MODELLING MODIFIED ATMOSPHERE PACKAGING FOR FRUITS AND VEGETABLES USING MEMBRANE SYSTEMS

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Abstract: As living materials, post-harvested fruits and vegetables continue their metabolic activity, exhibiting progressive biochemical changes. Optimisation of environmental conditions during storage of these fresh commodities is required in order to increase their shelf life. In this work we use P systems to abstract molecular interactions that occur between plant organ, film and surrounding atmosphere factors involved in fresh fruit and vegetable package designs. The proposed model constitutes a general framework to simulate the dynamical behaviour of these systems, specially due to gas exchanges and temperature fluctuations. Moreover, the model can be extended introducing other variables and processes that affect quality of such produces. This can be considered, to the best of our knowledge, the first contribution of Membrane Computing in Food Engineering.

1 INTRODUCTION

Membrane systems (Păun and Rozenberg, 2002), also called P systems, had emerged to assist in the modelling of systems of concurrent reactions taking place in compartments, so as occur in biological systems. In this paper we use P systems as membrane structures delimiting compartments that contain multisets of objects representing molecules. Compartments configuration changes over time (evolve) according to given rules that represent biochemical reactions and diffusions. In contrast to ODE-based approaches, each single molecule within the entire system is represented explicitly as individual entity. Capturing aspects of structural dynamics (changes in the membrane structure as well as in the composition of complex molecules) is seen as an advantageous feature of P systems. Inclusion of reaction kinetics into this formalism can be done by discretised kinetic laws (Hinze et al., 2006). We applied this mathematical formalism to a real known problem in fruits and vegetables post-harvest processing.

Fresh fruits and vegetables are living materials that continue to respire after harvesting exhibiting progressive biochemical changes. Food Engineering methods to preserve freshness of post-harvest produces include low temperature storage and special packaging technologies, mainly Modified Atmosphere Packaging (MAP). MAP of fresh fruits and vegetables refers to the technique of enveloping the produce in a sealed container of polymeric film in order to modify the $O_2$ and $CO_2$ concentrations inside the package, reducing metabolic activity and increasing shelf life (Paul and Clarke, 2002).

Designing MAP systems is a complex task that involves considerations about many interrelated environmental (as temperature and atmosphere composition), biological and package technology factors. Basic biological processes are respiration, transpiration, ethylene production and compositional changes due to metabolism. The variability of responses to internal and external signals depends on the characteristic of each plant organ type, developmental stage and physiological condition. In addition, much of the behaviour of a MAP system at cellular level are not fully understood. As examples we can refer to the little knowledge about the effect of $CO_2$ on the activity of respiratory enzymes (Ho et al., 2008). Moreover, the contribution of the biochemical changes that alters physical properties of cell walls and tissues modifying the texture of the produce is not known in detail (Gross et al., 2004). On the other hand, the mechanism of ethylene
signal transduction that coordinates fruit ripening processes, is another aspect subject to study (Alexander and Grierson, 2002).

The difficulty to test different combination of gases and temperatures and the complexity of experimental setup for MAP systems had lead to the development of various mathematical models (Exama et al., 1993; Tijskens et al., 2001; Paul and Clarke, 2002; Ho et al., 2008) and software (Mahajan et al., 2007). In the literature, many respiration models are empirical fits of experimental data, based on one particular type and variety of fruit or vegetable, and most of them are based on the principles of enzyme kinetics and are represented using ODEs (for reviews see (Fonseca et al., 2002; Rodriguez-Aguilera and Oliveira, 2009)). However, there exists some lack on studies about the dynamical behaviour of these systems in terms of changes in environmental conditions, so as produce composition and physiology due to developmental processes (Fonseca et al., 2002). It is worth mentioning that post-harvested fruits and vegetables, unlike other living materials, can be considered as less robust systems, as their responses on environmental fluctuations depends mostly on their actual configuration of biochemical components. In this context, some authors (Génard et al., 2007; Mahajan et al., 2007) have considered the potential benefits of a systematic analysis or process-based modelling approach for fruits and vegetables. Considering that understanding the reaction network underlying MAP systems can give food experts more knowledge about emergent properties of packaged fruits and vegetables, we propose a framework based on membrane systems that abstracts basic biochemical reactions that occur in MAP systems. In the future, the proposed model can serve as a predictive tool to simulate changes in fresh produce on the molecular level, due to changes in environmental conditions.

This paper is organized as follows: in Section 2 we present a P system framework for MAP, including the description of components, reaction kinetics and evolution of the system. Section 3 shows an application of the framework considering a package under modified atmosphere containing two produces. Finally, in Section 4 we point out some benefits of using our framework and future extensions of it.

2 A P SYSTEM-BASED MAP

We abstract a fruit or vegetable as a graph of cells or modules, like tissue P systems (Martín-Vide et al., 2003). Each cell represents a compartment that contains species, and at a specific time, the contents of the compartment determine the cell configuration. This serves as a mechanism to differentiate one cell from other, given the possibility to creating diverse tissue types, as occurs for example in fruits epicarp, mesocarp and endocarp tissues (Génard et al., 2007). Additionally, as gas consumption-production occur inside the cells, at the mitochondria level, and is stated that gas diffusion between cells depends on the geometry of the produce (Ho et al., 2008), differences in gas content in cells that conform a determinate region can adequately be represented. This is also in accordance to the idea that the ripening process usually starts in one region of a fruit and spreads to neighbouring regions, due to ethylene diffusion starting from promoter cells (Alexander and Grierson, 2002). Produces into the package are represented as a population of membranes, giving the advantage that the model can deal with distinct fruits and vegetables within the same film, or the same produce in distinct developmental stages, varieties and/or presentations. Figure 1 shows as example, the schematic representation for such a system. In the next section we present the formal specification of our model.

Figure 1: A schematic representation for the MAP system model. In this case, two produces share a package: plant$_1$ is formed by three connected cells, and plant$_2$ is formed by a single cell. Arrows represent paths for molecules (spheres) diffusions

### Multiset Prerequisites

Let $A$ be an arbitrary set and $\mathbb{N}$ the set of natural numbers including zero. A multisets over $A$ is a mapping $F : A \rightarrow \mathbb{N} \cup \{\infty\}$. $F(a)$, also denoted as $[a]_F$, specifies the multiplicity of $a \in A$ in $F$. Multisets can be written as an element-wise enumeration of the form \{$(a_1,F(a_1)),$\,$(a_2,F(a_2)),$\,...\} since $\forall (a,b_1),(a,b_2) \in F : b_1 = b_2$. The support $\text{supp}(F) \subseteq A$ of $F$ is defined by $\text{supp}(F) = \{a \in A : F(a) > 0\}$. A multiset $F$ over $A$ is said to be empty iff $\forall a \in A : F(a) = 0$. The cardinality $|F|$ of $F$ over $A$ is $|F| = \sum_{a \in A} F(a)$. Let $F_1$ and $F_2$ be multisets over $A$. $F_1$ is a subset of $F_2$, denoted as $F_1 \subseteq F_2$, iff $\forall a \in A : (F_1(a) \leq F_2(a))$. Multisets $F_1$ and $F_2$
are equal iff $F_1 \subseteq F_2 \land F_2 \subseteq F_1$. The intersection $F_1 \cap F_2 = \{(a, F(a)) \mid a \in A \land F(a) = \min\{F_1(a), F_2(a)\}\}$, the multiset sum $F_1 \uplus F_2 = \{(a, F(a)) \mid a \in A \land F(a) = F_1(a) + F_2(a)\}$, and the multiset difference $F_1 \ominus F_2 = \{(a, F(a)) \mid a \in A \land F(a) = \max\{F_1(a) - F_2(a), 0\}\}$ form multiset operations. Multiplication of a multiset $F = \{(a, F(a)) \mid a \in A\}$ with a scalar $c$, denoted $c \cdot F$, is defined by $\{(a, c \cdot F(a)) \mid a \in A\}$.

**P system components**

Let $\mathbb{N}_+ = \mathbb{N} \setminus \{0\}$ be the set of natural numbers without zero, and $m, n \in \mathbb{N}_+$. We define a P system for a MAP system as a construct:

$$\Pi_{\text{MAP}} = (\mu, S, \text{plant}_1, \ldots, \text{plant}_m, G, L_0, D_1, \ldots, D_d, f_1, \ldots, f_d, \Delta \tau)$$

where:

- $\mu = \{\text{cell}_{i_1} \cdots \text{cell}_{m_1} | \text{plant}_1 \cdots \text{plant}_m\}$ package is the spatial system structure composed of three inner levels: package, plants, and cells,
- $S$ is a set of chemical species,
- $\text{plant}_1, \ldots, \text{plant}_m$ represent the produces into the package,
- $G$ is a set of global parameters,
- $L_0: S \rightarrow \mathbb{N}$ is a multiset of axioms representing the initial molecular configuration,
- $D_\alpha$ is a diffusion (communication) rule among package and external environment ($\forall = 1, \ldots, d$),
- $f_\xi : (S \rightarrow \mathbb{N}) \rightarrow \mathbb{N}$ is a kinetic function attached to diffusion rule $D_\alpha$,
- $\Delta \tau \in \mathbb{R}_+$ is the time discretisation interval.

A diffusion rule $D_\alpha$ can be of the form $[s] \rightarrow [\_]s$ for molecules $s \in S$ leaving the package and released to the external environment, and $[\_]s \rightarrow [s]$ for molecules entering the package, respectively.

Furthermore, each $\text{plant}_i$ is defined as a tuple:

$$\text{plant}_i = (N_i, E_i, G_i, D_{i_1}, \ldots, D_{i_d}, f_{i_1}, \ldots, f_{i_d})$$

where:

- $N_i = \{\text{cell}_{i_1}, \ldots, \text{cell}_{i_k}\}$ defines a set of cells within plant $i$,
- $E_i \subseteq N_i \times N_i$ specifies a set of directed edges (diffusion channels between cells),
- $G_i$ is a set of plant (organ) specific parameters,
- $D_{i_k}$ represents a diffusion rule inside plant $i$ and between plant $i$ and package ($k = 1, \ldots, d$),
- $f_{i_k}$ is a kinetic function attached to diffusion rule $D_{i_k}$.

Here, a diffusion rule can be of the form $[s]_{\text{cell}_{p,q}} \rightarrow [\_]\text{cell}_{p,q}s$ for molecules $s \in S$ leaving $\text{cell}_{p,q}$ and spread out into the package. A rule of the form $[\_]\text{cell}_{p,q}s \rightarrow [s]_{\text{cell}_{p,q}}$ describes molecules entering $\text{cell}_{p,q}$ from the package. Finally, a rule of the form $[s]_{\text{cell}_{p,q}} \rightarrow [s]_{\text{cell}_{i,j}}$ formulates the directed transport of molecule $s$ along the edge $(\text{cell}_{p,q}, \text{cell}_{i,j}) \in E_i$.

Each $\text{cell}_{i,j}$ is defined as a tuple $\text{cell}_{i,j} = (L_{i,j,0}, R_{i,j,1}, \ldots, R_{i,j,r_i,j}, f_{i,j,1}, \ldots, f_{i,j,r_i,j})$ where:

- $L_{i,j,0} : S \rightarrow N$ is a multiset of axioms representing its initial molecular configuration,
- $R_{i,j,k} = (A_{i,j,k} R_{i,j,k})$ with $A_{i,j,k} : S \rightarrow N$ (multiset of reactants) and $R_{i,j,k} : S \rightarrow N$ (multiset of products) specifies a reaction rule including its stoichiometric factors,
- $f_{i,j,k} : (S \rightarrow \mathbb{N}) \rightarrow \mathbb{N}$ is a function corresponding to kinetics of reaction $R_{i,j,k}$.

**System evolution**

A P system of the form $\Pi_{\text{MAP}}$ evolves by successive progression of its configuration at discrete points in time $t \in \mathbb{N}$ for what we assume a global clock. Two consecutive dates $t$ and $t + 1$ specify a time span $\Delta t$. A system step at time $t$ consists of three modification stages carried out from outer to inner spatial components of the system. Firstly, the diffusion between package and its environment is considered. To this end, the rules $D_1$ up to $D_d$ are employed. Afterwards, the diffusion between package and cells as well as the intracellular diffusion is utilised by employing the rules $D_{i,k}$ for each plant $i = 1, \ldots, m$. The last modification stage concerns application of the reaction rules specified in each cell. To cope with conflicts that can occur if the available amount of substrate cannot satisfy all matching diffusion and reaction rules, we prioritise all rules by their index: $D_1 > D_2 > \ldots > D_d$. Moreover, for each plant $i$: $D_{i,1} > D_{i,2} > \ldots > D_{i,d_i}$ and for each cell $i, j$: $R_{i,j,1} > R_{i,j,2} > \ldots > R_{i,j,r_{i,j}}$. Thus, we keep determinism of the system evolution and enable mass conservation. An alternative method for coping with conflicts is randomisation in selection and sequentialisation of diffusion and reaction rules.

The application of an arbitrary rule is organised into two consecutive steps. The first step identifies all molecules from the rule’s left hand side acting as sources for diffusion or reactants. These molecules are removed from the current configurations. Corresponding molecules from the right hand side (destinations in case of diffusion and products in case of reactions) are then added. We formulate discretised reaction-diffusion kinetics by specification of scalar functions $f : M \rightarrow \mathbb{N}$ based on a multiset $M : S \rightarrow \mathbb{N}$. Each function $f$ converts the current configuration ($L_i$ or $L_{i,j,k}$), a multiset of objects, into the number of turns for application of the corresponding diffusion or reaction rule. Here, kinetic laws $f(s)$ for each species $s \in S$ employ the multiplicity of its occurrences to formulate the corresponding reaction rate. For updating the entire system configuration, we define an iteration scheme as shown in Figure 2.
Stage 1 (diffusion between package and external environment):
\[ \forall \alpha = 1, \ldots, d \]

<table>
<thead>
<tr>
<th>diffusion rule</th>
<th>conditions</th>
<th>action</th>
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</thead>
<tbody>
<tr>
<td>[ D_\alpha = \sigma \rightarrow {G} ]</td>
<td>( \sigma \in S ) &amp; ( {G, f_\alpha} \subseteq L_\alpha )</td>
<td>[ L_\alpha := L_\alpha \cup {G, f_\alpha} ]</td>
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<tr>
<td>[ D_\alpha = {G} \rightarrow \sigma ]</td>
<td>( \sigma \in S )</td>
<td>[ L_\alpha := L_\alpha \setminus {G, f_\alpha} ]</td>
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with \( f_\alpha(L_\alpha) = \left[k_\alpha(G) \cdot \Delta t \cdot \hat{\iota}(\{L_\alpha \cap \{G, \infty\}\})\right] \)

Stage 2 (diffusion between plant cells and package):
\[ \forall i = 1, \ldots, m \]
\[ \forall \alpha = 1, \ldots, d_i \]

<table>
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<th>action</th>
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<tbody>
<tr>
<td>[ D_{i,\alpha} = [\sigma]<em>{\text{cell}, i} \rightarrow {\sigma}</em>{\text{cell}, i} ]</td>
<td>( \sigma \in S ) &amp; ( {\sigma, f_{i,\alpha}} \subseteq L_{i,j,t} )</td>
<td>[ L_{i,j,t} := L_{i,j,t} \cup {\sigma, f_{i,\alpha}} ]</td>
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<tr>
<td>[ D_{i,\alpha} = {\sigma}<em>{\text{cell}, i} \rightarrow [\sigma]</em>{\text{cell}, i} ]</td>
<td>( \sigma \in S ) &amp; ( {\sigma, f_{i,\alpha}} \subseteq L_i )</td>
<td>[ L_i := L_i \cup {\sigma, f_{i,\alpha}} ]</td>
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<tr>
<td>[ D_{i,\alpha} = [\sigma]<em>{\text{cell}, i} \rightarrow [\sigma]</em>{\text{cell}, k} ]</td>
<td>( \sigma \in S ) &amp; ( {\sigma, f_{i,\alpha}} \subseteq L_{i,t} )</td>
<td>[ L_{i,k,t} := L_{i,k,t} \cup {\sigma, f_{i,\alpha}} ]</td>
</tr>
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with \( f_{i,\alpha}(L_{i,t}) = \left[k_{i,\alpha}(G_i, G_t) \cdot \Delta t \cdot \hat{\iota}(\{L_t \cap \{G_i, \infty\}\})\right] \)

Stage 3 (reactions occurring within each cell):
\[ \forall i = 1, \ldots, m \]
\[ \forall j = 1, \ldots, n_i \]
\[ \forall \alpha = 1, \ldots, r_{i,j} \]

<table>
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<tr>
<th>reaction rule</th>
<th>conditions</th>
<th>action</th>
</tr>
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<tr>
<td>[ R_{i,j,\alpha} = (A_{i,j,\alpha}, B_{i,j,\alpha}) ]</td>
<td>( f_{i,j,\alpha} \cdot A_{i,j,\alpha} \subseteq L_{i,j,t} )</td>
<td>[ L_{i,j,t} := L_{i,j,t} \cup {f_{i,j,\alpha} \cdot A_{i,j,\alpha}} ]</td>
</tr>
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with \( f_{i,j,\alpha}(L_{i,j,t}) = \left[k_{i,j,\alpha}(G_i, G_t) \cdot \Delta t \cdot \hat{\iota}(\{L_{i,j,t} \cap \{G_i, \infty\}\})\right] \prod_{c \in \text{supp}(A_{i,j,\alpha})} A_{i,j,\alpha} = (A_{i,j,\alpha}, B_{i,j,\alpha}) \)

Increment time \( t \):
\[ L_{t+1} := L_t \]
\[ \forall i = 1, \ldots, m \]
\[ \forall j = 1, \ldots, n_i \]
\[ L_{i,j,t+1} := L_{i,j,t} \]

Figure 2: Iteration scheme for the temporal evolution of \( \Pi_{\text{MAP}} \) system

### 3 SIMULATION

As a first application, we introduced as rules into the model only the basic processes involved in a MAP design: respiration and fermentation, so as gas diffusion between membranes. Respiration rate can be expressed in terms of \( O_2 \) consumed or \( CO_2 \) produced. The respiratory quotient (\( RQ \)), the ratio of \( CO_2 \) produced to \( O_2 \) consumed, ranges from about 0.7 to 1.4 depending on the substrate and its metabolic state (if the substrate is a lipid, \( RQ < 1 \), and \( RQ > 1 \) for organic acids)(Fonseca et al., 2002). When carbohydrates are aerobically respired, the \( RQ \) is near 1, and the reaction is represented by Eq. (1). The influence of gas composition on respiration rates of produce has been widely represented by Michaelis Menten-type equation (Fonseca et al., 2002). In this context, respiration rate is considered as a function of concentration in terms of enzymatic reaction, with \( O_2 \) in the place of substrate and the product \( CO_2 \) acting as inhibitor.

\[ C_3H_12O_6 + 6 O_2 \rightarrow 6 CO_2 + 6 H_2O + \text{energy} \quad (1) \]

Transpiration occurs due to the fact that fruits and vegetables internal atmosphere is saturated with water vapour, while external atmosphere contains lesser. Therefore, water loss rate depends on the external and internal water vapour pressure gradient.

Temperature dependence over respiratory rate and over film permeability was represented using Arrhenius equation (Eq. 2).

\[ k = F \times e^{-E_R/T} \quad (2) \]
where $E_a$ is the activation energy, expressed in joule per mol, defined as the energy that must be overcome for a chemical reaction to occur; $R$ is the gas constant ($\approx 8.314 \ J \cdot K^{-1} \cdot mol^{-1}$), $T$ the absolute temperature, $F$ is the pre-exponential factor that represents the total number of molecular collisions per second; and $k$ corresponds to the number of collisions per second that result in a reaction. This can be related to the probabilistic approach to P systems introduced by (Andrelean and Cavaliere, 2003) in order to obtain more biological-like models. In this context, the Arrhenius exponential term can be viewed as the probability per unit time that the reaction takes place.

In order to apply our model, we simulate the dynamical behaviour of an instance of a PMAP with two hypothetical fruits as it is shown in Fig. 1, using continuous film and passive MAP as package techniques. Rules that use symbol := between reactants and products must be interpreted as reversible reactions.

To formalize described in Fig. 2, a rule of the form $D_0 = [\sigma] = [\sigma] \rightarrow ^{\tau} [\sigma]$, for example, consists of the following two rules, in order of application: $[\sigma] \rightarrow [\sigma] \rightarrow [\sigma]$. Temperature is represented by $T$ and expressed in Kelvin ($K$). $O_2$, $CO_2$, and $H_2O$ abundances in the outside are represented by $O_{2ex}$, $CO_{2ex}$ and $H_{2Oex}$ respectively. $A$ and $E$ symbolise the surface area in $cm^2$ and the thickness of the packaging film in mil ($1mil = 0.00254cm$). $pO_2$ and $pCO_2$ represent the reference film permeability in $mL \cdot mil \cdot cm^2 \cdot hr^{-1} \cdot atm^{-1}$ for $O_2$, $CO_2$ and $H_2O$, respectively. $EaO_2$ and $EaCO_2$ symbolise the permeability activation energy expressed in $J \cdot mol^{-1}$ for $O_2$ and $CO_2$, respectively. $M_i$ symbolises mass of the produce $i$ in kg. For simplicity, we assume that each cell in a produce has the same mass. $rO_2$ and $rCO_2$ correspond to the preexponential factor for produce respiration and fermentation in $mL \cdot kg^{-1} \cdot hr^{-1}$. $ErO_2$ and $ErCO_2$ represent the respiration and fermentation activation energy for the produce expressed in $J \cdot mol^{-1}$. Most of the values for these symbols were taken from the literature (Exama et al., 1993). Symbols $O_2$, $CO_2$, $H_2O$, Ethanol and Glucose represent amounts of species $O_2$, $CO_2$, $H_2O$, Ethanol and Glucose. Initial values for these symbols in each compartment and the rest of the parameters were assigned empirically.

Figure 3 shows the corresponding courses of plant$_1$ internal gas composition, resulting from following parameter setting for the discrete iteration scheme: $A = 100$, $E = 1$, $M_i = 0.1$, $pO_2_i = 1620000$, $EaO_2_i = 43100$, $pCO_2_i = 238000$, $EaCO_2_i = 34300$, $rO_2_i = rCO_2_i = 3 \times 10^{14}$, $ErO_2_i = ErCO_2_i = 70700$, $pH_{2O_i} = 1$, $\Theta_{i,j,k} = 1$, for $i \in \{1, 2\}$ and $j \in \{1, 2, 3\}$ and $k \in \{1, 2\}$: $k_{i,j} = 0.2$ for $j \in \{1, \ldots, 9\}$, $k_{2,j} = 0.2$ for $j \in \{1, \ldots, 3\}$. A fixed value $T = 277.15$ was considered for a constant temperature scenario, and transient values for 273.15 $\leq T \leq 293.15$ were obtained through a sigmoid function to represent changes in temperature over time in another scenario. Simulations have been performed using Cospasi (Hoops et al., 2006). Differences in internal gas composition of plant$_1$ have been observed during time due to the interplay between cellular respiration and fermentation processes and intercellular diffusion. Those differences could determine the form of maturation of the produce, in this case, from the center to the skin. An equilibrium is reached in the package gas composition, while respiration rates of the produces diminished.

4 CONCLUSIONS

Using a membrane based model for MAP, we presented a framework that is able to abstract packaging for different fruit and vegetable types, varieties or developmental stages. Respiration of the produce
is considered as the basic process when modelling MAP, and predictions about the dynamical behaviour of such systems can be improved taking into account environmental, biological and technical factors. Our approach allows extensions including other low level processes, such as ethylene signaling pathway, cell/tissue rupture due to produce cutting and transport of other molecules, that can be easily modeled using P systems. When the formalism showed in Figure 2 is hidden in a software, the specification is intuitive an accessible for an expert focussing on MAP modelling. Finally, the quality of the packaged produce (taste, texture, colour and appearance) is based on some subjective consumer evaluation. These traits are based on specific product properties, such as sugar content, volatile production and cell wall structure (Tijakens et al., 2001), and therefore can be introduced into the model through reactions, as a mechanism to obtain more knowledge about the impact of packaging conditions over product quality.

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Figure 3: Dynamical behaviour for gas composition for plant_1 in constant and varying temperature scenarios.