Thermal inactivation kinetics of L-carnitine
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ABSTRACT
The objectives of this work were to study the thermal inactivation kinetics of L-carnitine and to develop a procedure for thermal inactivation kinetics determination, from dynamic temperature profile experiments. Experiments were contacted at isothermal conditions in a temperature range of 80 to 130°C. Remaining L-carnitine concentration was measured at predescribed time intervals at each temperature tested. The kinetic parameters \(D_{ref}\) and \(z\) were determined after the first order kinetic behavior of L-carnitine thermal inactivation was verified. Furthermore, L-carnitine concentration data were collected during a non-isothermal experiment and the \(D_{ref}\) and \(z\) values were determined through appropriate methodology. From the isothermal experiments, the parameters \(D_{120°C}\) and \(z\) were calculated equal to 50.6 min and 30.2°C, respectively. Similar values were obtained from the measurements during the dynamic temperature profile experiment. Based on the agreement between the parameters estimated using isothermal or non-isothermal temperature profiles, and given the reduced number of experimental data required by the latter approach, kinetic parameter estimation from experiments at dynamic conditions is recommended.

INTRODUCTION
L-carnitine (\(β\)-hydroxy-\(γ\)-trimethylaminobutyrate) is a quaternary ammonium compound biosynthesized in living cells from the amino acids lysine and methionine. Its primary function is to facilitate the transport of activated long chain fatty acids from the cytosol into the mitochondria and, thus, it is essential for the production of energy from lipids. In addition, L-carnitine helps to remove toxic compounds from within the cells [1, 2]. The deficiency of L-carnitine produces several disorders such as cardiomyopathy, skeletal myopathy, hypoglycemia, and hyperammonemia [3]. Although small amounts of L-carnitine can be synthesized by adults, the majority of L-carnitine needed in humans is taken through food consumption. Exogenous supply of L-carnitine is mainly supplied by foods of animal origin, where its concentration varies between 8 and 530 mg/kg of dry mass, whereas fruit and vegetables, with few exceptions such as avocado and some fermented soy products (e.g. tempeh), contain very little, if any, L-carnitine [1, 4].

In addition, L-carnitine was found to confer both chill and osmotic tolerance to the pathogen \textit{Listeria monocytogenes} [5]. According to Smith [6], L-carnitine acts by counterbalancing the external osmotic strength without destabilizing the cellular protein structure, even at the high concentrations at which it is found in the cell. L-carnitine is not synthesized by \textit{L. monocytogenes} and must be transported from the environment. The rate and the extent of its transport can be controlled by regulation of the synthesis of the transport systems and by its biochemical activation by osmotic gradients, temperature, or other factors.

The development of accurate models able to predict the behavior of substances under specific environmental conditions is of main importance for the food industry and relies on the estimation of appropriate kinetic data. Existing procedures for kinetic parameter estimation are normally based on experiments under isothermal conditions. The main advantages of this approach are the easiness of the implementation and the fact that the model validity can be interpreted graphically [7]. However, temperature may vary extensively throughout a thermal process and transposition of results obtained from isothermal conditions to dynamic conditions may require adjustment of the initial mathematical structure in use [8].

Therefore, the need of estimation of inactivation kinetics considering time-varying temperature conditions arises. In this approach, kinetic parameters are estimated by studying the concentration of the component of interest during the tested dynamic environment. According to Van Boekel [9], confidence intervals for parameters estimated under the one step regression are narrowed due to the increased number of degrees of freedom. Nunes et al. [10] and Claeys et al. [11] estimated kinetic parameters for sucrose hydrolysis and for
enzymatic reactions in milk, respectively, using non-isothermal data, whereas Cunha and Oliveira [12] presented an optimal experimental design for estimating kinetic parameters under linearly increasing temperature. Studies on thermal inactivation of L-carnitine have not been found in the literature, whereas a few works approach the estimation of inactivation kinetics considering time-varying temperature conditions. Thus, the objectives of this work were the kinetic study of thermal inactivation of L-carnitine and the determination of its kinetic parameters from dynamic temperature profile experiments.

MATERIALS & METHODS

The inactivation experiments of L-carnitine took place in glass Pasteur pipettes in which a volume of 300 µL L-carnitine solution with a concentration of 0.015 g/100 mL was pipetted. Pipettes were then sealed by a gas flame and immersed in a water/oil bath (Memmert, Model WNE 7, Memmert GmbH Co KG, Schwabach FRG, Germany) set at specified temperatures (80, 85, 90, 95, 100, 110, 120, and 130°C) selected after preliminary experiments. Temperature was continuously monitored and recorded at 10 s intervals in a multichannel datalogger by a type T thermocouple placed inside a pipette used as a temperature indicator. At least two pipettes (duplicate samples) were drawn at predetermined time intervals (chosen for each temperature based on the expected inactivation rates) and were placed into an ice-water bath. The samples were then analysed for L-carnitine content. The L-carnitine content was spectrophotometrically determined using a Helios UV-Visible spectrophotometer (Helios gamma, Thermo Spectronic, Madison, USA). The method was based on the transformation of L-carnitine to acetyl-carnitine and coenzyme A, catalyzed by carnitine acetyltransferase (CAT), followed by the conversion of coenzyme A to succinoyl-CoA by adding 2-oxoglutarate. After 15-30 min the reaction ceased as indicated by a plateau in the sample absorbance value. The difference between the pre-reaction and the end-reaction absorbance reading is proportional to the L-carnitine concentration [13].

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Decimal reduction times ($D_T$) at each temperature tested were calculated with the progressive use of the experimental remaining concentration data (Eq. (1)) and afterwards these $D_T$ values were used for the calculation of the $z$ value (Eq. (2)) by successive linear regressions.

\[
\log C = \log C_o - \frac{t}{D_T} \quad (1)
\]

\[
\log D_T = \log D_{T_{ref}} + \frac{T_{ref} - T}{z} \quad (2)
\]

where $C_o$ is the initial L-carnitine concentration, $C$ is the L-carnitine concentration at time $t$, $D_T$ is the decimal reduction time at temperature $T$, $z$ is the thermal resistance constant, and $T_{ref}$ is a reference temperature. Furthermore, remaining L-carnitine concentration data were collected during a non-isothermal experiment for a step-wise increasing temperature profile. Estimates of the kinetic parameters were obtained by non linear regression fitting Eq. (3) to the non-isothermal experimental data.

\[
\frac{C}{C_o} = 10^{-\frac{\int_{0}^{T(t)} - T_{ref}}{z} \, dt}{D_{T_{ref}}} \quad (3)
\]

RESULTS & DISCUSSION

Data on remaining L-carnitine concentration against processing time as a function of processing temperature are shown on Fig. 1. Higher inactivation rates were observed as processing temperature increased. From the distribution of the points we can see that the plots are linear ($R^2 = 0.961–0.999$) indicating that thermal inactivation of L-carnitine followed first order kinetics. Based on Eq. (1), decimal reduction times were determined at each temperature. The temperature dependence of $D_T$ values was expressed by Eq. (2), as illustrated on Fig. 2, and the thermal resistance constant, the $z$ value, was found equal to 30.2°C, whereas the $D_{T_{ref}}$ at a reference temperature of 120°C was determined as 50.6 min.
Since isothermal experiments represent an idealized situation, and most processes usually involve a heating and a cooling phase, kinetics were re-evaluated under more realistic, variable temperature conditions (Fig. 3). Parameter optimization was performed using the Gauss-Newton method. In this method, a first and second derivative of the change in sum squared errors (SSE) with respect to the parameter value is used to estimate the direction and distance the next iteration step should go within the optimization algorithm to reach a better point on the SSE surface. For this optimization approach, as the true parameters are a priori unknown, initial guesses are usually based on previous information. Based on the remaining L-carnitine concentration data as a function of processing time for the variable temperature conditions shown on Fig. 3, and Eq. (3), the kinetic parameters, that is, the $D_{120^\circ C}$ and $z$ values were found equal to 48.8 min and 29.9°C, respectively.

In order to measure the reliability of the kinetic parameter estimates to predict thermal inactivation of L-carnitine, concentrations predicted by integration of the recorded time-temperature profile using parameter estimates from both isothermal and non-isothermal data were compared with the experimentally observed concentrations (Fig. 4). As it can be seen, the two methods resulted in not significantly different results. In addition, both methods yielded precise and accurate predictions. Correlation between experimentally determined concentrations of L-carnitine after non-isothermal treatment and those calculated by means of kinetic parameter estimates form dynamic and isothermal data gave $R^2$ and SSE values of 0.999 and 8.19, respectively, for the dynamic method and 0.998 and 10.28 for the isothermal experiments.

**Figure 1.** Experimental (symbols) and predicted (lines) values of remaining L-carnitine concentration as a function of processing time and temperature.

**Figure 2.** Effect of temperature on the decimal reduction time.
CONCLUSION

Based on the agreement between the parameters estimated using isothermal or non-isothermal temperature profiles, and given the reduced number of experimental data required by the latter approach, kinetic parameter estimation from experiments at dynamic conditions is recommended. Although in the present study L-carnitine thermal inactivation followed first order kinetics and the methodology used was focused on such kinetic behavior, deviations from first order kinetics can be handled.

REFERENCES


