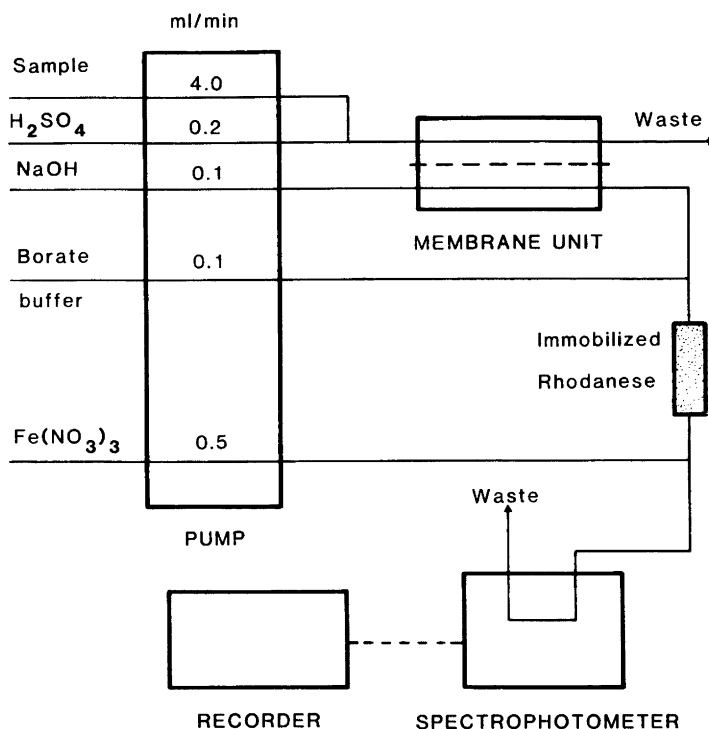


Fig. 2. Experimental set-up of the cyanide analyzing system with membrane enrichment, enzymic conversion and spectrophotometric monitoring. The $\text{Fe}(\text{NO}_3)_3$ contained 10 v/v % HNO_3 .

The determination of amygdalin in crude samples is interfered with by naturally occurring β -glucosidase. This β -glucosidase activity could, however, be eliminated by denaturation of the enzyme by heating the sample or by acid treatment as the following experiment showed. Amygdalin was added to a concentration of 10 mM to an amygdalin-free extract of almond kernels (20 % dry material). The hydrolysis of amygdalin in the extract was followed by enzyme thermistor analysis of 0.5 ml samples withdrawn every 5 min.

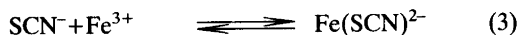
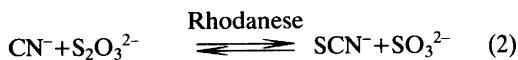
In the untreated extract the amygdalin content was reduced by 50 % in 17 min, whereas initial treatment of the almonds in boiling water for 5 min prior to extraction, decreased the decomposition rate to 5–10 % in 30 min. Treatment with phosphoric acid at pH 2 had an even larger denaturing effect as only 5 % of the amygdalin was hydrolysed in 50 min. It should be noted that these treatments had no direct effect on the amygdalin concentration. A combination of these two methods virtually eliminated the unwanted decomposition of amygdalin in crude samples.

Different samples obtained from a food indus-

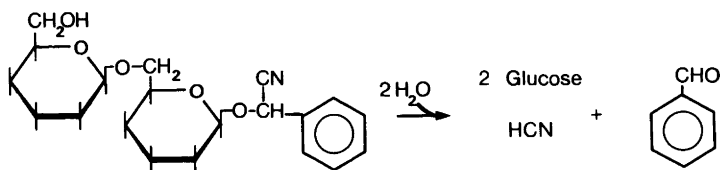
try (Viktoria fabriken, Helsingborg) were analysed for amygdalin by the present method and by a spectrophotometric technique based on determination of cyanide with picric acid.⁵ Data given in Table 1 show a good agreement between the two techniques. In conclusion, the enzyme thermistor method for determination of amygdalin described here turned out to work well with crude food samples. It is a rapid and direct method with sufficient sensitivity for such samples. Furthermore, it was capable of adequately monitoring the debittering process employed in the factory. The useful concentration range was 0.1–20 mM.

Since the highest permissible cyanide concentration in sewage water is set very low, enrichment of the cyanide present in the waste water samples had to be made prior to analysis. This was accomplished with an on-line filter unit containing a hydrophobic, porous teflon membrane. Such membranes are highly permeable to gases, including hydrogen cyanide. The membrane was mounted in a rectangular membrane holder exposing 400 mm² of each side of the membrane in 100 mm long channels with the

cross section $1 \times 4 \text{ mm}^2$. A multichannel peristaltic pump (Gilson, Minipulse, France) was used for administration of the different flow streams according to Fig. 2. Cyanide present in the sample was protonated to hydrogen cyanide by lowering the pH to below 1. The pH at the other side of the membrane was kept high ($\text{pH} > 12$). A low flow rate on this side of the membrane compared to that of the sample side resulted in considerably higher cyanide concentration than in the sample. Different hydrophobic membranes from Millipore (Bedford, Mass. USA.) were investigated using a spectrophotometric cyanide assay.⁶ We found that a Fluoropore membrane with $0.2 \text{ }\mu\text{m}$ porosity gave the highest enrichment factor (8 times), whereas 5-fold enrichment was seen with $1 \text{ }\mu\text{m}$ Fluoropore and $5 \text{ }\mu\text{m}$ Mitex membranes. Advantages of this membrane technique are that loss of cyanide is eliminated by the use of a closed system and that the sample is cleaned up, with minimised interference and increased specificity as consequences. Determination of cyanide was performed by converting the cyanide to thiocyanide (rhodanide) with use of immobilised rhodanese followed by reacting the thiocyanide with the Fe(III) ions to give the coloured complex, FeSCN^{2+} :



Rhodanese (E.C. 2.8.1.1; 10 units per mg; Sigma Chemicals, Mo, USA) was immobilised on CPG (5 mg rhodanese/ml CPG) using the same procedure as for β -glucosidase.³ The Fe(SCN)^{2+} complex was measured at 460 nm by a Zeiss PM6 spectrophotometer equipped with a 0.030 ml, 10 mm flow cell. Under the conditions given, the change in absorbance was linear to the cyanide concentration of aqueous standard solutions up to 15 mg CN^-/l (0.6 mM). The limit of detection was 0.2 mg/l (0.008 mM) and each analysis took 10–15 min.



Scheme 1. Eqn. (1).

Table 2. Waste water analyses.

Sample		Rhodanese method (mg CN^-/l waste water)	Official method
Untreated	I	3.3	2.9
Waste water	II	1.1	1.5
Treated	I	0.25	0.5
Waste water	II	<0.2	<0.1

Waste water samples from the factory (Viktoria fabriken) collected before and after detoxification were analysed for cyanide content by the present method and by a conventional, calorimetric technique (performed by an official control laboratory, Swelab, Sweden). Table 2 compares some of the results obtained in this study. We also found good agreement in results obtained with the present method, and with a Beckman cyanide electrode. The electrode was more sensitive, but its operation with crude samples was less reliable. Alternatively an enzyme thermistor can be used for cyanide determination,⁷ although it is more susceptible to interferences (non-specific heats) in work with crude solutions.

In conclusion, two analytical procedures have been developed for specific, on-line analyses of crude process solutions. Both of them have adequate sensitivity and time response to be used for process control in the situations they were investigated for.

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