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Multi-response modeling of reaction-diffusion to explain alpha-galactoside behavior during the soaking-cooking process in cowpea

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Abstract

A modelling approach was developed to better understand the behavior of the flatulence-causing oligosaccharides in cowpea seeds during isothermal water soaking-cooking process. Concentrations of verbascose, stachyose and raffinose were measured both in the seed and in the soaking water during the process ($T = 30$, 60 and 95 °C). A reaction-diffusion model was built for the three considered alpha-galactosides both in the seed and in the soaking water, together with a model of water transport in the seed. The model reproduced coupled reaction-diffusion of alpha-galactosides during the soaking-cooking process with a good fit. Produced, diffused and degraded alpha-galactoside fractions were identified by performing a mass balance. During soaking at 30 °C, degradation predominated (maximum found for raffinose degradation rate constant of 3.22×10^{-4} s^{-1}) whereas diffusion predominated at higher temperatures (95 °C).

Keywords

Alpha-galactosides; cowpea; diffusion; reaction kinetics; cooking.

1. Introduction

Today, increasing food production is not sufficient to achieve food security for the growing population (Tscharntke et al., 2012). A shift towards fewer animal-based and more plant-based diets would be beneficial both from the point of view of climate change and the environment (De Boer & Aiking, 2011). In this context, legumes are excellent candidates to help ensure the sustainability of food systems since they are rich in proteins and need much less water and fertilizer than animals raised for meat (Crews & Peoples, 2004). In terms of physical-chemical composition, legume seeds contain several essential nutritional components. For instance, cowpea, which is extensively cultivated and consumed in the semi-arid regions of West and Central Africa (Langyintuo et al., 2003), is rich in proteins (20-25%), starch (45-55%), and micronutrients including vitamins (folate, thiamin, riboflavin) and minerals (Fe, Zn) (Sreerama, Sashikala, Pratape, & Singh, 2012).

Cowpea is used in over 50 different traditional African dishes in both whole grain and milled forms (Dovlo, Williams, Zoaka, & others, 1976). In Benin, cowpea can be cooked alone or mixed with cereals, roots or tubers, but often requires soaking and cooking in water (Madodé et al., 2011). Soaking often consists in leaving the cowpeas to soak at room temperature overnight before cooking in boiling water for from 40 to 60 min (Madodé et al., 2013). Unfortunately, cowpea seed also contains antinutrients such as alpha-galactosides. The latter accumulate during the development of the seed to prevent desiccation and are metabolized during germination (Peterbauer & Richter, 2001; Obendorf & Gorecki, 2012). Alpha-galactosides are composed of one unit of sucrose and (n) unit(s) of galactose. Cowpea seeds contain three main thermally stable and water-soluble alpha-galactosides: raffinose (0.5-1.0% w/w), stachyose (1.7-6.0% w/w) and verbascose (0.6-1.3% w/w) (n = 1, 2 or 3 galactose units for raffinose, stachyose and verbascose respectively) (Gonçalves et al., 2016). Because humans lack an alpha-galactosidase enzyme, these molecules ferment in the colon and are responsible for flatulence, which creates a consumption bottleneck (Martínez-Villaluenga, Frias, & Vidal-Valverde, 2008; Obendorf & Gorecki, 2012).

However, thanks to soaking and cooking, the amount of alpha-galactosides can be reduced in the seed. Abdel-Gawad (1993) showed that soaking cowpeas at room temperature for 12 h reduced raffinose by 42% and stachyose by 24%. In addition to leaching of alpha-galactosides into the soaking water (Akinyele & Akinlosotu, 1991), (Onyenekwe, Njoku, & Ameh, 2000) reported that endogenous alpha-galactosidase activity also reduces alpha-galactoside content. When cowpeas were cooked at boiling temperature for 60 minutes, Abdel-Gawad (1993) showed a 71% reduction in raffinose and a 42% reduction in stachyose. Similarly, after boiling for 40 min, (Onyenekwe et al., 2000) observed a 47% reduction in raffinose and a 37% reduction in stachyose. In most studies, alpha-galactoside content has only been assessed in the seed, not in soaking water, so the underlying physical-chemical mechanisms involving alpha-galactosides are not explained. Germination occurs during long periods of soaking, and is another way to reduce the proportion of alpha-galactosides by increasing the hydrolysis of alpha-galactosides (Alani, Smith, & Markakis, 1990).

In the literature, only a few models are available that describe behavior of alpha-galactosides during soaking and cooking. Kadlec, Dostalova, Bernaskova, & Skulinova, (2008) modelled the degradation of alpha-galactosides during the germination of legume seeds (mung bean, chickpea, and lentil) using zero-order, first-order kinetic reactions as well as empirical equations. Their results showed that the best fit was obtained using empirical equations, which do not provide any information about the mechanisms underlying alpha-galactoside degradation. Rakshit, Sharma, Saha, & Sarkar (2015) identified the optimal soaking conditions (time, temperature, pH and water-to-seed ratio) that minimized the residual alpha-galactoside content in blackgram seeds using a classical experimental design. In addition to alpha-galactosides, water taken up by the legume seed also needs to be taken into account in the model, especially under the water-limited soaking-cooking conditions often used in Southern countries. For instance, Sopade & Obekpa (1990) described the transport of water in cowpea during soaking using an empirical model (Peleg's equation). To the best of our knowledge, no model available in the literature simultaneously considers mass transport and degradation of alpha-galactosides (and water) that occur during the soaking-cooking process.

The aim of the present study was to establish mass balance kinetics for the three alpha-galactosides between cowpea seed and soaking water at soaking-cooking temperatures of 30 °C, 60 °C and 95 °C to distinguish between diffusive and reactive (degradation or production) parts. The resulting model was used to simulate the cowpea soaking-cooking process and four scenarios were compared on the basis on their ability to diffuse and degrade alpha-galactosides.

2. Materials and methods

2.1 Material

The cowpea cultivar used in the study was the *Wankoun* brownish variety from Benin. It was sowed in November 2014 and harvested in February 2015. The seeds were kept in a vacuum pack and stored at 4 °C in the dark until use. Water (ISO 712: 1998), starch (NF V03-606), protein (NF EN ISO 20483), total dietary fiber (Prosky, Asp, Schweizer, DeVries, & Furda, 1987) and lipid (AOAC 2003.05) contents (expressed in dry basis) were respectively 12.12 ± 0.05 % (w/w db), 41.8 ± 1.6%, 24.0 ± 0.4%, 27.9 ± 0,8% and 1.63 ± 0.01%. The geometrical dimensions of the seed were measured using an electronic caliper and were length z = 8.24 ± 0.63 mm; width r = 6.38 ± 0.48 mm and thickness = 4.25 ± 0.28 mm.

Raffinose, stachyose and verbascose standards (purity ≥ 98%) were purchased from Sigma (USA). Ultrapure water was used throughout the experiments (Simpak MilliQsystem, Millipore, USA). Sodium hydroxide solution (50-52% in water) came from Sigma (Germany), and ethanol (purity ≥ 99.8%) from Honeywell Riedel-de-Häen (Germany).

2.2 Soaking-cooking experiments

Approximately 30 g of seeds were poured into a beaker filled with 120 mL thermally pre-equilibrated soaking water under agitation. The temperatures and times investigated were 30 °C /0.5−38 h, 60 °C/0.5−14 h and 95 °C/0.25−3 h. The bulk soaking temperature was regulated with an accuracy of ± 0.5 °C. To prevent bacterial growth during the long soaking period (≥ 24 h), the seeds were previously bleached in 1% (v/v) sodium hypochlorite for 20 min. The seeds were then rinsed with deionized water for 2 min. After soaking and cooking, the seeds were rapidly removed from the soaking-cooking water and centrifuged at 7 g for 5 min at 25 °C to eliminate residual water. Water content was measured for each experimental condition (i.e. each time and temperature) using twenty-five seeds. The difference between weight mass and dry mass (obtained after drying at 100 °C for 48 h) allowed to calculate water content on a dry basis and thus establish water content kinetics for all the investigated soaking temperatures. The other seeds were then freeze-dried and stored at – 80 °C for a maximum of two months before analysis. Each soaking-cooking experiment was performed in duplicate.

2.3 Alpha-galactoside contents

Freeze-dried seeds were ground with a ZM200 Retsch machine. The particle size of the flour is 0.5mm. Alpha-galactosides were extracted from 80 mg of ground cowpea flour placed in 10 mL of 80% (v/v) ethanol at 80 °C for 30 min (Johansen, Glitsø, & Bach Knudsen, 1996). A single 30-min extraction was sufficient to extract all the alpha-galactosides in the sample. The sample was then centrifuged at 9000 g for 15 min at 4 °C. The supernatant was dried with a Genevac centrifugal evaporator. The chamber was heated at 60°C with an infra-red lamp and the pressure was maintained at 20 mbar. Then the drying was solubilized in 2 mL of deionized water. The extract was then filtered using a 0.45 μm membrane filter. Alpha-galactosides were then separated by High-performance Anion Exchange Chromatography (HPAE) with model LC-20AB pumps and an SIL-20A Autosampler (Shimadzu, Kyoto, Japan), coupled with a PAD Decade 2 detector (Antec Leyden, Netherlands). The sugars were separated at room temperature in a 4 × 50 mm CarboPac PA100 pre-column and a 4 × 250 mm CarboPac PA100 column (Dionex, Germany). The injection volume was 1 µl. The mobile phase was 100 mM sodium hydroxide solution (Gangola, Jaiswal, Khedikar, & Chibbar, 2014). The flow rate was 0.4 ml/min. Raffinose, stachyose and verbascose were quantified using a calibration curve with external standards. Both extraction and measurements were done in triplicate for each soaking-cooking experiment.

~~*2.4 Water uptake kinetics*~~

~~Twenty-five soaked seeds were weighed just after centrifugation at 7 g at 25 °C for 5 min and then dried at 100 °C for 48 h. The difference between the dry and wet weights enabled calculation of water content. All the measurements were done in duplicate.~~

2.4 Starch gelatinization

The ground cowpeas were weighed (around 10 mg) in stainless steel pans. Deionized water (40 µl) was added to the flour and each pan was hermetically sealed. Samples were stabilized for 4 min at 20 °C, then heated from 20 °C to 160 °C at 20 °C.min^{-1}. Thermograms were recorded using a DSC 8500 (Perkin–Elmer, Norwalk, USA). The area under the peaks was integrated and the level of starch gelatinization was calculated by comparing the area under the peak of the sample to the area under the peak of raw cowpea. (Briffaz, Mestres, Matencio, Pons, & Dornier, 2013).

3. Multi-response soaking-cooking modeling

3.1 Assumptions

Two domains are considered in the model: a single cowpea seed (Ω_S) and the soaking water medium (Ω_{SW}). The cowpea seed is assumed to be a pseudo-ellipsoid (figure 1) revolving on a major axis z and having geometrical dimensions that result in a volume equivalent to real seed.

During the isothermal soaking-cooking process, the cowpea seed absorbs water, while alpha-galactosides leach out of the seed and/or are degraded or produced in the cowpea seed and in the soaking water. The alpha-galactosides have a sucrose-galactose(n) structure. For verbascose $\left[Verba\right]$: n = 3 galactoses $\left[Gal\right]$; stachyose $\left[Stach\right]$: n = 2 galactoses and raffinose $\left[Raffi\right]$: n = 1 galactose. Therefore the degradation of 1 mol of verbascose produces 1 mol of stachyose and the degradation of 1 mol of stachyose produces 1 mol of raffinose.

$$\begin{array}{ccccccc} Verba & \overset{k_{verba,\Omega}}{\rightarrow} & Stach & \overset{k_{stach,\Omega}}{\rightarrow} & Raffi & \overset{k_{raffi,\Omega}}{\rightarrow} & Sucrose \\ & \downarrow & & \downarrow & & \downarrow & \\ & Galac & & Galac & & Galac & \end{array} \quad (1)$$

where $k_{verba,\Omega}$, $k_{stach,\Omega}$ and $k_{raffi,\Omega}$ are respectively the rate constants of verbascose, stachyose and raffinose (s^{-1}) in $\Omega_i = \Omega_S$ or $\Omega_i = \Omega_{SW}$. The following assumptions were formulated for the model:

(A1) A seed is considered to be an homogeneous mixture of insoluble dry matter, water and alpha-galactosides.

(A2) The soaking water is assumed to be perfectly stirred.

(A3) The seed does not swell during soaking-cooking process and the shape of cowpea seed is assumed to be symmetrical along the *z* axis.

(A4) Except for alpha-galactosides, no dry matter is lost into the soaking water during the process.

(A5) Water as well as the transport of alpha-galactosides are described by Fick's laws of diffusion.

(A6) The degradation of alpha-galactosides follows first-order kinetics in both the seeds and the soaking water. The seven state variables studied were water content $\left[W\right]_{\Omega_S}$ in the seed, verbascose concentrations $[Verba]_{\Omega_i}$, stachyose concentrations $[Stach]_{\Omega_i}$ and raffinose concentrations $[Raffi]_{\Omega_i}$ expressed in (kg m^{-3}) in seed ($\Omega_i = \Omega_S$) or soaking water ($\Omega_i = \Omega_{SW}$).

3.2. The unsteady diffusion-reaction model

The mass balance equation for water in cowpea seeds (Ω_S) can be written as:

$$\frac{\partial [W]_{\Omega_S}}{\partial t} - \nabla\left(D_W \nabla [W]_{\Omega_S}\right) = 0 \qquad (2)$$

where D_W is the apparent diffusion coefficient of water inside the seed (m^2 s^{-1}). Cowpea seeds contain enzymes, including alpha-galactosidases, that hydrolyze alpha-galactosides by cutting their terminal galactose. Alpha-galactosides can be transported from the seed to the soaking water by diffusion. Convection is neglected because little water is taken up by cowpea seeds, compared to rice, for example. Hence the mass balance equations for alpha-galactosides in cowpea seeds (Ω_S, Eq. (3)) and soaking water (Ω_{SW}, Eq. (4)) can be written as:

$$\left.\begin{aligned}
&\frac{\partial [Verba]_{\Omega_S}}{\partial t} - \nabla\left(D_{Verba} \nabla [Verba]_{\Omega_S}\right) = -k_{verba,\Omega_S} [Verba]_{\Omega_S} \\
&\frac{\partial [Stach]_{\Omega_S}}{\partial t} - \nabla\left(D_{Stach} \nabla [Stach]_{\Omega_S}\right) = k_{verba,\Omega_S} [Verba]_{\Omega_S} - k_{stach,\Omega_S} [Stach]_{\Omega_S} \\
&\frac{\partial [Raffi]_{\Omega_S}}{\partial t} - \nabla\left(D_{Raffi} \nabla [Raffi]_{\Omega_S}\right) = k_{stach,\Omega_S} [Stach]_{\Omega_S} - k_{raffi,\Omega_S} [Raffi]_{\Omega_S}
\end{aligned}\right\} \qquad (3)$$

$$\left.\begin{aligned}
&V_{\Omega_{SW}} \frac{\partial [Verba]_{\Omega_{SW}}}{\partial t} = J_{Verba} - k_{verba,\Omega_{SW}} [Verba]_{\Omega_{SW}} V_{\Omega_{SW}} \\
&V_{\Omega_{SW}} \frac{\partial [Stach]_{\Omega_{SW}}}{\partial t} = J_{Stach} + k_{verba,\Omega_{SW}} [Verba]_{\Omega_{SW}} V_{\Omega_{SW}} - k_{stach,\Omega_{SW}} [Stach]_{\Omega_{SW}} V_{\Omega_{SW}} \\
&V_{\Omega_{SW}} \frac{\partial [Raffi]_{\Omega_{SW}}}{\partial t} = J_{Raffi} + k_{stach,\Omega_{SW}} [Stach]_{\Omega_{SW}} V_{\Omega_{SW}} - k_{raffi,\Omega_{SW}} [Raffi]_{\Omega_{SW}} V_{\Omega_{SW}}
\end{aligned}\right\} \qquad (4)$$

In the case of equations (3) and (4), the concentrations of species X are expressed in mol.m^{-3}. Equations (2), (3) and (4) have the following initial and boundary conditions (with $X = Verba$, *Stach* and *Raffi* and boundaries Γ_i shown in figure 1):

$$[W]_{\Omega_S} = [W]_{\Omega_S}^{\infty} \qquad \text{on } \Gamma_1 \tag{5}$$

$$[X]_{\Omega_S} = [X]_{\Omega_{SW}} \qquad \text{on } \Gamma_1 \tag{6}$$

$$\nabla[X]_{\Omega_S} \cdot \vec{n} = 0 \qquad \text{on } \Gamma_2 \tag{7}$$

$$[X]_{\Omega_S} = [X]_{\Omega_S,0} \qquad \text{in } \Omega_S \text{ for } t = 0 \tag{8}$$

$$[X]_{\Omega_{SW}} = 0 \qquad \text{in } \Omega_{SW} \text{ for } t = 0 \tag{9}$$

The outgoing mass fluxes J_X (kg s^{-1}) of alpha-galactosides crossing the cowpea seed/soaking water interface (Γ_1) can be expressed as:

$$J_X = -AD_X \nabla[X]_{\Omega_S} \tag{10}$$

where D_X is the apparent diffusion coefficient of species *X* (m^2 s^{-1}), V_{Ω_B} is the volume of soaking water (m^3), *A* is the surface area of the seed in contact with the soaking water (m^2), Γ_i is the boundary (i = 1: seed/soaking water interface; i = 2: Seed axial-symmetry), $[W]_{\Omega_S}^{\infty}$ is the equilibrium water content at the seed/soaking water interface, and $[X]_{\Omega_i,0}$ are the initial contents of component *X* in Ω_i. Model input parameters are listed in table 1. In order to perform a mass balance analysis of the diffusion-reaction processes, the cumulative fractions of alpha-galactosides produced by reaction ($m_{X,prod}$), degraded by reaction ($m_{X,degr}$), transferred by diffusion ($m_{X,diff}$) and residual

($m_{X,res}$) expressed in relation to initial mass ($m_{X,0}$) inside the seed, were obtained by solving the following integral equations:

$$\frac{m_{X,prod}}{m_{X,0}} = \int_t \left(\iint_{\Omega_S} k_{X,\Omega_S} [X]_{\Omega_S} \, dV_{\Omega_S} \right) dt \tag{11}$$

$$\frac{m_{X,degr}}{m_{X,0}} = \int_t \left(\iint_{\Omega_S} -k_{X,\Omega_S} [X]_{\Omega_S} \, dV_{\Omega_S} \right) dt \tag{12}$$

$$\frac{m_{X,diff}}{m_{X,0}} = \int_t J_X \, dt \tag{13}$$

$$\frac{m_{X,res}}{m_{X,0}} = \frac{m_{X,0} + m_{X,prod} - m_{X,diff} - m_{X,\deg}}{m_{X,0}} \tag{14}$$

3.3. Numerical solution

The system of four partial differential equations (Eqs (2)−(3)) and of three ordinary differential equations (Eqs (4)) was solved using the FEM-based commercial Comsol Multiphysics™ (version 5.2a, Comsol Inc., Stockholm, Sweden) with the initial conditions given by Eqs. (8) and (9), and boundary conditions given by Eqs. (5)−(7) and Eq. (10) . A 100-element mesh was created in Comsol. Lagrange polynomials (second order function) were the interpolation functions. The linearized problem was solved by the MUMPS time-dependent solver (Multifrontal Massively Parallel Solver) which implements a parallel distributed LU factorization of large sparse matrixes. The maximum time step was 0.05 s and the Jacobian was updated for each iteration. The typical simulation time was five minutes using a 3.25 Gb free memory (RAM) and 3-GHz Intel core Duo CPU computer (32 bits).

3.4. Parameter identification

The three apparent diffusivities (D_i) and three rate constants ($k_{i,\Omega}$) were simultaneously identified by regression analysis using a Bayesian approach. At 30 °C, both in seeds and soaking water, $k_{raffi,\Omega}$ was set to 2 × $k_{stach,\Omega}$ because of expected higher enzyme affinity for raffinose than for stachyose. Indeed, in the case of cowpea, the alpha-galactosidase Michaelis constant K_m for raffinose was found to be 4.6-5.0 mM versus 11-15 mM for stachyose (Alani, Smith, & Markakis, 1989). According to van Boekel, (2009), in the multi-response approach, the best-fit criterion is the minimization of the determinant of dispersion of matrix *C* with the elements:

$$C_{ij} = \sum_{u=1}^{n} \left(\frac{\tilde{X}_u^i - \hat{X}_u^i}{\max\left\{\tilde{X}^i\right\}} \right) \left(\frac{\tilde{X}_u^j - \hat{X}_u^j}{\max\left\{\tilde{X}^j\right\}} \right) \tag{15}$$

where *u* is the index of experimental runs ($u = 1, \cdots, 6$) corresponding to the experimental sampling times, $\tilde{X}_u$ are the experimental data points for experimental run *u*, and $\hat{X}_u$ the values predicted by the model. Here, X are 3 concentrations in the seed domain (Ω_S): $[Verba]_{\Omega_S}$, $[Stach]_{\Omega_S}$, $[Raffi]_{\Omega_S}$ and 3 concentrations in the soaking water domain (Ω_{SW}): $[Verba]_{\Omega_{SW}}$, $[Stach]_{\Omega_{SW}}$, $[Raffi]_{\Omega_{SW}}$. So, *i*, *j* are the indexes of the 6 concentration responses $(i, j = 1, \cdots, 6)$. As shown in Eq. (15), the residuals are evaluated according to the relative difference between the experimental and predicted values. In this approach, not only the sum of squares for each response is taken into account (diagonal elements of matrix *C*) but also the cross products of the responses (covariance). The model parameters were iteratively adjusted to the goodness-of-merit $\min\{\det(C)\}$ using a minimization procedure of the Nelder-Mead simplex with the “fminsearch” function of Matlab software. The standard deviation of each adjusted parameter was determined via Monte Carlo simulations (Hessler, 1997) with 200 draws.

$$X_{noise} = \tilde{X} + \sigma_y \, \delta \qquad (16)$$

where σ_y is the experimental standard deviation estimated for each experimental datum and δ is a random number between 0 and 1 and is normally distributed. The random draw was performed 200 times. The adjusted parameters follow a normal distribution.

The mutual correlation coefficients between adjusted model parameters were also estimated on the basis of the Monte-Carlo results.

4. Results and discussion

4.1. Water uptake kinetics

Figure 2 shows the water uptake kinetics of the cowpea seeds during the isothermal soaking-cooking process. The cowpea seeds reached a lower equilibrium water content at low temperatures (30 °C and 60 °C), than at 95 °C (average of 1.3 ± 0.01 versus 1.55 ± 0.03 kg.kg^{-1} (db)). These results are in agreement with results in the literature. For instance, in two different cowpea cultivars, Kaptso et al. (2008) found an equilibrium water content of 1.3−1.4 kg.kg^{-1} (db) reached after soaking at 35 °C for 6 h. In two other varieties, Taiwo, Akanbi, & Ajibola (1997) observed that cowpea seeds reached an equilibrium water content of about 1.1 kg.kg^{-1} (db) at 60 °C.

Increasing the soaking temperature from 30 °C to 60 °C reduced the time needed to reach equilibrium water content from 10 h to 2 h. This increase in the speed of water uptake was confirmed by the values of adjusted apparent water diffusivities where *Dw* increased 7 fold at 60 °C (8.2±1.1×10^{-10} m^{2}s^{-1}) in comparison with at 30 °C (1.2±0.013×10^{-10} m^{2}s^{-1}) (table2). These values are in the same order of magnitude as those cited in the literature. For example, Kaptso et al. (2008) identified a water diffusivity of 3.90×10^{-10} m^2.s^{-1} for cowpea being soaked at 35 °C.

The *Wankoun* cultivar exhibited starch gelatinization (figure 2) between 68 °C and 90 °C, which was close to the range observed by Okechukwu and Anandha Rao (1996) in another cowpea cultivar. Gelatinized starch has a higher water absorption capacity than native starch, which explains the higher water absorption capacity of cowpea seeds at 95 °C (1.55 ± 0.03 kg.kg^{-1} (db)) than at 60 °C (1.3 ± 0.05 kg.kg^{-1} (db)) as already observed in rice (Briffaz, Bohuon, Méot, Dornier, & Mestres, 2014). In addition, the starch gelatinization phenomenon can explain the lower water mobility, and consequently the lower water diffusivity observed in cowpea seed at 95 °C than at 60 °C (table 2), as also observed in rice (Briffaz et al.,2014). In agreement with the observed range of gelatinization temperatures, no gelatinization was detected during soaking at 60 °C, whereas after soaking at 95 °C for 15 min, 30 min and 1 h, the level of gelatinization was 11%, 42% and 100% respectively. From the point of view of gelatinization, cooking was thus complete after 1 h at a temperature close to boiling, in agreement with the traditional process (Madode et al., 2013).

4.2. Alpha-galactoside kinetics

4.2.1. Experimental changes in alpha-galactosides during the soaking-cooking process

Figure 3 shows the kinetics of the alpha-galactosides contents in both the seeds and the soaking water at soaking temperatures of 30 °C, 60 °C and 95 °C. The initial concentration of verbascose, stachyose and raffinose was 0.48 ± 0.014 g/100g (db), 3.8 ± 0.18 g/100g (db) and 0.38 ± 0.032 g/100 g (db), respectively.

After cooking at 95 °C for 3 h, the verbascose, stachyose and raffinose concentrations in seeds were reduced by 69%, 61% and 63%, respectively. Overall, these losses were transferred in the soaking water and the net contents (sum in the seeds and in the soaking water) remained almost constant during soaking at 95°C. The alpha-galactosides were thus not thermally degraded at 95 °C. These results are consistent with those in the literature. Onigbinde & Akinyele (1983) reported a

46% reduction in raffinose and a 50% reduction in stachyose contents after boiling for 40 min. Ibrahim, Habiba, Shatta, & Embaby (2002) reported that cooking in boiling water for 40 min with a water-to-seed ratio of 10:1 (w/w) resulted in a 100% loss of raffinose and an 81% loss of stachyose.

The data collected at 60 °C followed the same trend as that observed at 95 °C. Indeed, after soaking at 60 °C for 14 h, the concentration of verbascose, stachyose and raffinose in seeds was reduced by 63%, 76% and 74% of their respective initial values, and in most cases, were transferred to the soaking water with no net decrease. However, we observed a slight decrease in stachyose in the soaking water when the soaking period was > 5 h.

When soaked at 30 °C, the seeds germinated after 14 h as evidenced by the emergence of the radicle. After 38 h, the verbascose and raffinose concentrations did not change significantly in the seed, but the stachyose concentration decreased by 35%. The quantity of alpha-galactosides assessed in the soaking water was 10 to a 100-fold lower than in the seeds and, in addition, decreased after 24 h in the case of verbascose, 14 h for stachyose and 4 hours for raffinose. The net decrease in alpha-galactosides observed at 30 °C (29% after 38 h) cannot be imputed to thermal degradation but rather to enzymatic degradation. Endogenous alpha-galactosidase is indeed present in cowpea seeds (Alani et al., 1989), which produce raffinose from stachyose and stachyose from verbascose at low soaking temperatures. This enzyme mainly acts on low molecular weight alpha-galactosides, which explains the limited enzymatic degradation of verbascose. Alani, Smith, & Markakis (1990) also showed that 24 h of germination at 24 °C resulted in a 23% loss of stachyose. Nnanna & Phillips (1988) reported a 16% decrease in stachyose after soaking for 9 h without bubbling. However, the same authors also reported that oxygenation during soaking caused germination and a greater reduction in alpha-galactosides: 24 h of germination degraded 68% of stachyose and 13% of raffinose.

4.2.2 Modeling alpha-galactoside transport properties

Table 2 gives the adjusted apparent alpha-galactoside diffusivities ($m^2.s^{-1}$) in the cowpea seed. The fit of the model was satisfactory with a mean RMSE of about 12 %. At all the soaking temperatures investigated, including 30 °C, raffinose, stachyose and verbascose accumulated in the soaking water, so the adjusted apparent diffusivities were significantly different from zero.

At 30 °C, the adjusted apparent diffusivity was 41 fold higher for raffinose ($9.0 \pm 4.0 \times 10^{-12}$ $m^2.s^{-1}$) than for stachyose ($2.0 \pm 0.6 \times 10^{-13}$ $m^2.s^{-1}$) and 2.6 fold higher for stachyose than for verbascose ($0.8 \pm 0.2 \times 10^{-13}$ $m^2.s^{-1}$). Hence, the adjusted apparent diffusivities were ranked in decreasing order with an increase in the molar mass ($D_{Raffi} > D_{Stach} > D_{Verba}$). In water at 25 °C and infinite dilution, the same rank was observed according to molecular mass: raffinose and stachyose diffusivities have been reported as 4.35×10^{-10} $m^2.s^{-1}$ (Uedaira & Uedaira, 1985.) and 3.80×10^{-10} $m^2.s^{-1}$ (Craig & Pulley, 1962), respectively. By extrapolation with the Stoke-Einstein equation (assuming that the hydrodynamic radius of a molecule is inversely proportional to the cubic root of its molar mass), the verbascose diffusivity in water at 25 °C and infinite dilution was estimated at 3.15×10^{-10} $m^2.s^{-1}$ which is lower than for stachyose. In contrast, adjusted values of apparent diffusivities in the seed were much lower (factor of 50 to 4,000) than in pure water at 25 °C and infinite dilution. This is due to the cellular structure of cowpea seed that impedes the molecular mobility of alpha-galactosides. The cowpea coat is also a limiting factor to the diffusion of molecules, and dehulling could improve alpha-galactoside diffusivity (Madode et al., 2013). In the case of legumes, the spatial distribution of alpha-galactosides in the seed can be heterogeneous (Sreerama, et al. 2010). This may partly explain the difference between adjusted apparent diffusivities of each alpha-galactoside.

Temperature has a major impact on the diffusion of alpha-galactosides. Shifting from 30 °C to 95 °C resulted in a marked increase in their apparent diffusivities (table 2). For instance, verbascose apparent diffusivity increased from $0.8 \pm 0.2 \times 10^{-13}$ m^2.s^{-1} to $6.5 \pm 2.9 \times 10^{-11}$ m^2.s^{-1}. These temperatures are not the usual physiological conditions encountered by seeds and hence result in the degradation of the seed.

Due to high experimental standard deviations, the standard deviations of model parameters (including apparent diffusivities) that were estimated by means of Monte Carlo procedure are quite large. This noise may cause interference and hence render data interpretation more difficult.

4.2.3 Modeling alpha-galactoside reactivity

Table 2 gives the adjusted reactivity rate constants of the alpha-galactosides (expressed in s^{-1}) both in the seed and in the soaking water at soaking temperatures of 30 °C, 60 °C and 95 °C.

At 30 °C, non-null rate constants were found for all the galactosides in the soaking water. The adjusted degradation rate constant was 6.4 fold lower for verbascose (0.5×10^{-4} s^{-1}) than for raffinose. This is logical given the affinity of enzyme for these different substrates (see section 4.2.1). Indeed, alpha-galactosidases hydrolyze low molecular weight oligosaccharides, especially raffinose, more rapidly than higher molecular weight homologs (Peterbauer & Richter, 2001). In the same way, constraining k_{raffi,Ω_i} to be equal to $2\times k_{stach,\Omega_i}$ resulted in a good fitting performance. This is again due to the fact that, as explained in section 3.4, raffinose affinity is around twice that of stachyose (Alani, Smith, & Markakis, 1989). These results confirm that these rate constants are closely linked to endogenous alpha-galactosidase activity in both the seed and the soaking water. Moreover, it is interesting to note that at 30 °C, and irrespective of the alpha-galactoside considered, adjusted rate constants were much higher in the soaking water than in the seed. For example, the adjusted raffinose rate constant in the soaking water was 55 fold higher than in the seed. This may be explained by a molecular crowding effect in seeds that decreases the likelihood of a meeting between an enzyme and the substrate (Van Boekel, 2009).

At T = 60 °C, the adjusted rate constants in the soaking water and for any alpha-galactoside were lower than at 30 °C. For example, the rate constant of stachyose was $0.12\times10^{-4} \pm 0.04\ s^{-1}$ at 60 °C versus $1.6\times10^{-4} \pm 0.4\ s^{-1}$ at 30 °C. This result is in agreement with the optimal temperature range for endogenous alpha- galactosidase activity in legume seeds. Dey & Pridham (1969) reported that the alpha-galactosidase activity of *Vicia Faba* reached maximum at 45 °C and that, beyond this temperature, activity decreased until it stopped at 75 °C. Bhaskar, Ramachandra, & Virupaksha (1990) also found an optimal temperature around 50 °C for endogenous alpha-galactosidase originating from *Cassia Sericea SW.* seed with a marked decrease in enzyme activity at temperatures > 50 °C.

At 95 °C, the rate constant was fixed to zero both for seed and soaking water since we demonstrated that above this temperature, the total alpha-galactoside mass lost by the seeds was fully quantified in the soaking water (i.e. a pure diffusion process).

It is worth mentioning that, regarding the low alpha-galactosides concentrations in soaking water, particularly at 30 °C, assuming first-order kinetics (assumption A6) is equivalent to considering the Michaelis-Menten approach. Indeed, when $S << K_m$, the velocity of the enzymatic reaction is proportional to the concentration of S in the substrate with a constant of proportionality of $v_{\max} / K_m \approx k$.

The use of simplified first-order kinetics was a good way to explore the reactivity of alpha-galactosides in the context of a soaking-cooking process. But to optimize the enzymatic degradation of alpha-galactosides, cowpea alpha-galactosidase and the parameters that enable greater enzyme activity during soaking need to be explored. This work is currently in progress.

4.2.4. Alpha-galactosides: reaction vs. diffusion

Figure 4 shows changes in the predicted diffused (from the seed to soaking water), degraded, produced and residual alpha-galactoside fractions in the cowpea seeds as a function of soaking time and temperature. At 95 °C and 60 °C, the diffusion (Eq. (13)) phenomenon predominated, whereas at 30 °C, the production (Eq. (11)) and degradation (Eq. (12)) phenomena predominated, particularly for raffinose.

At 30 °C, the model demonstrated high turnover of raffinose; production due to the degradation of stachyose (as the initial quantity of stachyose was higher than that of raffinose, significant quantities of raffinose were formed in the seeds), representing 286% of initial raffinose content after 38 h, while at the same time, 231% of the initial concentration of raffinose diffused in the soaking water and 71% was degraded. As a result, the net concentration of raffinose in the seeds remained almost the same during soaking (85% of initial value after soaking for 38 h). In the case of stachyose and in the same soaking conditions (30 °C for 38 h), 46% of initial content disappeared and two thirds of the losses were due to the degradation phenomenon. The proportion of verbascose degraded, and consequently the production of stachyose, was negligible, while the reaction rate constant of verbascose was only 3 fold lower than for stachyose. This was linked to the much lower initial concentration of verbascose in seeds (10 times lower than stachyose); the reaction rate was thus 30 fold lower for verbascose than for stachyose. As a whole and according to the model, 8% of the initial alpha-galactosides was degraded into galactose and sucrose in the cowpea seed after soaking for 38 h at 30°C while 24% was degraded in the soaking water, resulting in a net degradation of 32%.

At 60 °C and 95 °C, neither degraded nor produced fractions of alpha-galactosides were detected in the seed, and the diffused fractions were much higher than at 30 °C. For instance, in the case of stachyose with a soaking time of 3 h, only 2.2% of initial content leached out of the seed at 30 °C whereas up to 54.3 % and 61.5 % of stachyose initial content leached out of the seed at 60 °C and 95 °C, respectively. This result confirms the major impact of temperature on the apparent molecular diffusivity of alpha-galactosides identified by the model (see section 4.2.2). As a consequence, the total diffused alpha-galactoside fraction was found to increase by a factor of 13.6 from 30 °C to 95 °C and after a soaking time of 3 h.

Constitutively, as shown in Eqs. (3) and (4), the model simultaneously accounts for two phenomena (mass transport and first-order reactions) that both reduce alpha-galactoside content in the seed. To get an idea of the degree of correlation between the adjusted model parameters considered two-by-two, the correlation coefficients were calculated on the basis of Monte Carlo results. The average correlation coefficient between parameters (rate constants and apparent diffusivities) was < 0.21, meaning that the model parameters we identified are quite independent. This can be explained by the fact that, by minimizing of the determinant of dispersion of matrix *C*, the method used for the identification of the model parameters limits the level of correlation between parameters.

4.3. Soaking-cooking simulations

In West African countries like Benin, cowpea seeds are traditionally prepared using two different methods: either by soaking seeds for 12 h followed by cooking for 25 min (method n°1), or by directly cooking the seeds in boiling water for 1 h with a limited water-to-seed ratio (method n°2) (Madodé, 2012). The total initial alpha-galactosides content in *Wankoun* cowpea seeds in our study was 4.66 kg/100kg (db). Different cooking processes were simulated using the model, and the final predicted alpha-galactoside concentrations both in the seeds and in the soaking water (expressed in kg/100kg (db)) were generated (see the Supplementary Table 2 for details). For processes including a draining step and for a given water-to-seed ratio, an additional volume of fresh water was used immediately after draining such that all water was absorbed by the seed. We also assume that a draining step removes all the alpha-galactosides present in soaking water. For low water-to-seed ratios, the residual volume of water at the end of processes estimated by the model, was assumed to be a highly concentrated solution of alpha-galactosides that adsorbed onto the seeds and was hence consumed.

Process 1 was cooking for one hour at 95 °C using a water-to-seed ratio of 4:1. In process 1, the large quantity of water (342 ml/100 g (db)) remaining after cooking was thrown away. After process 1, a residual concentration of 2.73 kg/100 kg (db) in the seeds and of 1.93 kg/100 kg (db) in the soaking water were observed. The expected consumption of alpha-galactosides in process 1 is 2.73 kg/100kg (db), that is, 59% of initial alpha-galactosides mass.

Process 2 resembled traditional method n°2, i.e., cooking for one hour at 95 °C with a low water-to-seed ratio (1:1) during which the water is completely absorbed by the seeds to reach equilibrium. After the traditional process n° 2, the final alpha-galactosides concentration in the seeds (2.85 kg/100 kg (db)) and soaking water (1.81 kg/100 kg (db)) was not much different from that in process 1. But as the water-to-seed ratio is much lower than in process 1, the final quantity of residual water that contains alpha-galactosides is consumed with the seeds. Thus, in this case, the expected consumption of alpha-galactosides is 4.66 kg/100 kg (db), i.e. 100% of the initial quantity of alpha-galactosides.

Process 3 was pre-cooking at 95 °C for 30 min using a low water-to-seed ratio of 1:1 (water fully absorbed by seeds to reach equilibrium) followed by boiling for 30 min. In the model, the soaking water was drained off between the pre-cooking and cooking steps. Process 3 resulted in a final concentration of alpha-galactosides in seeds of 2.75 kg/100 kg (db) and 0.54 kg/100 kg (db) in the soaking water. Draining after pre-cooking was the reason why the final alpha-galactosides content (seeds + residual soaking water) was lower than in process 2 (4.66 kg/100 kg (db)). Like in process 2, the ratio was limiting and the expected consumption of alpha-galactosides present in the seeds and residual water was 3.29 kg/100kg (db), i.e. 71 % of initial alpha-galactoside content.

Process 4 was close to traditional method n°1: soaking at 30 °C for 12 h using a low water-to-seed ratio of 1.2:1 (the water is fully absorbed by seeds to reach equilibrium) followed by draining and then cooking at 95 °C for 25 min. After process 4, the final concentration of alpha-galactosides in seeds was 3.09 kg/100 kg (db) and that in the soaking water was 0.86 kg/100 kg (db). In this process, the expected consumption of alpha-galactosides was 3.95 kg/100 kg (db), i.e. 85% of the initial concentration. This loss was essentially due to degradation by reaction (fig 4).

In parallel, these four processes (1 to 4) have been performed experimentally in order to assess total consumed alpha-galactoside content. The experimental results were close to the simulations with a mean relative deviation of 8.4%. The highest deviation was found for process 3 (24%). This might be attributed to the following reasons. In the simulation, leached alpha-galactoside content was always reset at zero after each draining step, which is not really the case for a draining performed experimentally at low water-to-seed ratio ratio (1:1 (w/w)), because of residual adsorption around the seeds. In addition, a high soaking temperature promotes diffusion out of the seed and draining (unlike rinsing) cannot remove this quantity from seed surface.

The most efficient way to minimize alpha-galactosides consumption is thus to profit from the diffusion of these molecules in the cooking water by discarding the excess cooking water (processes 1 and 3). The price to pay for this solution is however both loss of flavor of the cooked cowpea and of nutrients. Process 3 could be a compromise; precooking and changing cooking water before the end of cooking, is often used to cook hard-to-cook legume seeds. Another way is to enhance the enzymatic degradation of alpha-galactosides as observed at low soaking temperature (process 4).

5. Conclusion

Our study advanced our understanding of the contrasted behavior of alpha-galactosides in cowpea seeds as a function of the soaking-cooking conditions. A 2D axi-symmetric model was developed, considering a single cowpea seed being soaked in a limited amount of water. This approach simultaneously accounts for the diffusion and reaction of alpha-galactosides as well as for its transport of water. At higher temperatures (60 °C and 95 °C), the diffusion process predominated and no thermal degradation was observed. At a low temperature (30 °C), and assuming first-order reactions, the model satisfactorily described the production and degradation of alpha-galactosides both in the seed and in the soaking water, probably due to endogenous alpha-galactosidase activity. However, in these conditions, the diffusion of alpha-galactosides from the seed to the soaking water was very slow. The resulting model was then used to simulate some soaking-cooking scenarios with regards to final alpha-galactoside content. The simulations showed that including a draining step is one way to reduce the quantity of alpha-galactosides consumed. Another efficient way to reduce alpha-galactoside content is to use a relatively long soaking step at a low temperature to promote enzymatic degradation. Characterizing and modeling alpha-galactosidase activity as a function of the processing conditions, will enable the identification of optimized pathways to enhance enzymatic activity and hence reduce alpha-galactoside content more efficiently. This work is now in progress.

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Figure captions

Fig 1: (a) Microtomography of cowpea seed. (b) Simplified geometrical representation of cowpea seed (Ω_S) assuming a pseudo-ellipsoidal and symmetrical shape with specific dimensions (unit: 10^{-3}m). Ω_{SW} represents the soaking water domain.

Fig 2: Experimental (dots) and predicted (lines) water uptake kinetics (water content expressed on a dry basis) of cowpea seeds during the coaking-soaking process at 30 °C, 60 °C and 95 °C. Error bars represent the standard deviation (n = 4). The degrees of gelatinization (τ) are indicated for T = 95 °C.

Fig 3: Predicted (lines) and experimental data (symbols) for concentrations of raffinose [Raffi], stachyose [Stach], and verbascose [Verba] and total alpha-galactoside [Total] in cowpea seeds and in the soaking water (both expressed as g/100g on a dry basis) during the soaking-soaking process at 30 °C, 60 °C and 95 °C. Error bars represent standard deviations (n = 3).

Fig 4: Predicted algebraic residual, produced, diffused and degraded mass fraction kinetics for verbascose [Verba], stachyose [Stach] and raffinose [Raffi] species and total alpha-galactoside [Total] in cowpea seed during the soaking-cooking process at 30 °C, 60 °C and 95 °C.

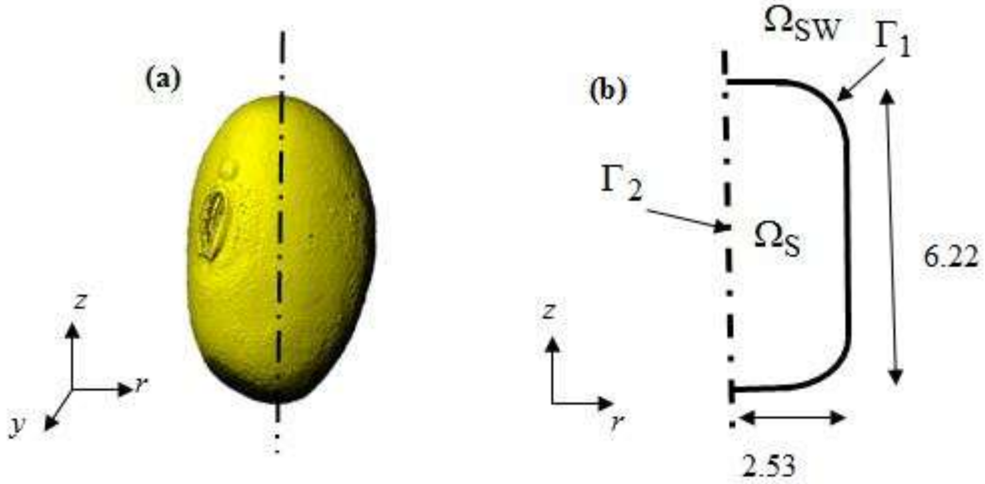
Table 1. Input parameters used in the 2D-axisymmetric reaction-diffusion model.

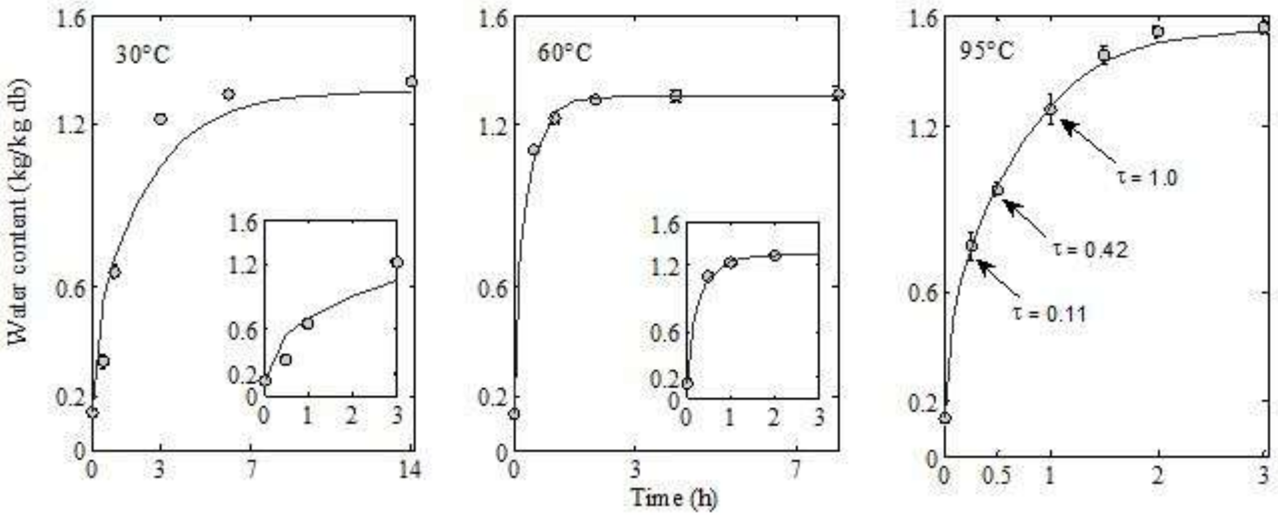
Table 2. Estimated apparent diffusion coefficients (D_X) and rate constants $(k_{X,\Omega_S}, k_{X,\Omega_B})$ for each alpha-galactoside in cowpea seeds (Ω_S) and in the soaking water (Ω_{SW}) at different soaking-cooking temperatures (T) (mean values ± standard deviations determined with Monte-Carlo simulations: 200 sets).

Supplementary Table1: Nomenclature.

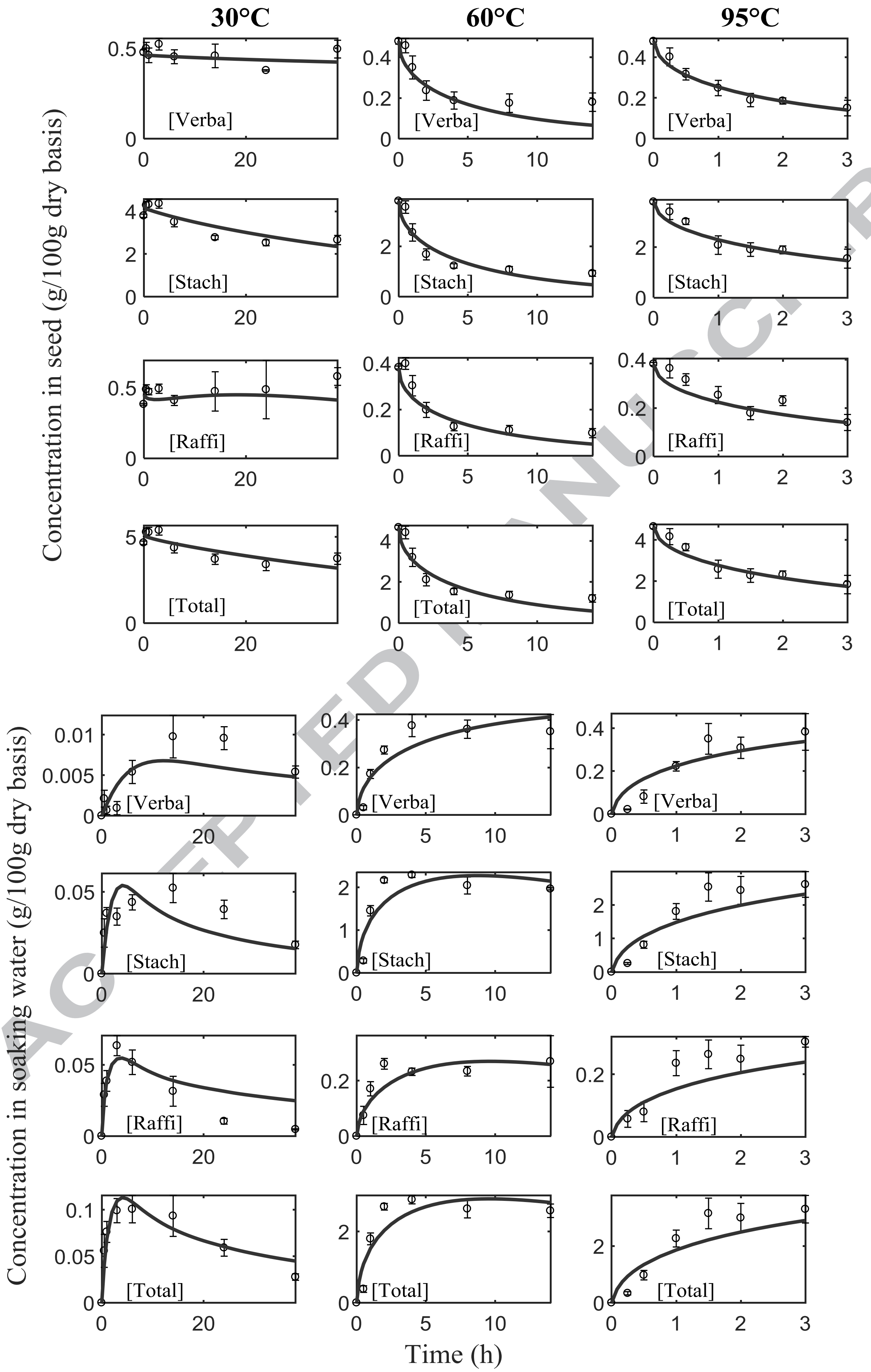
Supplementary Table2: Total alpha-galactoside contents (raffinose+stachyose+verbascose) in the seed and in the soaking water, and expected consumption determined by simulation and experimental assesment of different soaking-coaking processes. Process 1: cooking at 95 °C for 1 h (ratio 1:4 (w/w)). Process 2: Cooking at 95 °C for 1 h (ratio 1:1 (w/w)). Process 3: Pre-cooking at 95 °C for 30 min (ratio 1:1) followed by draining, and cooking at 95 °C for 30 min using the same volume of water as the discarded soaking water. Process 4: Soaking at 30 °C for 12 h (ratio 1:1 (w/w)) followed by draining, and cooking at 95 °C for 25 min using the same volume as the discarded soaking water.

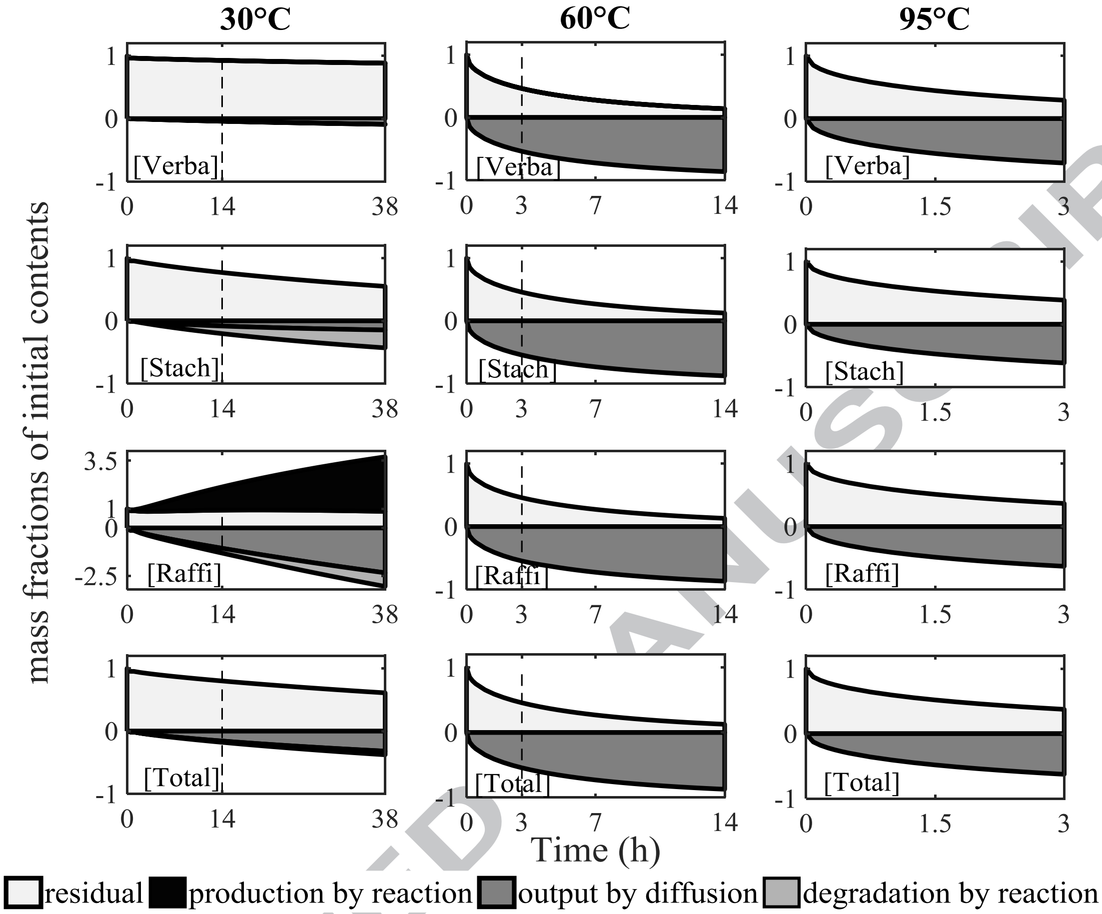
Supplementary Table3 : Predicted and experimental data for concentrations of raffinose [Raffi], stachyose [Stach], and verbascose [Verba] and total alpha-galactoside [Total] in cowpea seeds and in the soaking water (both expressed as g/100g on a dry basis) during the soaking-soaking process at 30 °C, 60 °C and 95 °C. (mean values ± standard deviations determined with (*n* = 6).


(a)
z
r
y
(b)
Ω_{SW}
Γ_1
Γ_2
Ω_S
6.22
z
r
2.53



Water content (kg/kg db)
Time (h)
30°C
60°C
95°C
τ = 1.0
τ = 0.42
τ = 0.11


30°C
60°C
95°C
Concentration in seed (g/100g dry basis)
[Verba]
[Stach]
[Raffi]
[Total]
Concentration in soaking water (g/100g dry basis)
Time (h)


30°C
60°C
95°C
mass fractions of initial contents
[Verba]
[Stach]
[Raffi]
[Total]
Time (h)
residual
production by reaction
output by diffusion
degradation by reaction

parameter	value	unit
$\left[Raffi\right]_{\Omega_{S,0}}$	4.44	$kg.m^{-3}$
$\left[Stach\right]_{\Omega_{S,0}}$	44.4	$kg.m^{-3}$
$\left[Verba\right]_{\Omega_{S,0}}$	5.61	$kg.m^{-3}$
ρ_{DM}^{0}	1302	$kg.m^{-3}$
ρ_{w}^{0}	1000	$kg.m^{-3}$
$\left[W\right]_{\Omega_S,0}$	150	$kg.m^{-3}$
V_{Ω_S}	$1.09*10^{-7}$	m^3

Species X	T (°C)	$D_X \times 10^{11} (m^2 s^{-1})$		$k_{X,\Omega_S} \times 10^6 (s^{-1})$		$k_{X,\Omega_{SW}} \times 10^4 (s^{-1})$		$10^2 \times$ RMSE* In seed (S)	$10^2 \times$ RMSE* In soaking water (SW)
Verbascose	30	0.008	0.002	0		0.5	0.2	5.4	0.3
	60	3.0	1.0[a]	0		0		7.3	6.9
	95	6.5	2.9	0		0		4.6	8.1
Stachyose	30	0.02	0.006	2.9	0.8	1.6	0.4	41.0	1.4
	60	3.0	0.9[a]	0		0.12	0.04	43.0	41.0
	95	4.3	2.0	0		0		39.0	56.0
Raffinose	30	0.9	0.4	5.8	1.5	3.2	0.7	10.0	1.7
	60	3.0	1.0[a]	0		1.1	0.4	5.8	6.7
	95	4.6	2.0	0		0		5.3	6.9
Water	30	12	0.5					12.0	
	60	82	11.0					3.6	
	95	43	2.0					1.8	

Values with same letters (a) were not significantly different ($p < 0.05$)

*RMSE: Root mean square error between experimental and predicted concentrations. Units: $(kg/100kg\ db)$ for alpha-galactosides; $(kg/kg\ db)$ for water.

Highlights

- A model describes α-galactoside behavior during cowpea soaking-cooking process
- No thermal degradation of α-galactosides was observed even at boiling temperature
- Overall, adjusted α-galactoside apparent diffusivities increased with temperature
- At 30 °C and 60 °C, rate constants were identified in the seeds and soaking water
- This approach can be used to identify scenarios that minimize α-galactoside content