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Numeric simulation can be used to predict heat transfer during the blanching of leaves and intact plants

J.F. Buyel [a,b,*]

[a] Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Forckenbeckstraße 6, 52074 Aachen, Germany
[b] Institute for Molecular Biotechnology, Worringerweg 1, RWTH Aachen University, 52074 Aachen, Germany

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ABSTRACT

Heat treatment is often used during the processing of plant-derived biomass to precipitate host cell proteins (HCPs) and thus simplify the purification of target proteins. Plants uniquely allow this precipitation step to be applied within intact leaves rather than large volumes of cell culture supernatant or extract. However, it can be expensive and time consuming to identify the optimal conditions for HCP removal even when applying statistical experimental designs, such as the design of experiments (DoE) approach. An alternative strategy that additionally yields mechanistic insights is the modeling of heat transfer within solid-state biological specimens, such as whole leaves. Here we present a method for the numeric simulation of heat treatment in tobacco (*Nicotiana tabacum*) leaves, and *N. benthamiana* leaves and whole plants, using input parameters such as the heating temperature, heat transfer coefficient, specific heat capacity and thermal conductivity, taking convective and conductive heat transfer into account. Incubation times of 0.5–1.0 min were sufficient to reach thermal equilibrium at typical heat transfer coefficients of 20–100 J s^{-1} m^{-2} K^{-1}, and convection rather than conduction was the limiting factor, in agreement with published empirical data. The model can thus form the basis of a quality-by-design approach to remove HCPs by heat precipitation. The limits and benefits of modeling are discussed and we provide examples of potential future applications.

1. Introduction

Plants have been used for many years to provide humans with food [1], animal feed, and the raw materials to manufacture diverse goods such as paper [2], textiles [3–5], rubber [6] and fuels [7]. More recently, genetic engineering has been used to produce fine chemicals and biopharmaceutical proteins from modified plants [8–11]. This development was driven by the inherent benefits of plants compared to microbes and mammalian cells, including inexpensive upstream production, scalability and a low human pathogen burden [12–14]. Tobacco (*Nicotiana tabacum*) and *N. benthamiana* are frequently used for recombinant protein expression due to their high biomass yield and compatibility with rapid transient expression protocols [10]. Because the target products usually accumulate inside plant cells, the initial processing operations are designed to disrupt the cells and release the target molecules into solution [15–17]. These operations are nonselective, so large numbers of soluble and insoluble impurities are also released into the feed stream, including host cell proteins (HCPs). The HCPs interfere with subsequent downstream purification steps [18] so their early removal is beneficial. We recently found that the heat treatment of crude plant extracts containing thermostable recombinant proteins can precipitate the HCPs and reduce the complexity of the feed stream [19–21]. This also helps to reduce downstream processing costs, which can account for up to 80% of the overall costs of production [17,22]. One unique advantage of plants is that heating can also be carried out by blanching, i.e. the immersion of intact leaves into a hot water bath (Fig. 1A). This minimizes the quantity of biomass and liquids that require heating and cooling compared to extracts or cell culture supernatants. Blanching whole plants instead of individual leaves simplifies this step even further.

The efficient implementation of a heating step requires information about the thermal properties of the biological material in order to model heat conduction, i.e. the specific heat capacity c_p and thermal conductivity λ. It also requires a suitable model describing thermal conduction and convective heat transfer, including the heat transfer coefficient h [23]. It is therefore important to

Abbreviations: DoE, design of experiments; GMP, good manufacturing practice; HCPs, host cell proteins; QbD, quality by design.

* Correspondence address: Institute for Molecular Biotechnology, Worringerweg 1, RWTH Aachen University, 52074 Aachen, Germany. Fax: +49 241 6085 10000.
E-mail address: johannes.buyel@rwth-aachen.de

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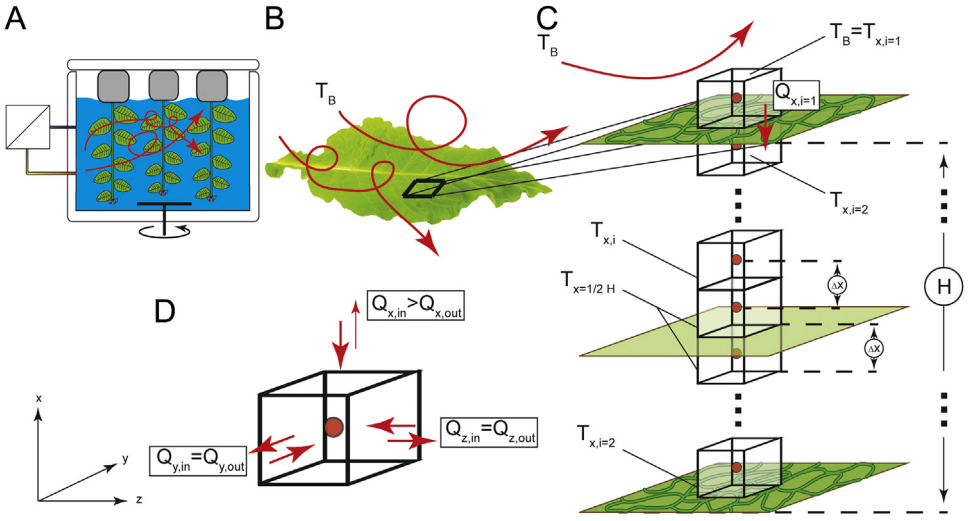

Fig. 1. Conversion of the macroscopic leaf appearance into a finite volume model used for numeric simulation. (A) Schematic representation of leaf blanching for the precipitation of HCPs, where plants are submerged into a blanching buffer (blue) kept at a defined temperature by a heat exchanger. (B) Schematic representation of a tobacco leaf with a turbulent flow of blanching buffer at temperature T_B. (C) Finite volumes (black cubes) with a dimension of Δx^3 are aligned along the *x*-axis of a leaf covering its entire height H (i.e. its thickness). Red dots indicate the centers of finite volumes for which the temperature is calculated during numeric simulation using Eq. (15) ($i=2$) or Eq. (5) ($i>2$). The buffer temperature T_B is assumed to be equal to that of the outermost volume ($i=1$), i.e. the buffer that is in direct contact with the leaf surface. Heating proceeds from the top and bottom surfaces simultaneously and hence the temperature of the finite volumes above and below the central leaf plane (light green) is equal ($T_x = H/2$). Thus, simulating one half of the leaf is sufficient. (D) The *y* and *z* dimensions of a leaf are three orders of magnitude larger than its *x* dimension. Thus, heating effects from the sides along with the heat flux Q in the *y* and *z* directions are negligible compared those from top and bottom.

characterize the unprocessed plant biomass in terms of size and shape, because this will affect the flow of liquid in a blanching bath and hence the efficiency of the heating procedure. Empirical and theoretical formulae have been developed to predict the thermal properties of plants and plant-derived products [24,25], in some cases based on their molecular composition [26–28]. The direct measurement of thermal properties has also been described [29–32]. Heating steps are often used to improve the properties and quality of plant-derived commodities [33–36].

Here we report the development of a numeric simulation that models the blanching of tobacco and *N. benthamiana* leaves as well as entire *N. benthamiana* plants. Energy transport at the leaf surface and within the leaf tissue are taken into account to calculate the time required to reach thermal equilibrium based on the environmental conditions, e.g. the temperature of the blanching fluid. The results are compared with recent empirical data and we discuss the application of the model in the context of a quality-by-design (QbD) compliant process design as recommended by the FDA [37]. The model will facilitate the design of processes and equipment for leaf/plant blanching during the manufacture of plant-derived biopharmaceuticals according to good manufacturing practice (GMP).

2. Theory

Convection describes energy transfer from an external medium into a specimen, whereas conduction describes the heat flux within that specimen [23]. Conduction in a solid body depends on the specimen's density ρ (kg m^{-3}), specific heat capacity c_p (J kg^{-1} K^{-1}) and thermal conductivity λ (W m^{-1} K^{-1}), and in the absence of internal energy sources can be described by Eq. (1) [23].

$$\frac{\partial T}{\partial t} = \frac{\lambda}{\rho c_p} \nabla^2 T \tag{1}$$

where ∂T is the temperature change (K) as a function of elapsed time ∂t (s), ∇ is the Laplace operator [38] and T is the temperature (K).

The numeric simulation of temperature gradients within a sample can be achieved using a finite volume approach [39,40], in which the leaf is discretized into a finite number of volumes with defined dimensions of height, length and width (Fig. 1B and C). The space/time-dependent temperature gradient within a two-dimensional specimen can then be calculated using Eq. (2) [23].

$$\lambda \frac{\Delta b}{\Delta a}\left(T_{i-1,j} + T_{i+1,j} - 2T_{i,j}\right) + \lambda \frac{\Delta a}{\Delta b}\left(T_{i,j-1} + T_{i,j+1} - 2T_{i,j}\right) = \left(\frac{\rho c_p T_{i,j|t+\Delta t} - \rho c_p T_{i,j|t}}{\Delta t}\right) \tag{2}$$

where Δa and Δb are the spatial increments (m) in the first and second dimensions, respectively, $T_{i,j}$ is the temperature (K) at position i,j and Δt is the time increment (s).

The complexity of the equation can be reduced if the volumes used in the numeric calculations are defined as cubes ($\Delta x = \Delta y = \Delta z$), and further simplification is possible if symmetries can be identified in the specimen and the number of dimensions can be reduced [41], i.e. heat transfer in certain directions can be ignored. The *y* and *z* dimensions of a tobacco leaf (150–300 mm) are about three orders of magnitude larger than the *x* dimension (<0.3 mm) so the latter is defined as the leaf height H. The *y* and *z* dimensions can therefore be treated as infinite with a zero net heat flux compared to the *x* dimension, i.e. the heat flux from the leaf edges has a negligible effect (less than 0.5%) on the resulting temperature gradient in the sample. The *x* dimension thus remains the only dimension with a relevant temperature gradient and investigating the temperature in different "slices" of the *x* dimension is sufficient (Fig. 1C and D). This reduces Eq. (2) to Eq. (3), which can be solved for the temperature after a time increment $T_{i|t+\Delta t}$.

$$\frac{\lambda}{\Delta x}\left(T_{i-1j|t} + T_{i+1|t} - 2T_{i|t}\right) = \left(\frac{\rho c_p T_{i|t+\Delta t} - \rho c_p T_{i|t}}{\Delta t}\right) \Delta x \Leftrightarrow T_{i|t+\Delta t}$$

$$= \frac{\lambda}{\rho c_p}\frac{\Delta t}{(\Delta x)^2}\left(T_{i+1|t} + T_{i-1|t}\right) + \left(1 - \frac{2\lambda}{\rho c_p}\frac{\Delta t}{(\Delta x)^2}\right) T_{i|t} \tag{3}$$

Using the equality in the stability criterion for numeric simulations [42] shown in Eq. (4) by selecting a suitable time increment