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Simultaneous Modelling of the Thermal Degradation Kinetics of Pectin Methylesterase in Lettuce (*Lactuca sativa* L.) and Carrot (*Daucus carota* L.) Extracts: Analysis of Seasonal Variation and Tissue Type

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The thermal degradation kinetics of pectin methylesterase (PME) from carrot and lettuce were studied. Fresh extracts were exposed to temperatures from 55 to 70 °C until the enzyme was inactivated. A model based on the presence of two forms of the enzyme, one active and one non-active, is proposed. The natural variability of the PME activity was taken into the model in the form of normally distributed random effects. The common model parameters obtained (cleavage constant ($0.0395 \pm 0.0062\,s^{-1}$), degradation constant ($0.556 \pm 0.112\,s^{-1}$), cleavage energy of activation ($469 \pm 23\,kJ\,mol^{-1}$) and degradation energy of activation ($488 \pm 18\,kJ\,mol^{-1}$)) show that the PME degradation kinetics of the two vegetables can be explained with a single set of parameters.

Key words: lettuce (*Lactuca sativa* L.); carrot (*Daucus carota* L.); pectin methylesterase; mixed-effects modelling; random effect

The textural changes in fruits and vegetables are related to certain enzymatic and non-enzymatic processes. The enzymatic degradation of pectins is catalysed by pectin methylesterase (PME) and polygalacturonase (PG).[1)] Pectin is first partially demethylated by PME, and later depolymerised by PG to polygalacturonic acid, causing a loss of firmness.[2)] However, the controlled activation of PME results interesting to maintain textural properties, as the partial demethylation of pectins increases the cross-linking with cations.[3)] This effect is favoured in the case of carrots, as endogenous PG activity is almost non-existent.[4)] The stimulation of PME activity at temperatures between 50 °C and blanching conditions has been correlated with textural maintenance.[1,5–8)] Low-temperature blanching (LTB), alone or combined with other agents, has also been used to prevent loss of quality in fresh-cut vegetables, especially in leafy vegetables (*e.g.*, fresh-cut lettuce). The time of exposure in these cases is very limited.[7–11)] Although there are studies on carrot PME and the effect of LTB,[6,12)] no information on lettuce PME has been reported.

The thermal kinetics of PME inactivation have been extensively studied, and there are several studies that propose different models that are being employed. In the area of juice processing, thermal inactivation experiments on PME have generally yielded an apparent first-order kinetic mechanism working with both the juice and the purified enzyme.[13–17)] However, Collet *et al.*[18)] (orange juice) and Anthon and Barrett[19)] (carrot and potatoes) have proposed two forms of enzyme with different thermal inactivation parameters.

When working with purified PME, Nguyen *et al.*[20,21)] and Castro *et al.*[22)] have proposed a similar two fraction biexponential model for the mild thermal and high-pressure inactivation kinetics of purified strawberry, carrot and green pepper pectin-esterase. Similar parameters between the models were found in all studies, leading to the hypothesis that the enzymes of different species might respond similarly to heat and pressure stresses. The inactivation of commercial PME by a pulsed electric field treatment has been studied by Giner *et al.*[23)] who proposed the Weibull model as the most appropriate (over first-order kinetics) to describe the kinetics of the process by using a Bayesian model selection procedure.

Finally, Tijskens *et al.*[24)] have found that the thermal inactivation kinetics of PME activity in carrot and potatoes samples showed the characteristic biphasic shape of enzyme degradation kinetics and proposed a

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Abbreviations: PME, pectin methylesterase; pro_PME, non-active pro-pectin methylesterase form; LTB, low temperature blanching; ODE, ordinary differential equations; T, temperature of the extract; T_b, bath temperature; T_r, reference temperature; t, time; R, universal gas constant; $k_{cle\text{-}ref}$, reference cleavage constant; $k_{deg\text{-}ref}$, reference degradation constant; k_{eff}, effective thermal conductivity; $E_{a\text{-}cle}$, cleavage energy of activation; $E_{a\text{-}deg}$, degradation energy of activation; AIC, Akaike information criterion; BLASTX, Basic Local Alignment Tool

two-step reaction mechanism, with an active bound enzyme that was released into an active soluble enzyme, and used a two-step nonlinear regression procedure to estimate the parameters and overcome the problems with convergence created by experimental variability. A similar model has been presented by the same authors in an investigation on the blanching of peach pieces, and the seasonal variability of the PME activity showed comparable parameters between seasons.[25)]

PME activity has recently been suggested as an indicator of thermal processing in fruit and vegetables on the basis of its apparent first-order inactivation kinetics and the fact that it is present in most of those products.[26)] From the foregoing evidence presented, there is still a need to obtain more information on the thermal inactivation of this enzyme and the fundamental governing mechanism and to particularly study similarities in the thermal inactivation kinetics of the enzyme between different vegetables.

PMEs are extracellular isoenzymes in higher plants that are produced in an inactivated form by the plant cell and then excreted to the cell wall where their perform demethylesterification of the pectins. Micheli,[27)] based on the work of Bordenave[28)] and Shinde,[29)] has reviewed the classification of PME enzymes of different origin and proposed a model mechanism for PME isoform excretion that is generally agreed to happen in higher plants. With this mechanism the PME gene encodes not only the protein sequence, but also the pre- and pro-regions. The pre- region is a signal peptide that is generally cleaved early in the intracellular space. Pro-PME is then secreted to the apoplasm *via* the Golgi cisternae, and only active mature PME is found in the cell wall. It is speculated that the pro-PME sequence is not active or has very reduced activity and that the enzyme is only activated with cleavage of the pro-sequence by extracellular proteases. Under this mechanism, vegetable extracts produced by tissue lysis and assayed for PME activity possibly contain the two forms of PME (pro-PME and mature PME).

Many plants acclimatise in response to changes in temperature. This adaptation process produces physiological and biochemical changes in the plants.[30,31)] The predictable variations in initial concentrations of PME between two vegetable, season and tissue type, as well as the natural product variability, may influence the behaviour of this enzymatic degradation and affect the post-harvest treatment efficiency. Tijskens *et al.*[25)] have studied the seasonal variation of the enzyme and its variation between batches, and have already proposed that the thermal degradation of PME in peaches was governed by a model in which the rate parameters were constant between seasons and only the initial enzyme species concentration in each of the batches varied. An assessment of the different components of the variability affecting a fresh vegetable product can help to define if the effect that different measures (*e.g.*, heat-shock treatment) will have on the quality of the final product is going to be important when compared with the natural variability of the product. This is of particular importance when translating technological advances from the laboratory (a low variability scenario) to the retail sector (a high variability scenario).

Statistical models to estimate components of variance in non-linear experiments, namely in the form of non-linear mixed-effect modelling, are tools that have recently started to become available to the life scientist working bench.[32,33)] This modelling technique has recently started to be used in the area of food phenomena kinetic modelling.[23,34–37)] A non-linear mixed effect model presents two components, a fixed effects part with a model that describes the deterministic knowledge of how the average population behaves in respect to the responses studied and a random-effect part which represents the stochastic component of the model and accounts for unexplained inter- and intra- individual variability in the form of statistical distributions.

The objective here was to study the thermal kinetics of PME from carrot and lettuce and to propose a common thermal inactivation kinetic model based on physiological assumptions and the high similarity of the two enzymes. The use of mixed-effect modelling to describe the thermal degradation of the enzyme from different tissues, vegetables and seasons, together with an estimation of the batch-to-batch variability inherent to a natural product was also part of the objective of this work.

Materials and Methods

Experimental design. The experiments were conducted from March to September 2004. Iceberg lettuce (*Lactuca sativa* L.) and carrots (*Daucus carota* L.) were used in the assays as models of leafy and root vegetables. A study of the distribution of PME in the tissues was conducted. PME isolated from photosynthetic and vascular tissues was measured in lettuce and in the cortex (phloem) and core (xylem) of carrot.

Raw material and sample preparation. Iceberg lettuce (*Lactuca sativa* L.) and carrots (*Daucus carota* L.) were obtained from a local supermarket, brought to the laboratory within 12 hours after the harvest and stored at 4 °C before processing. The outer leaves (damaged or wilted) from the lettuce heads were discarded, and the core was excised with a stainless steel knife. The rest of the leaves were separated and used. The carrots were peeled with a stainless steel peeler and cut into discs (~5 mm thickness) for easy tissue identification. Distilled water at room temperature (18–20 °C) was used to wash the fresh-cut vegetables by immersion with constant agitation for one minute. After this, the excess water was removed with a salad spinner (Bonjour 3500-70, France) for 5 min. Finally, segregation of the different tissues was carried before extract preparation and thermal treatment. With the lettuce, samples from the

Table 1. Experimental Thermal Treatment Conditions
Heating temperature (°C) and time (min) for the lettuce and carrot extracts.

Temperature (°C)	Thermal treatment time (min)
55 °C	0–33
60 °C	0–7
65 °C	0–3
70 °C	0–2

green (photosynthetic) and white midrib (vascular) parts of the leaves were selected. With the carrot, separated samples of the core (xylem) and cortex (phloem) were obtained.

Each extract was prepared by homogenising (5500 rpm) 10 g of tissue in 20 ml of an extraction solution (a 0.2 M sodium phosphate buffer at pH 7.5 containing 1 M sodium chloride and 10 mM dithiothreitol) for 2 min. The macerate was incubated for 30 min with agitation and centrifuged at 12,500 *g* for 30 min. The supernatant was collected as the final extract. The extracts were kept at 4 °C during all the processing procedure.

For the thermal treatment, 1 ml aliquots of the PME extracts were transferred to plastic Eppendorf tubes (polypropylene). The samples were heated in a circulating oil bath (Lauda E-300, Könisghofen, Germany) at 55, 60, 65 and 70 °C. Table 1 shows the experimental thermal inactivation time ranges for each temperature at which the remaining PME activity was 5% less of the initial value. After a preliminary screening, sampling points were selected for the experiments, aiming to have an equal number of these in both the activation and inactivation phases (Fig. 1). Each sampling point was replicated three times. All the trials at each temperature were replicated at least four times to complete a set of 1831 observations, whereby 16 batches of carrot core and peel and 17 batches of lettuce leaf and rib were studied.

Pectin methyl esterase (PME) activity measurement (E.C.3.1.1.11.). The PME activity was measured by using the method described by Kimball.[38] After the thermal treatment, the PME activity of each extract was measured. A 1-ml amount of the extract was mixed with 40 ml of a substrate solution (0.1% pectin). The solution was adjusted to pH 7.0 with 1.0 M NaOH, and the pH of the solution was re-adjusted to pH 7.5 with 0.05 M NaOH. After the pH had reached 7.5, 0.2 ml of 0.05 N NaOH was added. The time required to return to pH 7.5 was recorded. Activity was quantified by the carboxyl groups formed by the hydrolysis of methyl esters of pectin and was tritrimetrically measured with a pH electrode to monitor the production of H^+. All the enzymatic assays were carried out at 20 °C. PME activity was calculated by using the equation described below.[38] The enzymatic activity unit (EAU) is expressed as μmol COO^- g^{-1} min^{-1}

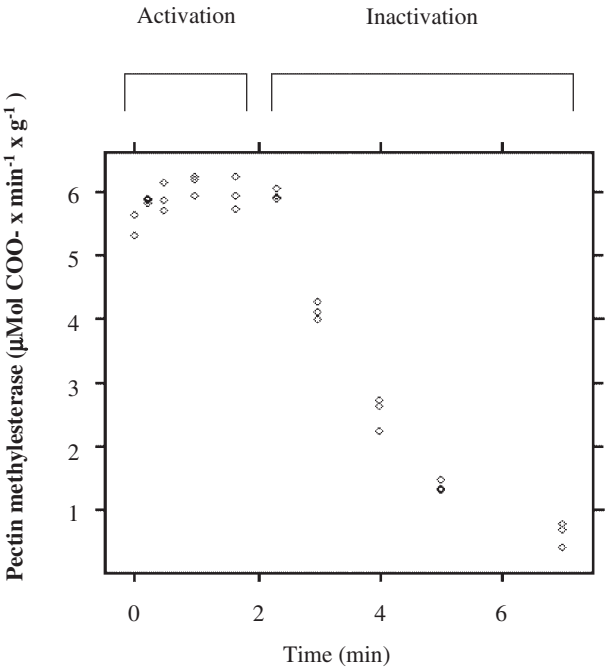

Fig. 1. Pectin Methylesterase Activity (EAU: μMol COO^- x min^{-1} x g^{-1}) *vs.* Time (min) in Extracts from Lettuce (◇) Heated at 60 °C, Showing an Activation Phase Prior to Inactivation.

$$PME = \frac{0.2[\mathrm{ml}]NaOH \cdot 0.05[\mathrm{mol \cdot l^{-1}}]NaOH \cdot X[\mathrm{ml}] \cdot 10^6[\mu\mathrm{mol \cdot mol^{-1}}] \cdot 10^3[\mathrm{l \cdot ml^{-1}}]}{Y[\mathrm{ml}] \cdot Z[\mathrm{g}] \cdot time[\mathrm{min}]}$$

where X is the total volume of extract obtained, Y (1 ml) is the volume assayed and Z (10 g) is the total weight of the sample.

Thermal conductivity of the vegetable extracts and vials. Due to the temperature at which the enzymatic extracts were kept (3 °C) before to the thermal degradation experiments, there was a significant warm-up time until an extract reached the bath temperature (55–70 °C). The non-isothermal characteristic of the experiments was integrated into the model and fitted to the data in the form of Newton's law of heating and cooling:

$$\frac{dT}{dt} = -r \cdot (T - T_b)$$

where the rate of heating is proportional to the difference between the temperature of the vial (T) and the temperature of the bath (T_r). The proportionality constant, r, was estimated to $0.020551 \pm 0.000204\ s^{-1}$. In order to estimate this, a set of eight experiments

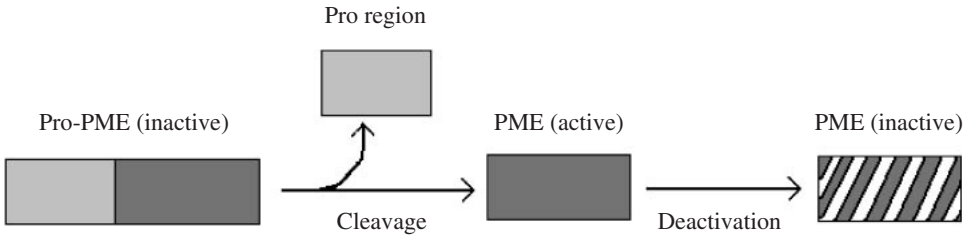

Fig. 2. Mechanistic Model Used to Obtain the Ordinary Differential Equation System (ODE) for Mathematical Modelling.

measuring the temperature warm-up of a typical vegetable extract inside the Eppendorf tube with a thermocouple was performed.

Model building for pectin methyl esterase thermal degradation. The construction of the model was based on the following assumptions:

i) Two PME forms were present in the vegetable extract before thermal deactivation, pro-PME and PME.[23,24,27)]

ii) Only the PME species presented enzymatic activity, and not the non-active pro-PME form.

iii) The thermal degradation of the PME/pro-PME mixture followed a two-irreversible-step mechanism: pro-PME was cleaved (by proteases present in the extract or by the effect of the thermal process) and produced mature PME which was then subsequently degraded to an inactive form. The amount of pro-PME directly degraded to an inactive form was considered negligible (Fig. 2).

iv) The carrot and lettuce PME degradation parameters were assumed to be of similar nature, although presenting a different initial PME/pro-PME mixture. Therefore, the model-building process was aimed at a general model that would explain both the degradation kinetics (lettuce and carrot tissue PME) by a single set of parameters.

v) Initial pro-PME and PME concentration variability in the tissues was assumed to follow a normal distribution. If differences in initial PME activities were found between vegetables, tissues or season, these could be built in the fixed part of the model.

vi) Finally, the thermal treatment of the viscous vegetable extracts was a non-isothermal process due to the limited thermal diffusivity of each extract and the vial container.

Following these assumptions, the kinetics of PME activity degradation with temperature were modelled by using a group of ordinary differential equations (ODE) based on the mechanistic model proposed (Fig. 2). The ODE system describes the transformation of pro-PME into active PME as

$$\frac{d\mathrm{PR}o\_PME}{dt} = -k_{cle\_ref} \cdot \mathrm{Pro\_}PME$$

the degradation of the active form as

$$\frac{dPME}{dt} = k_{cle\_ref} \cdot \mathrm{Pro\_}PME - k_{\mathrm{deg}\_ref} \cdot PME$$

and the equation, described previously, to integrate into the model the non-isothermal characteristic of the heating experiment as

$$\frac{dT}{dt} = -r \cdot (T - T_b)$$

The initial conditions for the ODE system are as follows:

$$t = 0;\ pro\_PME = \mathrm{Pro\_}PME_{0i}$$

$$t = 0;\ PME = PME_{0i}$$

$$t = 0;\ T = 3\,^{\circ}\mathrm{C}$$

where PME_{0i} stands for the initial concentration of PME at batch i.

The cleavage and degradation constants were assumed to follow an Arrhenius dependence on temperature:

$$k_{cle} = k_{cle\_i} \cdot e^{-\frac{Ea_{cle}}{R} * \left[\frac{1}{T} - \frac{1}{T_r}\right]}$$

$$k_{\mathrm{deg}} = k_{\mathrm{deg}\_i} \cdot e^{-\frac{Ea_{\mathrm{deg}}}{R} * \left[\frac{1}{T} - \frac{1}{T_r}\right]}$$

Similar models have been proposed for showing observations of PME activity in carrots.[24,25)] The model proposed in the present work takes into account the non-isothermal nature of blanching and considers that the pro-PME fraction is inactive.

To cope with the natural variability between batches of the initial enzyme concentration, random effects[32)] between extracts were included in the PME_{0i} and pro-PME_{0i} initial conditions for each batch (Fig. 3). The resulting non-linear mixed-effects model was composed of a) fixed effect parameters in the system of three ordinary differential equations as a deterministic model and to describe the differences in initial activities between tissues (carrot) and seasons (lettuce), and b) random effects expressed as a normally distributed batch-to-batch variability added to the initial activities of pro-PME and PME.[33)]

Similarity of carrot and lettuce PMEs obtained by amino-acid sequence alignment. A comparison of the two PME enzymes studied (lettuce and carrot) was

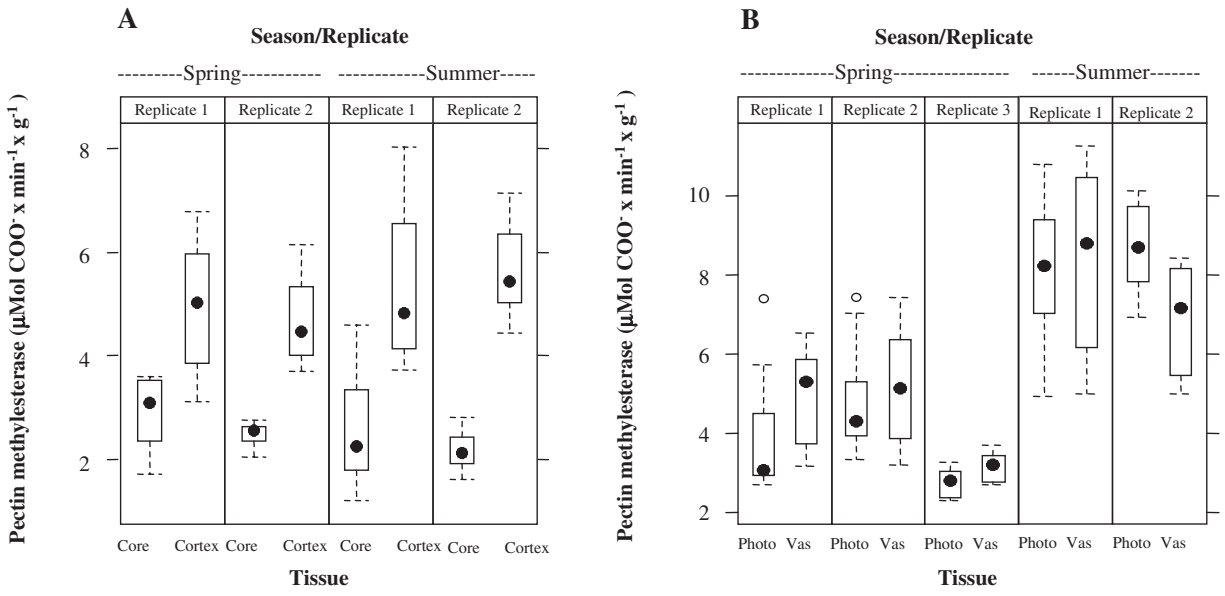

Fig. 3. Initial Pectin Methylesterase Activity (EAU: µMol COO^{-} x min^{-1} x g^{-1}) in Fresh Extracts of Carrot (A) and Lettuce (B). Effect of season (spring-summer) and tissue type (core and cortex for carrot and photosynthetic (Phot) and vascular (Vas) for lettuce). Median (●), inter-quartile range (box), extremes (⊤, ⊥) and out-layers (○) are presented.

performed by using bioinformatics. Carrot PME sequence P83218 (NCBI database: http://www.ncbi.nlm.nih.gov) was employed for comparison purposes. For the lettuce, a list of nucleotide short sequences was obtained from the Compositae Genome Project (http://compgenomics.ucdavis.edu/) labelled by its similarities to the pectin methylesterase sequences from other plants and microorganisms. All the sequences in this list (49 in total) were translated to amino-acid sequences and compared for similarity with the carrot amino-acid sequence by using a Basic Local Alignment Tool (BLASTX).[39)]

Statistical analysis. An analysis of the variance, at confidence intervals at $p \leq 0.05$, *post hoc* tests and additional regression analyses were carried out with R software.[40)] The model was simulated by using the odesolve R package,[41)] and parameter estimation was performed by using Nlme library.[42)]

Results and Discussion

Initial PME activity of fresh lettuce and carrots

Effect of seasonal variation and tissue type on the enzymatic activity. Before the thermal treatment (time = 0), the initial PME activity in the extracts was different between both vegetables, being significantly higher ($p \leq 0.05$) with lettuce than with carrots. The average PME value for lettuce was 4.650 ± 0.158 EAU and for carrot was 2.941 ± 0.109 EAU. These results are in agreement with previous work which has reported that the activity in leafy vegetables was higher than that in root vegetables.[6)] The high initial activity of the lettuce extracts indicates the potential interest of targeting this enzyme with a mild thermal treatment to affect the texture/crispness of the lettuce.

The distribution of PME in the tissues of carrot (core and cortex) and lettuce (vascular and photosynthetic) was evaluated (Fig. 3). The initial activity values of the enzyme, prior to the thermal treatment, showed significant differences ($p < 0.05$) between the tissues in carrots, while no differences were apparent for lettuce tissues. The PME activity values for raw carrot tissue were significantly higher ($p < 0.05$) in the cortex than in the core. Nieslen and Christensen[43)] have found levels of PME activity 2- to 5-fold higher in peel extracts than in flesh extracts (orange, lemon, lime, grapefruit and clementine). McMillian and Pérombelon[44)] have also measured higher PME activity in external cells of different species of tubers than in cells from the internal parts. However, in Iceberg lettuce, the distribution of PME activity in the tissues was not significantly different, there being no significant difference ($p < 0.05$) between the activities of the PME enzyme found in vascular tissue and in photosynthetic tissue.

High variability within replicates was found in the initial values of PME activity. This may have been due to unavoidable differences in the metabolic stage of the plant tissue, time of harvest, plant maturity, *etc.* (Fig. 3). De Assis *et al.*[45)] have studied PME activity in acerola fruits at different maturity stages: immature green, green, mature green/yellow, pale red and ripe mature. They found that the highest level of PME was in the green stage, this being followed by the mature green/yellow stage.

Carrots (Fig. 3A) did not show any differences between seasons. Lettuce (Fig. 3B) grown and harvested in spring (March-May) had significantly lower PME

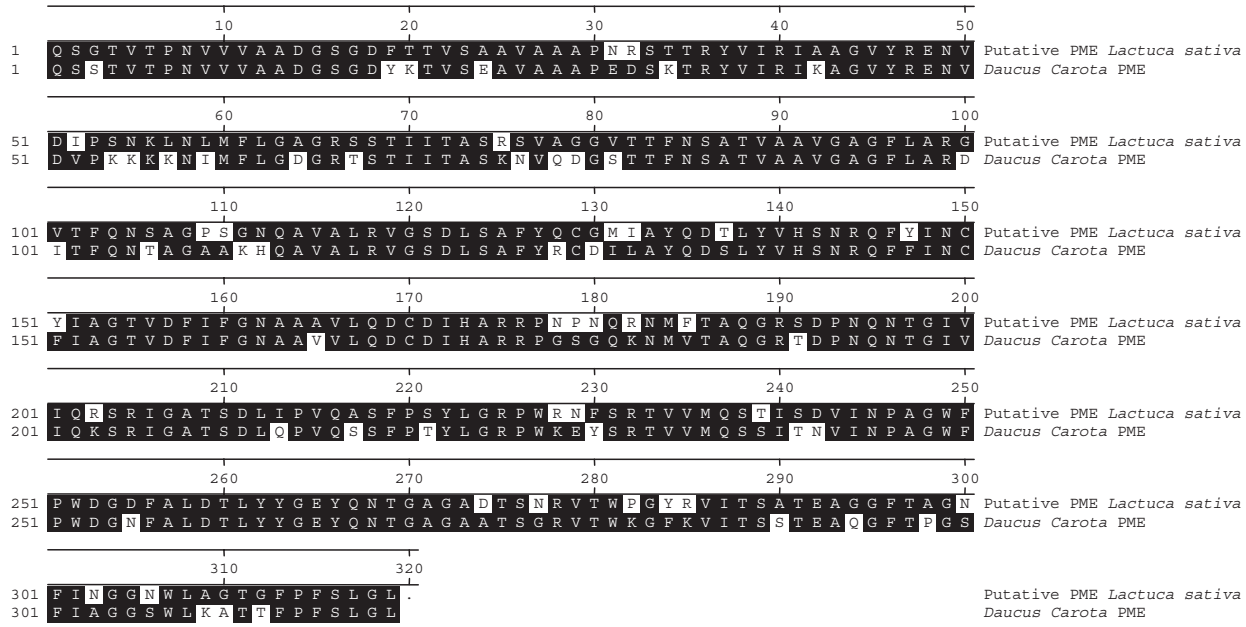

Fig. 4. Comparison of the Aminoacid Sequence from Carrot and Lettuce (putative) Pectin Methylesterases (PME).

values ($p < 0.05$) than lettuce harvested in summer (June-September). Perhaps the lower PME activity in spring in lettuce might be associated with a reduction in the metabolic rate as a response to the acclimatisation to lower temperatures and the capacity to avoid unfavourable changes in the environment.[30,31] A possible reason for the lack of seasonal variability in the carrot can be differences in production; the carrot is a seasonal product that undergoes storage during longer periods, unlike the lettuce which is produced all year round under very different cultivation temperatures.

Similarity of carrot and lettuce PME sequences

The nucleotide sequences of lettuce (*Iceberg lettuce sp.*) DNA obtained from the Compositae Genome Project were translated and compared (BLASTX) with the protein sequences for pectin methylesterase of carrot (*Daucus carota* L.). This comparison of lettuce and carrot PME amino acid sequence showed a similarity of 83% (Note: The lettuce sequence used was QG_CA_Contig1563). In Fig. 4, the amino acid composition of both sequences is compared and the differences between them highlighted.

Inactivation kinetics

As reported previously, the activity in the extracts did not immediately decrease with the temperature. There was an initial phase in which the activity increased to a value higher than the initial one, describing an activation phase (Fig. 2). This phenomenon could not be explained with the warm-up time of the inactivation experiment (3 min maximum).

The model was initially fitted with kinetic parameters particular to each vegetable data set as a preliminary step and was then converged with the data with kinetic parameters common to all the data sets in a single-model fit. Once the model had been fitted, a search with random initial estimates was initiated to ensure that the optimal point found for the system of ODEs was the best one. Figure 5 shows four examples of the experimental data and the model predictions, using the best linear unbiased predictors (BLUP). The residual plot for this final model showed a homogeneous variance close to normally distributed (Fig. 6A), and the quantile-quantile plot was close to linear (Fig. 6B).

Figure 7 shows the residual distribution of the data fit to the general model, for the four groups that showed significant ($p < 0.05$) differences in the initial PME activity, *i.e.*, the tissues in the case of the carrot and seasons in the lettuce. The residuals apparently follow a normal distribution in all cases. There is a slightly higher dispersion in the case of the lettuce residuals, but all the groups followed a normal distribution. Also, no dependence of the model was observed on the other variables studied of temperature, season and tissue (data not shown), indicating the model reliability to describe the effect of those variables on the PME activity.

The parameters obtained for the common model (lettuce and carrot) are shown in Table 2. All parameters were statistically significant ($p < 0.05$). The standard error associated with each of the parameters is improved in respect of the previous kinetic determination of the thermal degradation of pectin methyl esterase,[24,25] showing the suitability of the nonlinear mixed-effect model in a situation where variability is going to affect parameter estimation. Without this variability component extracted from the experimental error, the parameter standard error would be increased. There was no relevant correlation (>0.95) between any of the estimated parameters.

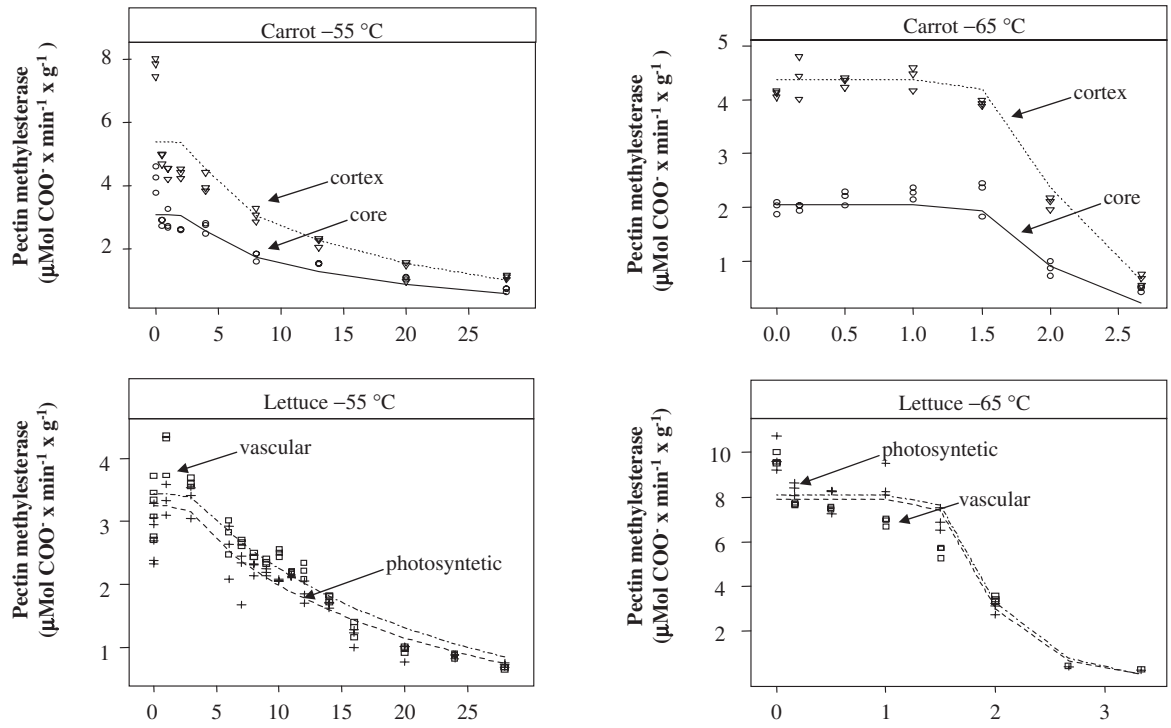

Fig. 5. Experimental Data and Best Linear Unbiased Prediction (BLUP) of Pectin Methylesterase Activity (EAU: µMol COO^{-} x min^{-1} x g^{-1}) *vs.* Time (min) in Fresh Extracts of Carrot Cortex (▽), Carrot Core (○), Lettuce Vascular (□) and Lettuce Photosynthetic (+) Tissues Heated at 55 °C and 65 °C.

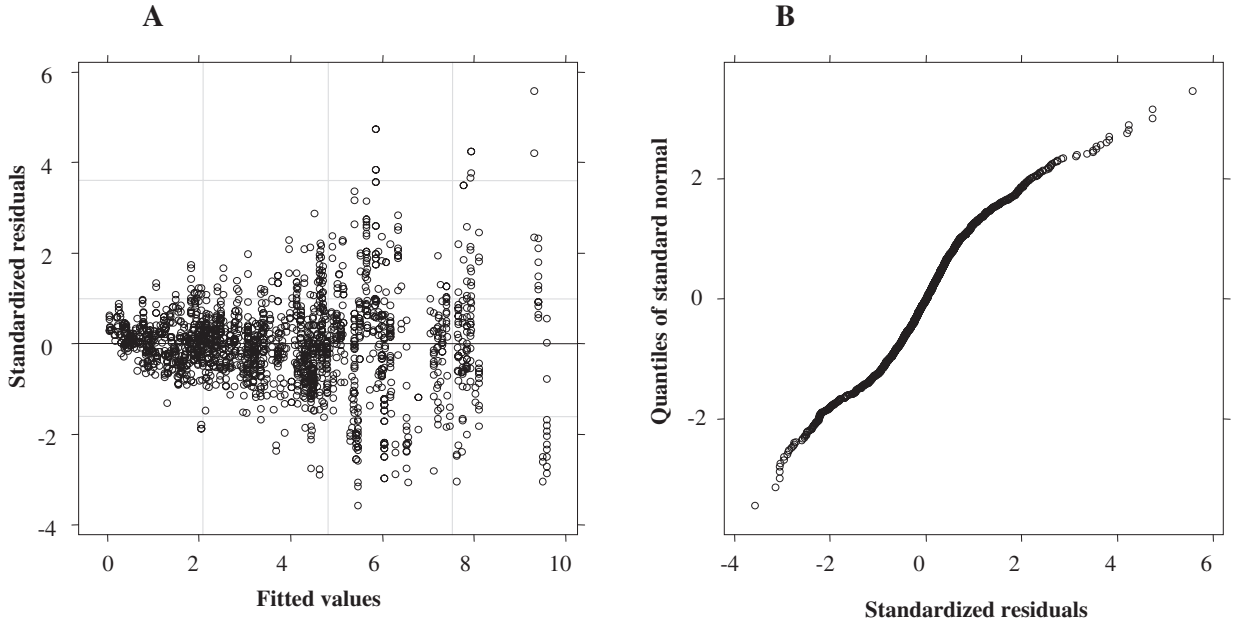

Fig. 6. Standarised Residual Figure (A) and Quantile-Quantile Figure (B) for the Mathematical Model.

Variability estimation

Figure 8 shows the distribution of the initial values of pro-PME and PME for each data set. An apparent lower variance in the initial PME activity in carrot was observed. Model fits taking into consideration different variance components for carrot and lettuce did not significantly improve the model (comparing AIC and log-likelihood differences under REML estimations). The variability between batches of the initial enzymatic activity (PME) and precursor (pro-PME), expressed as a standard deviation from a normal distribution with zero average, was estimated to be 0.975 EAU and 12.179 EAU, respectively, meaning that variations in the order of 30–40% in initial PME and between 50–

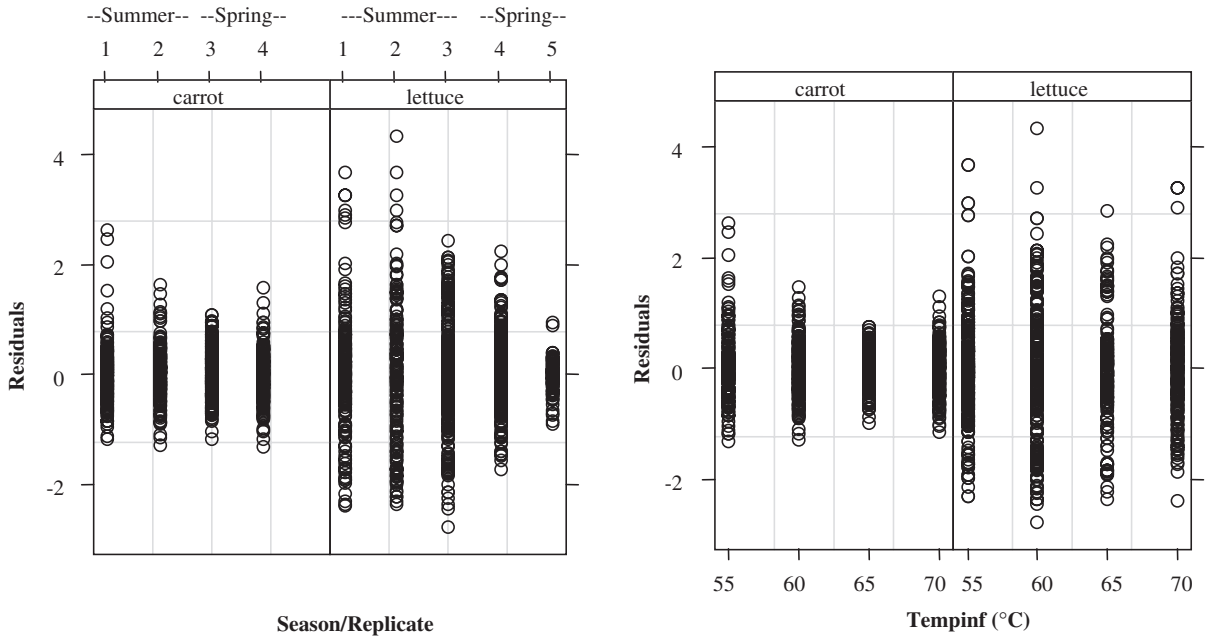

Fig. 7. Residual Distribution in Groups Presenting Significantly Different ($p < 0.05$) Initial PME Activities: Carrot Tissues and Lettuce Seasons.

Table 2. Model Estimated Parameters and Their Respective Standard Errors together with the Random Effect Distributions and Model Fitting Criteria

Model parameter	Value ± Std. error
$k_{cle\text{-}ref}$	$0.0395 \pm 0.0062\ s^{-1}$
$k_{deg\text{-}ref}$	$0.556 \pm 0.112\ s^{-1}$
$E_{a\text{-}cle}$	468.105 ± 23.124 Kj mol^{-1}
$E_{a\text{-}deg}$	489.363 ± 18.383 kJ mol^{-1}
T_r	335.5 K
PME_0(Carrot/Phloem)	2.667 ± 0.251 (μmol COO^- x min^{-1} x g^{-1})
PME_0(Carrot/Cortex)	2.325 ± 0.073 (μmol COO^- x min^{-1} x g^{-1})
PME_0(Lettuce/Photosynt.)	3.263 ± 0.414 (μmol COO^- x min^{-1} x g^{-1})
PME_0(Lettuce/Vascular)	3.626 ± 0.430 (μmol COO^- x min^{-1} x g^{-1})
Pro-PME_0(Carrot/Phloem)	19.845 ± 4.964 (μmol COO^- x min^{-1} x g^{-1})
Pro-PME_0(Carrot/Cortex)	18.492 ± 3.566 (μmol COO^- x min^{-1} x g^{-1})
Pro-PME_0(Lettuce/Photosynt.)	20.194 ± 6.345 (μmol COO^- x min^{-1} x g^{-1})
Pro-PME_0(Lettuce/Vascular)	23.544 ± 7.500 (μmol COO^- x min^{-1} x g^{-1})
Between batches variation PME_0 (σ)	1.0 CI [0.8 1.3] (μmol COO^- x min^{-1} x g^{-1})
Between batches variation Pro-PME_0 (σ)	12 CI [8 17] (μmol COO^- x min^{-1} x g^{-1})
Residual (σ)	0.781 (μmol COO^- x min^{-1} x g^{-1})
AIC	4531.128
BIC	4613.817
logLik	−2250.564

Note: All fixed and random parameters significant ($p < 0.05$).

65% in pro-PME values are to be expected in a normal retail situation. The residuals from the model had a standard deviation of 0.781 EAU. As it can be seen, the variability of the precursor between batches seems to be the most important source of variability in the system, having most of the influence on the shape of the kinetics in the process (Fig. 5).

Although the model showed good-fit results with the data, it did not manage to follow the increase of activity described during the initial part of the heating time. The model parameters obtained with the best-fit results create a flat stage before the activity decrease. There is a certain limit to the thermal treatment time until an extract maintains the initial activity level, after which the activity starts to decrease. This time could be useful as a reference for treatment design to maintain the PME activity at a maximum level for textural enhancement, after adapting the values to the actual vegetable (the extract in this case). Table 3 shows the time and temperature at which an extract could be heated before starting to loose PME activity.

In summary, the thermal degradation of carrot and lettuce PME was well described by the proposed model, and supporting arguments found in previous works and

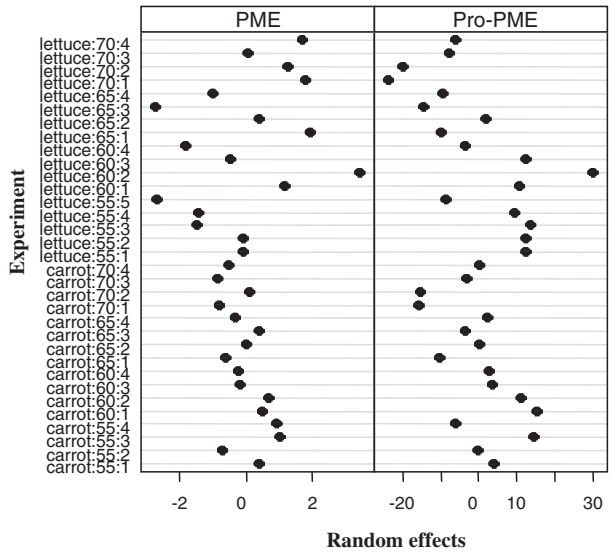

Fig. 8. Distribution of Initial Active (PME) and Non-Active (Pro-PME) Pectin Methylesterase Forms (EAU: μMol COO^- x min^{-1} x g^{-1}), Predicted with the Lettuce and Carrot Mathematical Model in the Different Experimental Data Sets.

Table 3. Temperature of Treatment and Model Predicted Time at Which the Extracts Maintain PME Activity at the Same Level as Prior to Heating

Temperature (°C)	Vegetable	Time (min)
55	Lettuce/Carrot	2.6
60	Lettuce/Carrot	1.7
65	Lettuce/Carrot	1.2
70	Lettuce/Carrot	1.1

the use of bioinformatics tools to assess underlying the physiological mechanisms were presented. The initial variability between tissues and season was accommodated and assessed. The validity of a common mathematical model for lettuce and carrot was supported by the high similarity found in the sequence data obtained from external sources. The use of the model presents a reliable tool for predicting the PME activity with low-temperature blanching in lettuce and carrot and for assessing the expected variability that will arise when processing an agricultural product.

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