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Although Fe-oxides are more easily dissolved in the more recent sediments, Cu is leached more slowly, suggesting that other processes determine the leaching of Cu. For example, more research is needed on the influence of aging on organic matter and dissolved organic carbon.

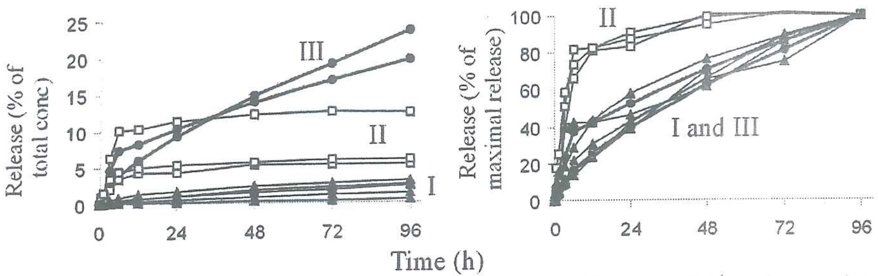

Figure 2: Release of Fe at pH 2 from the sediments of group I (*), group II (◊) and group III (•)

CONCLUSION

During pH_{stat} titrations on dredged sediments of different age since disposal on land, 4 types of metal leaching behaviour were observed. For most elements, leaching patterns in aged and recent sediments were very similar, except for Fe and Cu. Differences in the amount of Fe released and the leaching patterns of Fe in recent and aged sediments were attributed to differences in crystallinity of Fe-oxides. However, other processes probably explain the different leaching pattern of Cu in aged and recent sediments.

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THERMAL INACTIVATION OF PECTIN METHYLESTERASE FROM PEPPERS (*CAPSICUM ANNUUM*)

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ABSTRACT

Pectin methylesterase (PME) from green bell peppers (*Capsicum annuum*) was extracted and purified. Isothermal inactivation of purified pepper PME at pH 7.5 could be described by a fractional conversion model for lower temperatures (55-57°C) and a biphasic model for higher temperatures (58-70°C).

INTRODUCTION

Peppers are becoming more popular in recent years due to their chemical composition (e.g. vitamins), and a wide variety is nowadays available in the market. Most varieties belong to Capsicum annuum species and they can be consumed fresh or processed, as immature (i.e. green) or as mature fruit (i.e. yellow, orange, red), as spice or as vegetable in food, because of their distinct colours, pungency and flavour. The objective of this research work was to investigate the inactivation kinetics of purified pepper PME under isothermal conditions (55-70°C).

MATERIALS AND METHODS

Green bell peppers (*Capsicum annuum*) were purchased from a local auction (Mechelen, Belgium). PME was extracted from peppers according to the procedure as described by Ly-Nguyen *et al.* (2002a). Isothermal treatments were performed in a temperature-controlled water bath using glass capillaries to enclose the enzyme solution at pH 7.5.

Kinetic Data Analysis

Inactivation of enzymes can often be described by a first-order kinetic model. When there are several isozymes present, which show different behavior towards temperature, i.e., labile and stable fraction, and both inactivating according to a first order kinetic model (Indrawati *et al.*, 1999; Van den Broeck *et al.*, 2000), a biphasic kinetic model, or distinct isozyme model (equation 1) can be used.

$$A = A_L \exp(-k_L \cdot t) + A_S \exp(-k_S \cdot t) \qquad (1)$$

Where the subscript L and S means labile and stable enzyme fraction, respectively. The residual activity from the labile and stable fractions as well as the inactivation rate constants can be estimated by non-linear regression analysis. When only the labile fraction inactivates, whereas the activity of the stable fraction does not change with respect to time, a fractional conversion kinetic model is to be applied (Van den Broeck *et al.*, 1999). A fractional conversion model takes into account the residual activity after prolonged thermal treatment (equation 2):

$$A = A_{\infty} + (A_0 - A_{\infty})\exp(-k \cdot t) \quad (2)$$

Where A_{∞} is the residual activity after prolonged treatment time. The inactivation rate constant (k) and the residual activity (A_{∞}) are estimated by non-linear regression analysis. The temperature dependence of inactivation rate constants can be estimated using the Arrhenius model (equation 3):

$$\ln(k) = \ln(k_0) + \left[\frac{E_a}{R}\left(\frac{1}{T_0} - \frac{1}{T}\right)\right] \quad (3)$$

Where T and T_0 are the absolute temperature (K) and the reference temperature (K), respectively; k_0 is the rate constant at T_0, E_a is the activation energy ($kJmol^{-1}$), R (8.314 $Jmol^{-1}K^{-1}$) is the universal gas constant. The activation energy can be estimated by linear regression analysis on equation 3.

RESULTS AND DISCUSSION

A detailed kinetic study of thermal inactivation of purified pepper PME dissolved in 20 mM Tris buffer (pH 7.5) was performed in the range from 55°C-70°C at atmospheric pressure. Figure 1 presents the thermal inactivation curves of pepper PME at pH 7.5, in the temperature range from 55°C to 57°C. A fractional conversion model could accurately describe this inactivation behavior indicating the presence of a temperature resistant enzyme fraction that is not affected after a prolonged heating at the preset temperatures. The temperature dependence of the inactivation rate constants in the temperature range (55-57°C) was estimated by linear regression analysis (equation 3) as 369.2 kJ/mol. The residual activity after prolonged heating (A_{∞}) was approximately 62% of the total PME activity, independent on the temperature applied. Figure 2 illustrates the thermal inactivation curves of pepper PME in a temperature range from 58°C-70°C. The thermal inactivation of pepper PME at pH 7.5 in this temperature range exhibits a biphasic model, indicating the presence of a heat labile and a heat resistant fraction of PME, both showing first order inactivation mechanisms.

Labile and resistant forms of PME have been shown to occur in a number of other fruits and vegetables including oranges (Van den Broeck *et al.*, 2000), grapefruits (Seymour *et al.*, 1991), sweet cherries (Alonso *et al.* 1996), persimmon (Alonso *et al.*, 1997), and green beans (Laats *et al.*, 1997). Purified pepper PME, like carrot PME, is less stable toward thermal treatment when compared to other PMEs from different sources. The inactivation rate constant for labile and stable PME fraction of purified pepper PME at 60°C were, respectively, 0.8084 and 0.0107 min^{-1}, and for stable PME fraction at 70°C was 0.7161 min^{-1}. Ly-Nguyen *et al.* (2002b) reported a $k_{60°C}$ 0.6814 min^{-1} for purified carrot PME, while Anthon and Barret (2002) obtained inactivation rate constants $k_{70°C}$ between 0.0109 s^{-1} and 0.0114 s^{-1} for carrot juice.

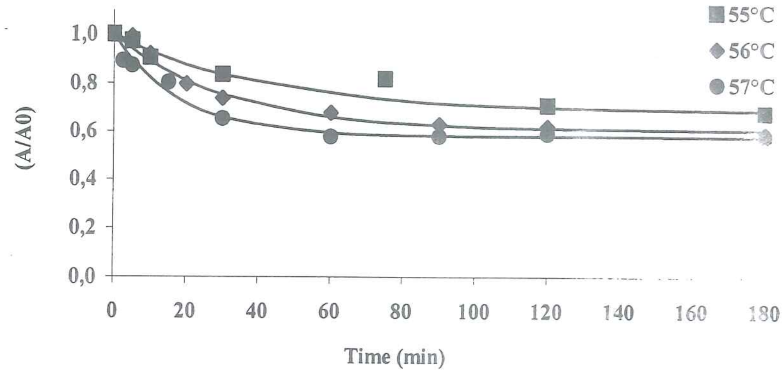

Figure 1. Thermal inactivation of purified green pepper PME dissolved in 20 mM Tris buffer (pH 7.5), for 55-57°C temperature range.

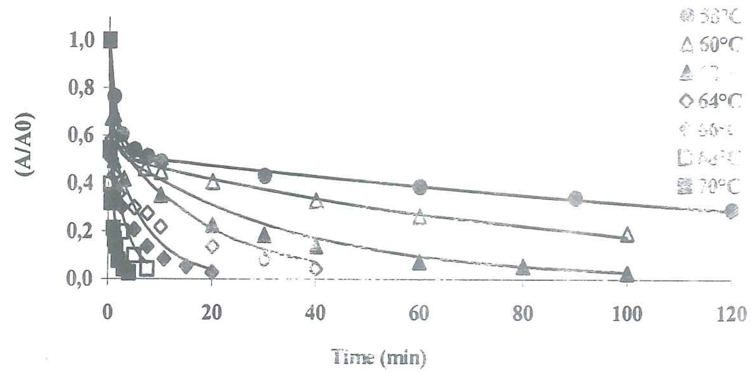

Figure 2. Thermal inactivation of purified green pepper PME dissolved in 20 mM Tris buffer (pH 7.5), for 58-70°C temperature range.

The Arrhenius plots for the thermal inactivation of the thermoresistant fraction in the temperature range of 58-70°C (Figure 3), showed no obvious deviation from linearity (r^2 = 0.998), with an activation energy of 388.9 kJ/mol. This value is in the same range (301.4-350.5 kJ/mol) found for commercial orange PME (Van den Broeck *et al.*, 2000), but higher than the one found by Ly-Nguyen *et al.* (2002) for purified carrot PME.

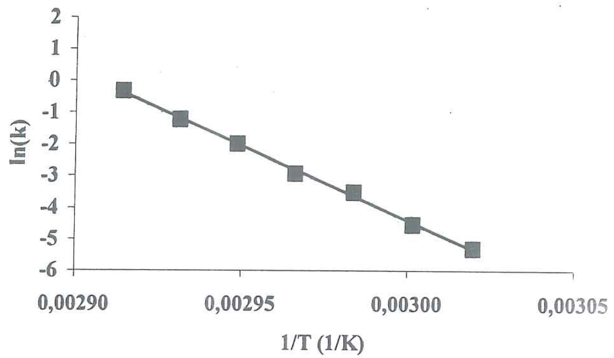

Figure 3. Temperature dependence of inactivation rate constant for thermal inactivation of the thermostable fraction of purified pepper PME.

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BAYESIAN BELIEF NETWORKS FOR THE PREDICTION OF MACROINVERTEBRATE TAXA IN THE ZWALM RIVER BASIN

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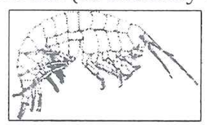
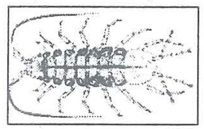
INTRODUCTION

Predictive models have become important instruments to support decision makin in water management. Besides mathematical modelling techniques, based o partial differential equations and multivariate statistics, also other techniques such as artificial neural networks, fuzzy logic and Bayesian belief networks (BBN's (Trigg *et al.*, 2000; Borsuk *et al.*, 2002) are recently used for this purpose. BBN's (Pearl, 1988) are models with a network structure which focuses on the explici representation of cause-and-effect relationships between variables, representing i this case ecosystem components. The network architecture is linked to probability distributions that allow to deal with variability and uncertainty in the models. Thi is in particular very useful for the description of ecological systems (Regan, 2002).

MATERIALS AND METHODS

In this study BBN's were constructed to predict the abundancy classes o *Gammarus* and *Asellus* (the most important Crustaceans in freshwater ecosystems) based on causal links between abiotic variables (that describe river conditions) and the mentioned macroinvertebrate taxa. For the selection of the abiotic variable (Table 1) and the development of the network structures, literature knowledge has been used. The specification of the probability distributions, however, was base on a dataset, that consisted of 232 samples, collected in the Zwalm river catchmen (Figure 1) during summer from 2000 till 2002.

Table 1. Selected variables for the development of the BBN's.

Biotic (predicted) variables	Abiotic variables
• Gammaridae (abundancy classes) 	• Conductivity (COND, µS/cm) • Stream Velocity (SV, m/s) • Width (WIDTH, cm) • Sand (SAND, %) • Gravel (GRAVEL, %)
• Asellidae (abundancy classes) 	• Suspended Solids (SS, mg/L) • Chemical Oxygen Demand (COD, g/L) • Temperature (T, °C) • Dissolved Oxygen (DO, mg/L)