Extraction of Phenolic Compounds from Grape Fruit. A Comparison between a 3D FEM Model and Experimental Results.

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Abstract: Fresh fruits and vegetables are gaining importance in the human diet because they contain many beneficial compounds. Among these compounds, phenols are of vital importance due to their antioxidant properties. For example, the famous sociological phenomena called “French paradox” may be explained by diet that associates regular intakes of vegetables, fruits and wines. It is well-evident from previous researches that the skin of red grapes is considered a good source of phenols. That’s why, wine producers are nowadays more focused on understanding the mechanism of extracting phenols from the grape skin and incorporating it into the wine. The aim of this work is to simulate the extraction procedure of phenols *in vitro* and model its diffusion process from the skin into the wine using a 3D Finite Element Method scheme. Results show the dynamical behaviour of the concentration. Comparing our theoretical approach to the experimental findings lends supports to the continuous nature of phenols localisation in the grape skin.

Keywords: Diffusion, Finite Element Method, Phenolic compounds, Biological tissue, Grape fruit

1. Introduction

Polyphenols designate a broad family of bio-molecules such as anthocyanins, flavonoïds, caratenoïds...In recent years, many researches have been devoted to phenolic compounds present in wine (Cadot *et al.*, 2006; Di Majo *et al.*, 2008; Kennedy, 2008; Maury *et al.*, 2001). They are justified by two main reasons. First, these compounds give some specific physical and sensory properties to wine and, particularly the red one. For examples, colour, bitterness and astringency of the red and rosé wines are influenced by anthocyanins and tannins (Flanzy, 1998; Stintzing and Carle, 2004). The second reason is that these compounds contribute to consumers' health and well-being. Numerous works have highlighted their antioxidative and immunomodulatory activities (Di Majo et al., 2008; Dolara *et al.*, 2005; Rivero-Pérez *et al.*, 2008; Stintzing et al., 2004). Therefore, polyphenols are regarded with interest as possible chemopreventive agents against cancer. The sociological phenomenon known as the "French Paradox" is very often evoked. This paradox refers to the fact that in France mortality from heart disease is low compared to other countries, despite high consumption of satured fat. This is explained by a rich diet in fruits, vegetables and regular moderate consumption of wine (de Lange, 2007; Ducimetière, 2000; Law and Wald, 1999). Indeed, polyphenols are mainly present in raw fruits and vegetables. Therefore, it appears relevant to design storing and processing fruits and vegetables processes not to destroy these compounds. It is also crucial to optimize their extraction for juices and other beverages such as tea and wine.

In the case of grape berries, polyphenols are located in the skin and the seeds. In addition, a lot of recent works had improved the understanding of their chemical structure, their action as a healthy agent and, their interaction with saliva compounds when generating feeling such as bitterness and astringency (Chevalier *et al.*, 2003; Quideau *et al.*, 2005; Vidal *et al.*, 2004). For example, the latter is due to precipitation of phenolic compounds by some proteins in saliva. However, nowadays, there is a lack of knowledge about the mechanism whereby phenolic compounds are transferred from the skin to the wine or from the seed to the wine.

In the past few years, diffusion theory has been advantageously used to describe phenomena occurring in food processing. Therefore, several workers have dealt with a wide range of food such as tomatoes, apples, pears, Gouda cheese…

(Cruz *et al.*, 2008; Derossi *et al.*, 2008; Ho *et al.*, 2006)

This work is a part of a program aiming at improving the extraction of polyphenols during wine-making. Therefore, the first approach focuses on anthocyanins which are the main phenolic compounds present in grape berries. Thus, this work studies the mechanism by which anthocyanins migrate from grape berries skin to wine. More specifically, on one hand, it models the transfer through the skin to alcoholic liquid using a three-dimensional Fickian-based-model by examining two geometric configurations; it solves the governing PDEs by means of a FEM scheme. Next, both outcomes of models are compared to experimental results.

2. Experimental design

Grape berries came from grapevines regularly monitored by our team. Immediately after harvest, phenols extraction was conducted into a hydro-alcoholic mixture in the following way. The freshly peeled skins were cut up in 1 cm x 0.5 cm x 0.15cm parallelepipeds. Each piece of skin was then plunged into a 5 cm high mixture of alcohol contained in glass cylinder (diameter 5 cm). This mixture was made with alcohol (12% ethanol), tartaric acid (3g/l) and SO_2 (100 mg/l). The pH was set to 3.5 and the samples were stored at 20°C in argon atmosphere for 7 days.

For the first 12 hours, absorbance spectrophotometric measurements at 520 nm were carried out every 3 hours to determine the concentration in total anthocyanins.

Governing Equations and Numerical Model

Two geometric configurations were studied in this work. In the first one, anthocyanins were supposed to be distributed uniformly over the skin of fruit; forming a thin parallelepiped layer (Figure 1-a). Conversely, the second configuration is a discontinuous distribution. Anthocyanins were supposed to be located in spherical nodes (Figure 1-b).

Therefore, in each geometric configuration, we dealt with the diffusion in three bodies; the initial anthocyanins location, the skin and the hydro-alcoholic mixture.

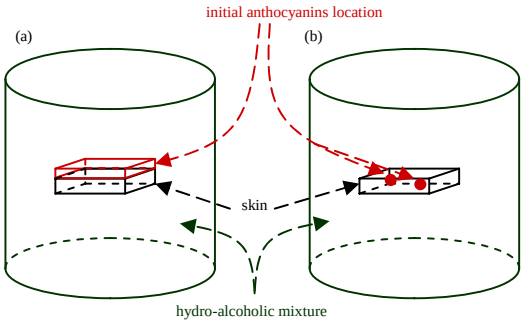


Figure 1 The two studied geometric configuration: (a) the initial anthocyanins location is continuous and constitutes a parallelepiped slab (b) the initial anthocyanins location is discontinuous in spherical nodes.

In both cases, if D_1, D_2 and D_3 represent respectively the diffusivities of the anthocyanins location, the skin and the hydro-alcoholic mixture, the set of governing and boundary equations to be solved was:

$$t = 0; c_1 = c_0$$

$$\frac{\partial c_1}{\partial t} = \nabla \bullet (D_1 \nabla c_1) \text{ inside } \Omega_1$$

$$D_1 \frac{\partial c_1}{\partial n} = D_2 \frac{\partial c_2}{\partial n} \text{ at } \Gamma_{12}$$

$$t = 0; c_2 = 0$$

$$\frac{\partial c_2}{\partial t} = \nabla \bullet (D_2 \nabla c_2) \text{ inside } \Omega_2$$

$$D_2 \frac{\partial c_2}{\partial n} = D_3 \frac{\partial c_3}{\partial n} \text{ at } \Gamma_{23}$$

$$t = 0; c_3 = 0$$

$$\frac{\partial c_3}{\partial t} = \nabla \bullet (D_3 \nabla c_3) \text{ inside } \Omega_3$$

$$\frac{\partial c_3}{\partial n} = 0 \text{ at } \Gamma_3$$

where c_1, c_2 and, c_3 are the concentration in total anthocyanins within the initial anthocyanins location, the peel and the hydro-alcoholic mixture. The initial concentration within the

initial anthocyanins location was set at c_0 whereas it was fixed to zero elsewhere. In both geometric configurations, the c_0 values we used were adjusted so that the total amount of anthocyanins present in the studied system was identical.
The 3D FE simulations were performed using version 3.4 of COMSOL Multiphysics™ software package. Thus, on the basis of the governing PDEs on the 3 parts of the system and the specified boundary conditions, a grid of elements was constructed. The number of nodes in the FE grids was approximately 72900.

Results and Discussion

The models presented here were acquired using as diffusivities values: D_1=7x10^{-9}m^2.s^{-1}, D_2=5x10^{-10}m^2.s^{-1} and, D_3=1x10^{-8}m^2.s^{-1}.

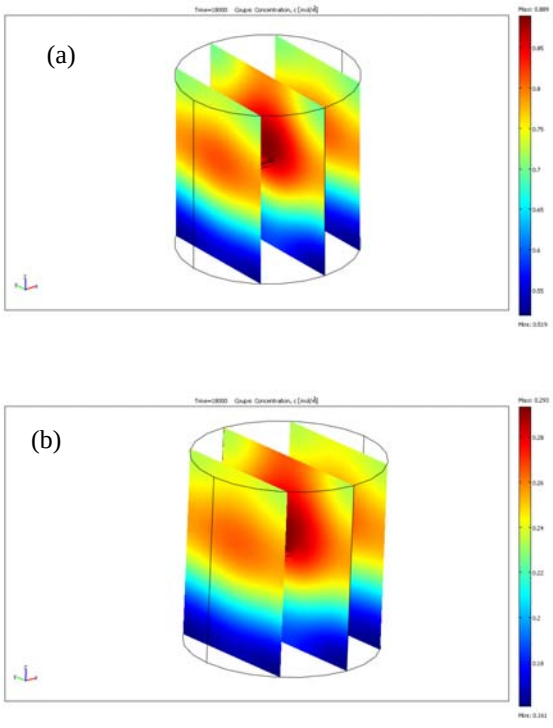


Figure 2 : Concentration distribution after 21600 s for (a) a continuous initial anthocyanins distribution; (b) a discontinuous initial anthocyanins distribution.

These numerical values came from preparatory tests conducted at the laboratory and not yet published.

Figure 2-a and 2-b show the concentration distribution drawn for t = 18000s (5 hours). Both were established for an equal initial quantity of total anthocyanins into the grape skin. We observe that in the first case, the concentration varies from 0 to 0.9 mol.m^{-3} while this interval is from 0 to 0.3 mol.m^{-3} in the second configuration. It is also interesting to notice the shape of the iso-values notably in the central horizontal plan. This "flame of candle" shape is due to the little conductive character of the skin (D_2=5x10^{-10}m^2.s^{-1}) compared to hydro-alcoholic mixture. Thus, the skin almost screens off the anthocyanins source-mixture exchange. Moreover, this screen off effect can be better observed by considering the whole figure. The lowest values are situated in the bottom of the hydro-alcoholic mixture and this asymmetry remains throughout the simulation process.

To compare both models outcomes to experimental results, we calculated the average concentration in the hydro-alcoholic mixture by:

$$\bar{c} = \frac{1}{V}\int_V c \mathrm{d}\Omega$$

The figures 3-a and 3-b show $\bar{c}$ evolution versus time for each of both studied configurations. Experimental values are also plotted on both figures. We can notice the global shapes of the curves are similar. Therefore, both systems seem to behave like a first-order one. However, concerning the maximal and asymptotic values, both models lead to two different orders of magnitude. The first one is much higher than the second one.

As the hydro-alcoholic mixture behaves globally as a first-order-system, this evolution is described using the equation (Fournand *et al.*, 2006):

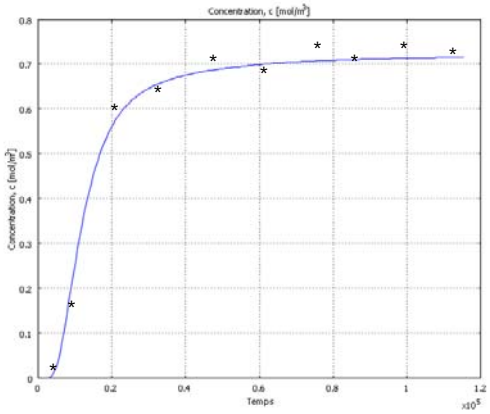
$$\bar{c} = \overline{c_0} + \Delta c(1 - e^{t/\tau})$$

where $\overline{c_0}$ and Δc indicate respectively the initial value of the concentration and the amplitude of its variation during the process. The dynamics of the system is then characterized by the relaxation time τ.

Glancing at the curves suggests to introduce a very short delay time. Thus, the evolution of the concentration was described by the equation:

$$\overline{c} = \overline{c_0} + \Delta c\left(1 - e^{\frac{t-t_0}{\tau}}\right)$$

(a)



(b)

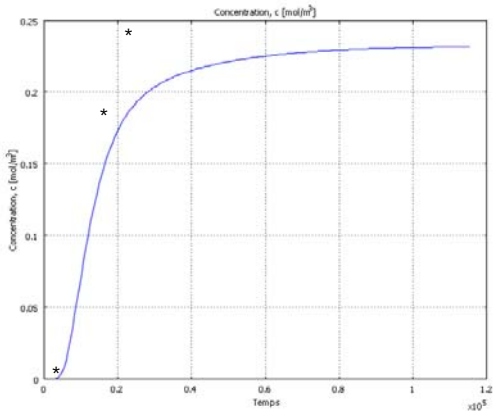


Figure 3: Average concentration in anthocyanins into the hydro-alcoholic mixture versus time for
(a) a continuous initial anthocyanins location;
(b) a discontinuous initial anthocyanins distribution.
Solid lines represent the model. Experimental values are indicated as (*)

Table 1 Fitting values of models and experimental results

	$\overline{c_0}$ mol /m³	Δc	t_0	τ	*RSME*
Continuous distribution	0	0.75	0.05e5	0.15e5	0.02
Discontinuous distribution	0	0.23	0.05e5	0.19e5	0.5
Experimental value	0	0.78	0.06e5	0.14e5	

The fitting was achieved thanks to "Cftool", toolbox of Matlab® (version 7.6.0.324; R2008a). In fact, t_0 value was very low. Visually, the differences between both configurations are convincing; the model corresponding to the continuous distribution of the initial distribution of anthocyanins seems more in compliance with the experimental results. Furthermore, two criteria confirm these observations: the relaxation time and the gap with the experimental values measured by *RSME* such as:

$$RSME = \frac{1}{N}\sqrt{\sum_i (\overline{c}_i - \overline{c}_{iex})^2}$$

where N represents the number of observations, $\overline{c}_i$ and, $\overline{c}_{iex}$ respectively, the values from the model and those from the experiment.

Table 1 sums up all the results. They confirm the previous observation; the continuous configuration leads to values closer to experimental values and *RSME* is much weaker.

Conclusion

This paper presents two numerical simulations the transport of anthocyanins diffusion through the grape berry peel to the wine. Continuous and discontinuous initial anthocyanins distribution are studied. Solving the Fickian-diffusion problem is achieved by using a FEM scheme. Next, both outcomes of models are compared to experimental results.

Findings show that the continuous distribution seems to be more consistent at the mesoscopic scale due to the good accordance between the corresponding model and the experimental values. However, the big variability that is inherent to biological tissue is worth borne in mind. Obviously, these initial results need to be confronted with experimentations involving large number of samples.

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