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Kinetics of Enzyme Activity in Peaches During Storage and Processing

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Summary

The behaviour and activity of the enzymes polygalacturonase (PG, endo- EC. 3.2.1.15 and exo- EC. 3.2.1.67) and of pectin methyl esterase (PE, EC. 3.1.1.11) in peaches, measured during storage and processing, was modelled, based on the fundamental laws of kinetics. For both enzymes the activity was susceptible to senescence (PG) or denaturation (PE) counteracting a formation out of precursor or a bound and inactive form of the enzyme. For PE the behaviour was clearly based on two isoenzymes with, at a certain temperature, a conversion between the two configurations.

Keywords: enzyme denaturation, apparent activity, storage, processing, kinetics, generic model, peaches

Introduction

Some enzymes present in fruits and vegetables exert a positive effect on the final quality (e.g., pectin methyl esterase PE, decreasing the loss of firmness after sterilisation), whereas other enzymes have a negative effect (e.g., polygalacturonase PG, decreasing the firmness, and lipoxygenase LOX, generating off-flavours) (*1*). Of other enzymes the effect on quality is not entirely known (e.g., peroxidase POD). The level of the activity of enzymes may change during the storage period prior to processing. These changes will also affect the product quality obtained after heat treatments.

The aim of blanching fruits and vegetables prior to sterilisation is among others, the activation and/or inactivation of enzymes present in the plant tissue. The apparent activity of different enzymes at different temperatures exhibits a well-known behaviour: at low temperature, up to about 40 °C, a steady increase in activity is observed. This can frequently be described and analysed by the well-known law of Arrhenius. Eventually, a maximum activity is reached. This is often referred to as the optimal temperature for enzymatic action. At still higher temperatures a rather steep decline in activity is observed (*2–4*) due to denaturation.

The action of these enzymes will have a pronounced effect on the observed quality of stored and processed products. A consistent philosophy on quality perception and assignment relates product properties to quality attributes (*5,6*). Applying a consistent framework of quality is of utmost importance in the modelling and prediction of enzyme activity and of their specific effects on those product properties. It greatly helps to understand the interactions occurring during processing and storage, to direct the research in that field and to make appropriate decision regarding the processing and storage conditions and regarding the obtainable product quality.

Raw Data

Storage experiments

Peaches (cv. Red Haven) were harvested in July 1994 in Northern Greece (Verria) and stored at constant temperatures of 0, 5, 10, 15 and 20 °C up to 35 days. At regular intervals samples were taken and the activity of PG was assessed (at 25 °C) by a chemical method (*7,8*) measuring the formation of reducing groups from a poly-galacturonic acid substrate. The experiments were conducted on three different batches. From each batch duplicate samples were used. So, in total each point represents a mean value for 6 observations. Only very few

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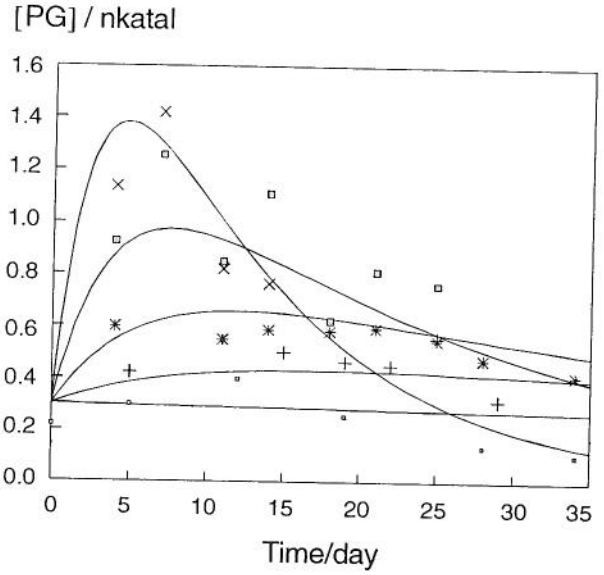

Fig. 1. PG activity during storage at different temperatures: ▪ 0, + 5, ✱ 10, ■ 15, × 20 °C. Symbols are mean value of 6 replicate measurements, solid lines are simulated behaviour.

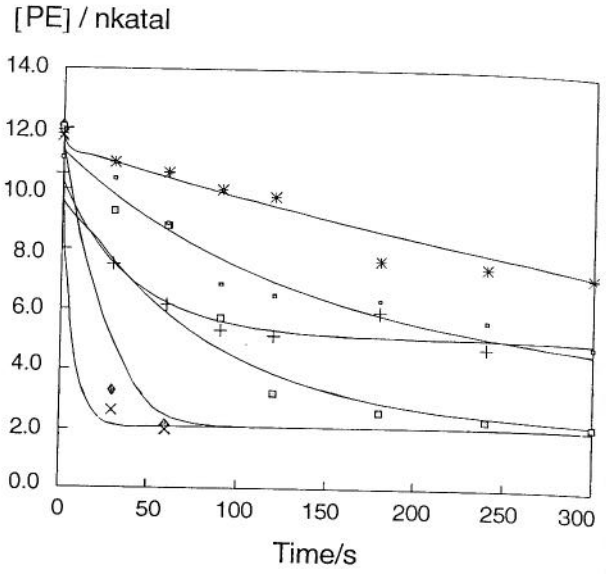

Fig. 2. PE activity during blanching at different temperatures: ▪ 45, + 50, ✱ 60, ■ 65, × 70, ◆ 75 °C. Symbols are mean value of 6 replicate measurements, solid lines are simulated behaviour.

values (less than 10% of the total number of measurements) were outliers and excluded from the calculation of the mean values.

The raw measured data on PG activity are shown in Fig. 1. The data clearly exhibit an increase in activity during the first two weeks of storage. The magnitude of the maximum reached depends on the storage temperature. The time at which this maximum activity is reached, also depends on temperature. This would indicate that the production of a new enzyme and deactivation of the already present enzyme are working against one another. The observed phenomenon may be traced to the well-known turnover normally present in living tissue for any enzyme present.

Blanching experiments

Peaches (cv. Andross) were harvested in August 1994 in Northern Greece (Verria) and processed within 24 h after harvesting. Samples of product were blanched at 45, 50, 60, 65, 70 and 75 °C. At regular times during blanching up to 300 s, samples were taken during the blanching process and PE activity was chemically assessed (at 25 °C) by titration of the carboxylic groups generated from the same fruit pectin. The experiments were conducted on three different batches. From each batch duplicate samples were used. So, in total each point represents a mean value for 6 observations. Only very few values (less than 10% of the total number of measurements) were outliers and excluded from the calculation of the mean values.

The raw data on PE activity during blanching treatments are shown in Fig. 2. The data clearly show a decrease in activity in time, due to denaturation, at the different constant temperatures. At higher temperatures the rate at which the activity decreases is greater than at lower temperatures. Below 50 °C there is a normal increase in the rate of enzyme denaturation. Also, above 65 °C a similar increase in rate exists. The striking anomaly in the data is, however, to be found in the effect of the blanching temperatures ranging from 50 to 65 °C. In that temperature region the rate of denaturation decreases with increasing temperature and simultaneously the range over which the activity decreases increases by lowering of the final level. As this phenomenon is measured again in a next season (data not shown), a measuring error can be excluded. Some physical or chemical process has to be responsible for this phenomenon, as a decrease in rate constant with increasing temperature is against all rules and laws of fundamental kinetics and physical chemistry.

Modelling

The models presented were developed using a system of problem decomposition (*9*). This system is oriented towards occurring and underlying processes that explain the occurring phenomena rather than towards the phenomena themselves. The models are based on kinetic mechanisms, assumed for that particular process, and were developed further by using the well-known rules of chemical kinetics (*2,3,10*). The mathematical development (formulation, testing and presentation) was carried out using MAPLEV, a computer algebra package, capable of handling algebraic equations. Simulations with the dynamic system were carried out by PROSIM, a modern modelling language, combining the benefits of discrete and continuous modelling.

It is generally accepted (*3,4,10,11*) that irreversible denaturation of enzymes behaves according to a first order reaction mechanism. Reversible denaturation has been indicated (*12*) to behave according to an equilibrium reaction. As a result of the different dependence on temperature of both opposing reactions, the normally observed enzyme activity, that is an increase in activity at low and medium temperatures followed by a more or less steep decrease in activity at still higher temperatures, can be explained (Tijskens, unpublished).

Based on this information, and the fact that enzyme activities do not recover after blanching, pasteurisation or sterilisation, a mechanism of irreversible denaturation was chosen as a start. In equation 1 the general reaction mechanism for irreversible denaturation is shown. This mechanism results in an exponential decrease in enzyme activity.

$$\mathrm{Enz} \xrightarrow{k_d} \mathrm{Enz_{na}},$$

$$[\mathrm{Enz}] = [\mathrm{Enz}]_0 \, e^{-k_d t} \qquad /1/$$

Specific rates of reactions depend on temperature, most probably according to Arrhenius' law (see equation 2).

$$k = k_{ref} \, e^{\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)} \qquad /2/$$

Storage process

The increase in PG activity during storage can be explained with a mechanism where an increasing enzyme activity is counteracted by a decreasing activity (by substrate accessibility or enzyme turnover?), with both reactions depending differently on temperature. The analogy with a consecutive reaction where one form of PG is generated from another enzymatically active precursor and a simultaneous decrease in activity by denaturation is immanent. The behaviour of PG activity can be depicted by the reaction scheme (equation 3).

$$\mathrm{PG_{pre}} \xrightarrow{k_f} \mathrm{PG},$$

$$\mathrm{PG} \xrightarrow{k_d} \mathrm{PG_{na}} \qquad /3/$$

This chemical set of reactions (equation 3) can be converted by fundamental kinetics to a set of differential equations and solved analytically for constant temperatures (equation 4). Like any chemical reaction, both reaction rate constants k_f, and k_d are assumed to depend on temperature according to Arrhenius' law (see equation 2).

$$[\mathrm{PG}] = [\mathrm{PG}]_{pre,0} \, k_f \left(\frac{e^{-k_d t} - e^{-k_f t}}{k_f - k_d}\right) + [\mathrm{PG}]_0 \, e^{-k_d t} \qquad /4/$$

Blanching process

The striking anomaly in the temperature dependence of PE activity during blanching experiments can be explained with a mechanism where an increasing enzyme activity is counteracted by a decreasing activity if both reactions depend differently on temperature. The activity of PE during blanching is clearly a combination of at least two isoenzymes both with their own specific rate of denaturation. The behaviour of both enzymes can be depicted by the reaction scheme (equation 5):

$$\mathrm{PE_1} \xrightarrow{k_c} \mathrm{PE_2},$$

$$\mathrm{PE_1} \xrightarrow{k_{d,1}} \mathrm{PE_{na}},$$

$$\mathrm{PE_2} \xrightarrow{k_{d,2}} \mathrm{PE_{na}} \qquad /5/$$

This chemical set of reactions (equations 5) can again be converted by fundamental kinetics to a set of differential equations and solved analytically for constant temperatures (equation 6). Like any chemical reaction, all reaction rate constants k_c, $k_{d,1}$ and $k_{d,2}$ depend of course on temperature according to Arrhenius' law (see equation 2). As the total activity of both isoenzymes is measured, the activities modelled for both isoenzymes have to be added. For the deduction of equation 6 a fixed invariable activity is already included, to account for the observed remaining activity, even after prolonged heat treatment. Apparently, there exists either an artefact, introduced by the measuring system, or a very heat-stable iso-enzyme is present in peaches.

$$[\mathrm{PE}]_{tot} = [\mathrm{PE}]_{1,0} \, e^{-(k_{d,1} + k_c) t} \left(\frac{k_{d,1} - k_{d,2}}{k_{d,1} + k_c - k_{d,2}}\right) +$$

$$+ e^{-k_{d,2} t} \left(\frac{[\mathrm{PE}]_{1,0} k_c + [\mathrm{PE}]_{2,0} (k_{d,1} + k_c - k_{d,2})}{k_{d,1} + k_c - k_{d,2}}\right) +$$

$$+ [\mathrm{PE}]_{fix} \qquad /6/$$

Statistical analysis

Statistical analyses were conducted using the iterative system for nonlinear regression analysis of the statistical package GENSTAT. No transformations were applied to the data to prevent errors during the estimation and the data were analysed as one integral set using time and temperature simultaneously as explaining variables.

The data on enzyme activities, collected at constant temperatures, were analysed statistically, by nonlinear regression, using equation 4 for PG and equation 6 for PE together with equation 2 (Arrhenius equation).

Table 1. Results of the analysis of PG activity in stored peaches, measured as amount of reducing groups

Parameter	Estimate	s.e.
$k_{f,ref}$/day^{-1}	.0173	.0100
$(E_{a,f}/R)$/K	13646	2614
$k_{d,ref}$/day^{-1}	.179	.107
$(E_{a,d}/R)$/K	5671	2386
$[PG]_0$/nkatal	.300	.106
$[PG]_{pre,0}$/nkatal	8.37	7.75
t_{ref}/°C	15	
R^2_{adj}	81.8	
n	31	

Storage process

In Table 1 the estimated parameters are shown for the model on PG activity. From the standard error of estimate it is clear that some parameters are more reliably estimated than others. Of the kinetic parameters of the formation reaction (index f), the energy of activation E_a is quite reliable, whereas the reaction rate constants at reference temperature and the parameters of the denaturation reaction (index d) are somewhat less reliable. The energy of activation ($E_{a,d}$) is altogether more or less zero, as it is just in the range of 2 times its standard error. In

results of later seasons it is confirmed that $E_{a,d}$ is more or less zero. For a real denaturation this is quite unusual, but here the denaturation can be interpreted as an inactivation due to senescence.

The estimating procedure puts almost all variability at initial level of the enzyme in the precursor form ($PG_{pre,0}$) which is completely virtual and only estimated, and not in the active already present form (PG_0) which is really present and measured. This implies that the pool of potential PG activity is larger than the pool initially present. In this way the model can explain the observed increase in activity dependent on the applied temperature as shown in Fig. 1.

The percentage variance accounted for by the model (R^2_{adj}) is, although not overwhelming, acceptably high. In the analysis of a later season, a R^2_{adj} of more than 90% was obtained (data not shown). The simulated behaviour of PG activity during storage at different temperatures is shown in Fig. 1 as solid lines. As can be seen, some series appear to be fitted rather badly (e.g. the series of 0 and 15 °C). The estimating procedure in multiple linear regression analysis, however, minimizes all deviance over time and temperature, that is all data point, together and simultaneously, but not separately over the individual temperature series.

To elucidate the three-dimensional behaviour, simulated data on PG activity are presented as a function of time and temperature in Fig. 3. Viewed along the time axis, the behaviour of PG activity is for each temperature of a bell-shaped form as in the raw data. Viewed along the temperature axis, not only the maximal level of enzyme activity as a function of temperature, but also the difference in apparent basic activity below and above 10 °C becomes clear. The latter effect is due to the temperature dependence of the conversion of the inactive iso-enzyme (PG_{pre}) into the active iso-enzyme (PG).

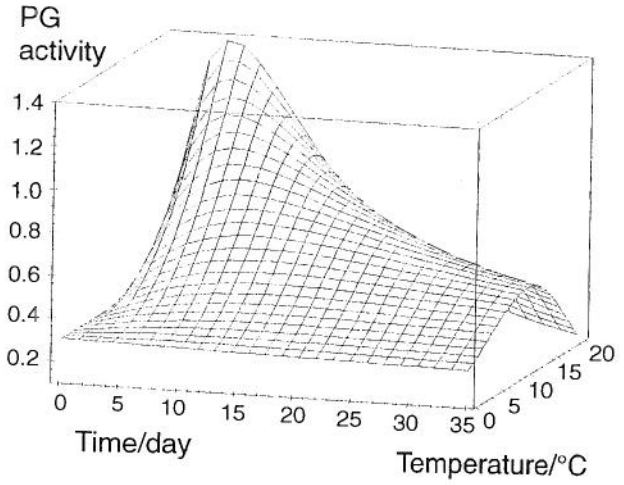

Fig. 3. Simulation results of PG activity as a 3D function of time and temperature, based on the estimated parameter values

Table 2. Results of the analysis of the thermal resistance of PE activity in peaches

Parameter	Estimate	s.e.
$k_{c,ref}/s^{-1}$	1.2	fixed
$(E_{a,c}/R)/K$	57000	fixed
$k_{d1,ref}/s^{-1}$	.1352	.0880
$(E_{a,d1}/R)/K$	20962	4400
$k_{d2,ref}/s^{-1}$	.001954	.000361
$(E_{a,d2,ref}/R)/K$	41833	4019
$[PE]_{fix}$/nkatal	2.071	.345
$[PE]_{1,45}$/nkatal	7.789	.856
$[PE]_{1,50}$/nkatal	6.81	1.04
$[PE]_{1,60}$/nkatal	8.60	.859
$[PE]_{1,65}$/nkatal	6.20	1.02
$[PE]_{1,70}$/nkatal	8.21	1.07
$[PE]_{1.75}$/nkatal	8.63	1.07
$[PE]_{2,0}$/nkatal	1.401	.900
t_{ref}/°C	60	
R^2_{adj}	95.5	
n	38	

Blanching process

In Table 2 the results of the analysis of PE activity during blanching treatments are shown. The kinetic parameters on the conversion reaction (index c) had to be fixed to plausible values, based on preliminary analyses, in order to make the complex estimation procedure successful.

A second peculiarity had to be taken into account in the statistical analysis. It had been noticed during the experimental determination of the PE activity of this and other products (carrots, potatoes), that the measuring method applied is susceptible to daily variations. The reason for this is not clear at the moment. A complete time series for each temperature was therefore measured in a single sequence. Each temperature series had consequently different initial levels of activity. This variation was completely attributed to the initial level of the first active form of PE for each of the temperature series ($PE_{1,T}$). The initial value of the second active form $PE_{2,0}$ was estimated in common for all the series. The standard errors for all the parameters are quite acceptable,

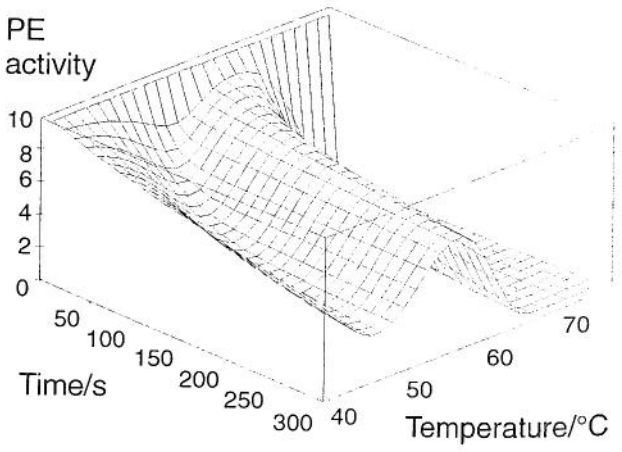

Fig. 4. Simulation results of PE activity as a 3D function of time and temperature, based on the estimated parameter values

and the R^2_{adj} is very high: 95.5%. The simulated behaviour of PE activity during blanching at different temperatures is shown in Fig. 2 (solid lines).

To elucidate the three-dimensional behaviour, including the anomaly observed between 50 and 65 °C, simulated data on PE activity are presented as a function of time and temperature in Fig. 4. Viewed along the time axis, the behaviour of PE activity is for each temperature of an exponential form. Viewed along the temperature axis, the abnormal behaviour of the enzyme becomes clear. The effect is due to the difference in denaturation susceptibility of the two forms of active enzyme, coupled with the conversion between the enzyme forms. Due to the very high energy of activation, this conversion reaction is almost non-existent at temperatures below 50 °C, and almost complete above 65 °C.

Discussion and Consequences

The direct consequences of these models for practical applications depend strongly on the situation. One has, however, to bear in mind that the solutions and the equation shown refer only to conditions with the same and constant temperature throughout the entire time of storage. These equations can only be applied for statistical analysis and simulation during conditions. Dynamic behaviour, with temperatures changing in time, are described by the model formulation in differential equations. These differential equations have been implemented in the modelling language PROSIM. It would be, however, too far outside the scope of this paper to explain the dynamics of this system.

With regard to the PG enzyme, it is interesting to see that a process like the normally encountered turnover of enzyme (symbolised by the conversion reaction index f) can exert such a strong effect on the amount of active enzyme present in living and respiring product. The somewhat abnormal behaviour of PG activity will exert a peculiar effect on the behaviour of the firmness of peaches during storage.

One interesting consequence of the analyses shown is that PE apparently occurs in peaches in two iso-enzymes. As a consequence of the conversion reaction (index c) between the two active forms of the enzyme, one may conclude that those two forms are isometric forms of the same enzyme, possibly different only in their stereoconfiguration. This type of information is commonly not available from data and interpretation in literature.

Acknowledgment

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Legend to the symbols

Indices	Description
c	conversion
d	denaturation
f	formation, conversion reaction
fix	the activity that remains even after intensive denaturation
na	inactivated, not active
pre	precursor
ref	reference (temperature)
tot	total (both iso-enzymes combined)
0	initial concentration
1,2	active enzyme isomers

Kinetika enzimske aktivnosti u breskvama tijekom skladištenja i proizvodnje

Sažetak

Djelovanje i aktivnost enzima poligalakturonaze (PG, endo-EC. 3.2.1.15 i egzo-EC. 3.2.1.67) i pektin-metil-esteraze (PE, EC. 3.1.1.11) u breskvama mjereni su za vrijeme skladištenja i proizvodnje u skladu s osnovnim zakonitostima kinetike. Aktivnost oba enzima ovisila je o starosti bresaka (PG) ili denaturaciji (PE), a suprotno nastajanju iz prekurzora ili vezanoga i inaktiviranoga oblika enzima. Djelovanje PE očito se zasnivalo na dvama izoenzimima kod kojih dolazi do pretvorbe između dviju konfiguracija na određenoj temperaturi.