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Thermodynamics study of linamarin sorption during its isolation from cassava

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ABSTRACT

Linamarin is an important biomaterial. The removal of linamarin from aqueous solutions at different contact times, temperatures and initial concentration of crude cassava extract during its isolation using activated carbon was investigated. The extent of linamarin removal increases with increase in initial concentration of extract, contact time and temperature of the solution. The adsorption isotherm is described by means of composite isotherm where U-shaped isotherm was observed. The mole of linamarin adsorbed per pore volume of activated carbon (n_1^s) at different temperatures (298, 303, 318, 328, and 338 K) was almost equal (0.99, 1.00, 1.01, 1.00 and 1.00 g mol/cm^3). The activation energy of the system (E_a) was calculated as 22.61 kJ/mol. The free energy of the adsorption (ΔG°), enthalpy (ΔH°), and entropy (ΔS°) changes were calculated to predict the nature of adsorption. The estimated values of ΔG° at 298, 303, 318, 328, and 338 K were −2.45, 1.69, 1.80, 1.70, and 1.73 kJ/mol, respectively, which are relatively high except at 298 K, indicating endothermic and non-spontaneous process.

1. Introduction

Cassava (Manihot esculenta Crantz) is known to be a storage root crop which serves as a staple foodstuff for more than 500 million people throughout tropical Africa [1] as a result of its carbohydrate-rich and low-in protein storage roots.

Linamarin (2-β-D-glucopyranosyloxy-2-methylpropanenitrile) is a major component of cassava which serves as a defense mechanism against predators and herbivores when linamarin undergoes hydrolysis (Scheme 1) by an endogenous enzyme, linamarase (β-glucosidase) to form a toxic compound known as prussic acid (HCN) [2]. The toxic characteristic of this compound has been harnessed to control one form of cancer or the other in the Chinese traditional medicine [1] though without scientific validation. Linamarin is known to have applications; as a standard in determining cyanide content in cassava-related food products like garri, cassava flour, and chips and in linamarin/linamarase/SnO_2 electrode biosensors [1] used for glucose measurement in biomedical applications or hydrogen cyanide in the environment [3].

Generally, when cassava is being processed, the cyanide released in excess concentration of 200 μm/l is known to be rapidly toxic to most species of fish as well as causing negative changes to plant community structure [4]. Although once released in the environment, cyanide activity provides numerous pathways for its degradation, but uncontrollable release of this compound to the environment has often amounted to over concentration and thus adversely impact on its immediate environment [5].

Reviews in the past have centered mainly on the biosynthesis, distribution and toxicology of this compound [6] in crude form because of the difficulty associated with its isolation. Recently we had a breakthrough in its isolation and its cytotoxicity to a selected cancer cell lines was investigated and reported [7]. It was observed during the isolation of linamarin with activated carbon that its purification preceded its adsorption but helped to ascertain optimum contact time of purification. In this work, focus is on studying the process of linamarin removal from the aqueous solution during its isolation, considering the effect of initial concentration of extract, contact time and temperature. Thermodynamic parameters and factors controlling the adsorption process were also evaluated and discussed.

2. Experimental

2.1. Chemicals

Analytical grade (98–99.5% purity). The following chemicals were obtained from Sigma–Aldrich (USA): sodium carbonate, picric acid, 0.1 M phosphate buffered solution (PBS) (pH 6.0) and 0.1 M acetate buffered solution (pH 5.5). Methanol and ammonium sulphate were obtained from Merck Chemicals (RSA), commercial granular activated carbon was obtained from Associated Chemical Enterprises (RSA) with the following physical properties: particle size of 8–30 mesh, apparent density of 483 kg/m^3, moisture content

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Nomenclature

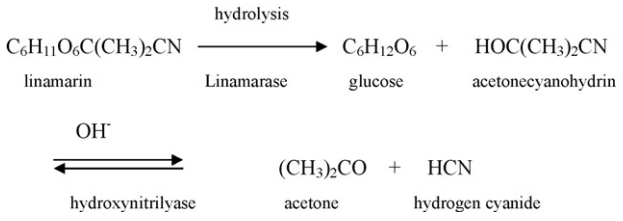
E_a	activation energy
ΔG°	standard Gibb's free energy change
ΔH°	enthalpy change
J	joules
K	temperature in Kelvin
K	equilibrium constant of adsorption
K_{pf}	equilibrium rate constant
kJ	kilojoules
n	Freundlish constant of adsorption
q_t	amount of solute adsorbed at a given time
q_{eq}	equilibrium concentration
R	universal gas constant
ΔS°	entropy change
T	absolute temperature
V	volume of solution

of 2.4 wt%, iodine adsorption of 1257 mg/g, specific surface area of 746 m^2/g and average pore size of 26 Å.

A MiniFlex UF system, built around a tubular module, was obtained from Schleicher and Schuell (Germany). It contained polyethersulfone membranes (0.2–0.45 μm) of polypropylene screens and silicone adhesives, together with a nominal molecular weight cut-off membrane of 50 kDa, all having a membrane surface area of 2.4 mm^2. Other parts of the system included: 3R VL 100 constant pressure variable speed peristaltic pump, pressure gauge (bar) for indicating retention inlet and outlet pressure, connecting tubes for feed flow, 2 ml × 140 ml graduated container for feed and permeate, and a VOLTCRAFT switching power supply.

2.2. Preparation of hydrolyzing enzyme (linamarse) solution from cassava root peel

The preparation of linamarase was carried out as described previously [8] with modification. Briefly, fresh cassava peel (locally produced) were diced and homogenised in 0.1 M acetate buffer (pH 5.5). The solution was centrifuged at 10,000 rpm (rounds per minute) for 30 min and the supernatant was brought into a saturated solution of ammonium sulphate (60%, w/v) and held at 4 °C for 16 h. This solution was then centrifuged at 10,000 rpm for 1 h. The precipitate thus obtained was dissolved in 0.1 M PBS (pH 6.0), and was dialysed using 0.45 μm membrane pore size. Further purification was achieved using 0.2 μm membrane (ultrafiltration) that permeated cyanogenic components. Purified enzyme was later collected as retentate over 50 kDa membrane pore size. The enzyme solution was stored at 4 °C. Immediately before use, a solution containing 0.42 units/ml of the enzyme was prepared to determine its specific activity (unit/mass of protein) using 0.08 g of protein. This produces a specific activity of 52×10^{-6} mol μg^{-1} min^{-1} of linamarase.



Scheme 1. Hydrolysis of cassava linamarin.

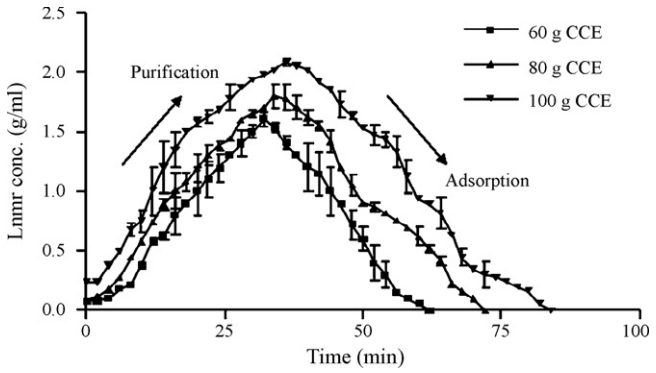


Fig. 1. Purification/adsorption of linamarin isolation at room temperature, at 190 rpm. CCE (crude cassava extract), Lnmr conc. (linamarin concentration).

2.3. Determination of the optimal amounts of linamarase and linamarin

Linamarase of varying amount (2–13 ml) were mixed with 1.5, 2.0 and 2.5 g of linamarin. The activity of linamarase on linamarin hydrolysis was found to be proportional to the amount of the enzyme used. The highest activity was achieved using 11 ml of enzyme solution with 2.0 g of linamarin. The absorbance readings represent HCN equivalence released [8,9] from linamarin, which at this point, did not increase as a result of saturation. Therefore 11 ml of the enzyme solution was determined to be the optimum hydrolyzing amount on linamarin, as against excess (2.5 g) of linamarin. With this, 11 ml of the enzyme solution was used in all subsequent runs as reported [7].

2.4. Preparation of crude cassava extracts (CCEs) and kinetic determination of purification/adsorption point of linamarin

Initially, alkaline picric paper being a useful physical tool for confirming the presence of linamarin in solution was prepared [8]. Cassava roots (1 kg) were diced and homogenised in boiling methanol (99.5%). The bulk solution was allowed to settle for 1 h, after which the relatively clear supernatant was decanted from the sediment. This was followed by ultrafiltration (0.2 μm). The isolated linamarin mixture was then evaporated at 45 °C. A dark brown jelly-like solid (60 g) was recovered. This preparation was repeated for 1.5 and 2.5 kg of cassava roots. During the isolation of linamarin from the aqueous solution it was observed that its purification is accompanied with adsorption from solution hence there was the need to establish initial contact time of linamarin removal. Here crude cassava extract (60, 80 and 100 g) was dissolved in 250-ml distilled water, which was then introduced into 80 g of activated carbon. The mixture was shaken (190 rpm) at room temperature (25 °C) for varying contact times until the solution turned colourless after filtration. After each contact time and filtration, 4 ml filtrate was collected and added to 11 ml linamarase. Picric paper, cut to size, was suspended above the samples and the vials immediately stoppered. The vials were placed at 30 °C overnight. The colour change of picric paper to orange and brown was observed confirming linamarin/linamarase activity. This is in accordance with previous studies conducted by Cooke [8]. The papers were removed and immersed in 50-ml distilled water. After 30 min, the elutes were collected and the absorbance read at 510 nm against a picric paper blank. This was repeated with longer contact times of 62, 72, and 84 min for 60, 80 and 100 g, respectively. With varying contact times, the highest concentration of linamarin obtained (purification process) before the concentration started to drop (adsorption process) (Fig. 1) was taken as the threshold concentration/contact