CHANGE IN TASTE OF TOMATO DURING THERMAL PROCESSING AND DRYING: QUANTITATIVE ANALYSES ON ASCORBIC ACID AND PYROGLUTAMIC ACID

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Abstract: Ascorbic acid and pyroglutamic acid (PCA) are important chemical markers for the nutritional quality and taste of concentrated and dried tomato products, respectively. Especially PCA contributes to the perceived loss of fresh taste and brings bitterness and sourness to the product. During thermal processing, ascorbic acid degrades while PCA is formed from the reaction between glutamic acid and glutamate. The quantitative changes of the acids during thermal processing are affected by both temperature and moisture content. While increase of temperature accelerates both reactions, decrease in moisture content retards ascorbic acid degradation, but enhances PCA formation. The developed kinetic models were applied in combination with a process model to evaluate the effect of co- and counter-current air drying on ascorbic acid and PCA levels in tomato-based products. This approach can offer opportunities for process optimization towards taste and nutritional quality.

Keywords: Kinetic modelling; Organic acids, Tomato, Taste, Thermal treatment

Introduction

Tomato (*Lycopersicum esculentum*) is amongst the most popular consumed vegetables globally [1]. Tomato is considered a useful source of fibres, proteins, minerals, vitamins, lycopene, and antioxidants and thus fits in a healthy diet [2-5]. Tomatoes are consumed fresh and incorporated in processed foods, such as juice, puree, sauce, canned varieties and dried products [6-8]. The processing of tomatoes into dried ingredients should have minimum effect on perceived freshness and taste. Numerous studies have reported on the impact of processing on tomato quality specifically in terms of nutritional quality and colour retention. Only few studies focused on retention of non-volatile taste characteristics, such as sour taste. The sour taste of tomato is an important organoleptic quality attribute connected to perceived freshness and is found related to the presence of specific organic acids [8-10].

One of the most abundantly present organic acids in tomatoes is ascorbic acid, which has been considered to play important roles in nutritional and taste retention of processed tomato products [10, 11]. Ascorbic acid, also known as vitamin C, is well known for its sensitivity towards heat and presence of oxygen and degrades during processing and storage [12, 13]. The degradation may be characterised as a thermo-accelerated oxidation. During thermal processing, some organic acids are also formed, which results in an overall increase of the total amount of organic acid. An important organic acid with large influence on taste in tomato products is 5-oxopyrrolidine-2-carboxylic acid, also named pyroglutamic acid (PCA). PCA is the reaction product of glutamine or glutamic acid [11, 14, 15]. The formation route of PCA in tomato juice can be both enzymatic and non-enzymatic. The enzymatic reaction is facilitated by γ-glutamylcysteine synthetase (γ-GCS) and Glutamate-5-Kinase (G5K) [16-19]. The non-enzymatic reaction is catalysed in a weak acid environment and is enhanced at elevated temperatures [20, 21]. Formation of PCA contributes to the perceived loss of freshness and gives the product a bitter and undesirable sour taste, and thereby leads to offflavour of processed tomato [8, 10]. Quantitative understanding of the changing levels of ascorbic acid and PCA in tomato products can provide better control to retain taste during processing.

Specifically, in this study we focus on kinetic modelling of the degradation of ascorbic acid and the formation of PCA to optimise heating and drying processes of tomatoes. In most previous studies the degradation of ascorbic acid and the formation of PCA were described by first order kinetics as a function of temperature only [22-24]. Major challenge here is to extend the kinetic modelling approach to describe the combined effect of temperature and moisture content. Only few studies attempted to do so for ascorbic acid in the case of kiwifruits during storage and during air drying of the fruit rosehip [23, 25], but no studies on tomato processing were carried out. To the best of our knowledge kinetic models for PCA formation have not yet been proposed considering the effect of both temperature and moisture content.

Therefore, the objective of this study is twofold: (1) to experimentally assess the levels of ascorbic acid and PCA of tomato after thermal processing; (2) to develop kinetic models that can predict ascorbic acid degradation and PCA formation and include dependency on both temperature and moisture content.

Mathematical models

A first order kinetics is applied to describe ascorbic acid degradation and PCA formation:

$$-\frac{C_{AA,t}}{C_{AA,0}} = \exp(-k_{AA}t) \qquad \text{Eq. 1}$$

$$C_{PCA,t} = -C_{Glu,0}\,(1-\exp(-k_{PCA}t)) \qquad \text{Eq. 2}$$

where $C_{AA,t}$ and $C_{PCA,t}$ are the concentrations of ascorbic acid and PCA after a specific time, respectively; $C_{AA,0}$ is the initial concentration of ascorbic acid in tomato; $C_{Glu,0}$ is the the total initial concentration of glutamic acid and glutamine in tomato; t is time and k is the reaction rate constant (s^{-1}).

The dependency on temperature and moisture content of both reactions can be described with the following modified Arrhenius equation [26]:

$$k(T,X_w) = k_w(T)\exp\left[\ln\left(\frac{k_s(T)}{k_w(T)}\right)\cdot\exp\left(-p\frac{X_w}{1-X_w}\right)\right] \qquad \text{Eq. 3}$$

where X_w is the mass fraction of water and p is an empirical parameter describing the effect of moisture content on the reaction rate constant. Specially, $k_w(T)$ and $k_s(T)$ are the reaction rate constants at infinite dilution (X_w=1) and in pure solid form (X_w=0), respectively, and can be expressed as a function of temperature:

$$k_w(T) = k_{ref,w}\exp\left[-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right] \qquad \text{Eq. 4}$$

$$k_s(T) = k_{ref,s}\exp\left[-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right] \qquad \text{Eq. 5}$$

The kinetic model includes four fitting parameters, namely$k_{ref,w}$, $k_{ref,s}$, E_a and p. It describes the combined effect of temperature and moisture content, which is especially relevant during drying processes. As an example, a co- and countercurrent air drying model was implemented in the present work [27]. The developed kinetic models are used to evaluate the changes on ascorbic acid and PCA levels during the air drying of a tomato-based product.

Materials and Methods

Frozen cut tomato cubes were kindly provided by Unilever (Vlaardingen, Netherlands). The cubes, with a volume of approximately 1 cm3, were blended by an electronic blender (Vorwerk Thermomix Tm 31, Vorwerk, Wuppertal Germany) and squeezed into tomato juice. The moisture concentration of the juice was 94.5 w/w%. To vary moisture contents of the tomato juice with minimized thermal influence, the squeezed tomato juice was concentrated by freeze drying. By varying the freeze-drying time, the tomato juice was concentrated to different moisture contents of 85.2 w/w%, 60.0 w/w%, 51.4 w/w%, 18.7 w/w% and 13.1 w/w%.

Samples were pipetted into 5 mL vials, sealed and heated in an Eppendorf Thermomixer®C (Eppendorf, Hamburg Germany), at a desired temperature, with combined mixing performance and accurate temperature control. The temperature range of the experiments in this study was between 60 and 100 ˚C and the mixing speed was fixed at 300 rpm. After heating for the required time, the vial was transferred immediately into an ice bath, in order to quickly decrease the temperature. Subsequently, the sample was used for extraction of organic acids. The extraction method for organic acids from tomato juice was used as described by Selli et al. (2014) [28]. The first step involved addition of 25 g of a 25 mmol/L KH_2PO_4 buffer (adjusted to pH 2.5 by H_3PO_4) to a 5g tomato sample and subsequent mixing at 300 rpm for 10 min to homogenise the sample. The homogenised mixture was centrifuged at 12,000 g and 10 ˚C for 8 min. Then the supernatant was filtered through a 0.45 µm Minisart® Syringe filter (Sartorius, Goettingen, Germany) and used for HPLC analysis.

An UltiMate® 3000 HPLC system equipped with a diode array detector (DAD) (Dionex, Dreieich, Germany) was used to simultaneously separate and detect organic acids. The system was run at 1.0 mL/min using a PrevailTM Organic Acid column with 150mm x 4.6 mm and 5 µm particle size (Grace, USA). The column temperature was maintained at 30 ˚C and the organic acids were detected with the DAD at a wave length of 210 nm. The injection volume was 5 µL. The mobile phase was a 25 mmol/L KH_2PO_4 buffer adjusted to pH 2.5. Peaks areas were quantified by comparison to peak areas of external standard peaks of reference organic acids. The concentrations of organic acids are expressed as mmol per gram Fresh Tomato (mmol/g FT) as the moisture content of fresh tomatoes is assumed constant.

Results and Discussion

The concentrations of ascorbic acid and PCA were followed during isothermal heating experiments. The initial concentrations of ascorbic acid in squeezed tomato juice were found to vary between 0.00036 and 0.00045 mmol/g FT. Figure 1 shows the normalized ascorbic acid and PCA concentrations as a function of heating time. The solid lines represent the kinetic models fitted to the experimental data (Eq. 1 and Eq. 2). It can be observed that the kinetic models fit the experimental data well, indicating that a first order kinetic model is a valid approach to describe both reactions of organic acids.

In **Erreur ! Source du renvoi introuvable.** the reaction rate constants of ascorbic acid degradation and PCA formation are shown for different combinations of temperature and moisture content. The reaction rate constants of ascorbic acid and PCA were calculated with **Erreur ! Source du renvoi introuvable.** and Eq. 2, respectively. The value of $C_{Glu,0}$ (0.032 mmol/g FT) representing the total concentration of glutamic acid and glutamine in the tomato juice was estimated from literature data [29, 30].

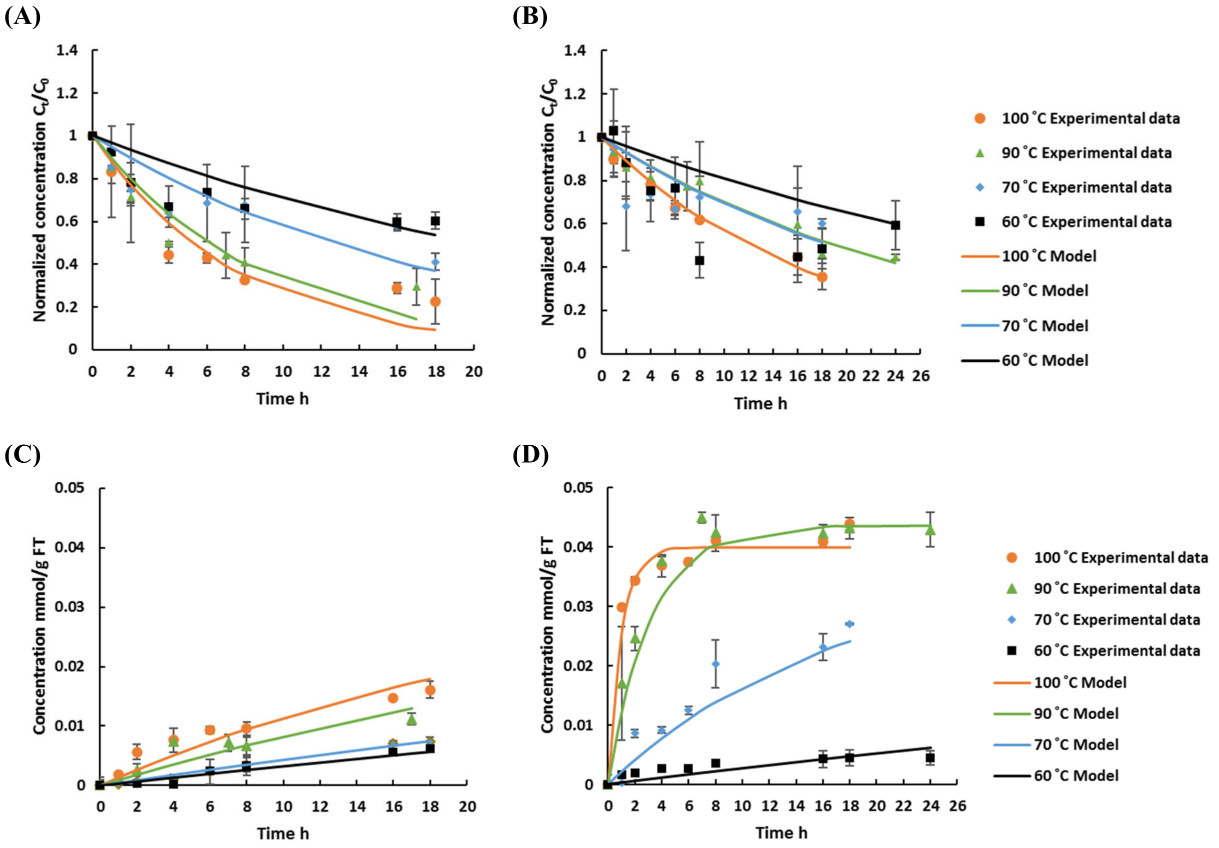


Figure 1 The concentration of ascorbic acid in tomato samples with (A) Xw = 0.95 and (B) Xw = 0.18; and PCA in tomato samples with (C) Xw = 0.95 and (D) Xw = 0.19, during heating. The error bars show the 95% confidence interval of the experimental data.

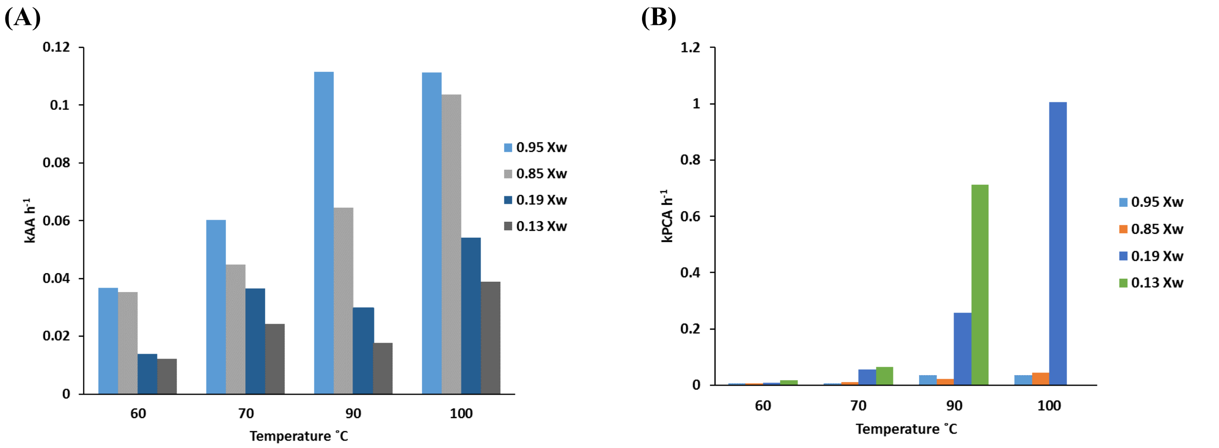


Figure 2 Reaction rates of: (A) ascorbic acid and (B) PCA at specific combinations of temperature and moisture content derived from experimental data.

In **Erreur ! Source du renvoi introuvable.** (A) it can be observed that the reaction rate constant of ascorbic acid degradation increases with increasing temperature. This may be explained because at higher temperature the reaction between ascorbic acid and oxygen is enhanced with increasing molecular mobility and collision frequency. The stability of ascorbic acid depends also on the moisture content of tomato juice. In **Erreur ! Source du renvoi introuvable.** (A) also a positive correlation between reaction rate and moisture content can be observed. This may be attributed to higher diffusivities of ascorbic acid and oxygen at increasing moisture contents [31]. Concentrated tomato samples with lower moisture content form a denser solid matrix retarding the penetration of oxygen. This may reduce the oxygen concentration inside the product and thereby reduce the ascorbic acid degradation. Uddin et al., 2001 reported similar results by investigating ascorbic acid degradation in dried kiwifruits with different water activity during storage [23]. Djendoubi Mrad et al, 2012 reported that during air drying of pears, ascorbic acid degrades slowly at higher moisture contents, followed by a sharper decrease at low moisture contents [32]. This can be explained by the phenomenon that during initial drying the temperature is equal to the wet bulb temperature. After decrease of the moisture content the temperature of the product increases; this explains the sharp decrease in ascorbic acid.

In **Erreur ! Source du renvoi introuvable.** (B) the reaction rate constant of PCA is shown at different combinations of temperature and moisture content. Similar to ascorbic acid degradation, it can be observed that PCA formation is enhanced at increasing temperature. However, at higher moisture the formation rate of PCA is decreasing. In the present study, PCA formation via the enzymatic route may be neglected, because the high heating temperatures (from 60 ˚C to 100 ˚C) lead to low activities of γ-glutamylcysteine synthetase (γ-GCS) and Glutamate-5-Kinase (G5K) [16-19]. It is therefore expected that non-enzymatic PCA formation in our system is dominant [33, 34]. During this reaction either glutamine or glutamic acid is converted into PCA via an unimolecular ring-closure reaction, which is catalysed by a weak acid. At lower moisture contents the increased concentrations of reactants, either glutamine or glutamic acid, and the weak acid are expected to promote PCA formation. Moreover, PCA formation can even occur in the dry state because the amino group and the carboxamide group of glutamine can easily condense in solid state [21]. Meanwhile, it can be observed that the effect of temperature is more significant at low moisture contents i.e. $X_w = 0.13$ than at high moisture contents, i.e. $X_w = 0.95$.

Figure 3 shows the experimentally obtained and predicted reaction rates at different moisture content and temperature for ascorbic acid degradation (A) and PCA formation (B) obtained by fitting. It can be observed that the model is in good agreement with the experimental data for the PCA reaction. For ascorbic acid there is reasonable agreement, which is at least partly explained by the higher variability in the HPLC analysis.

(A) **(B)**

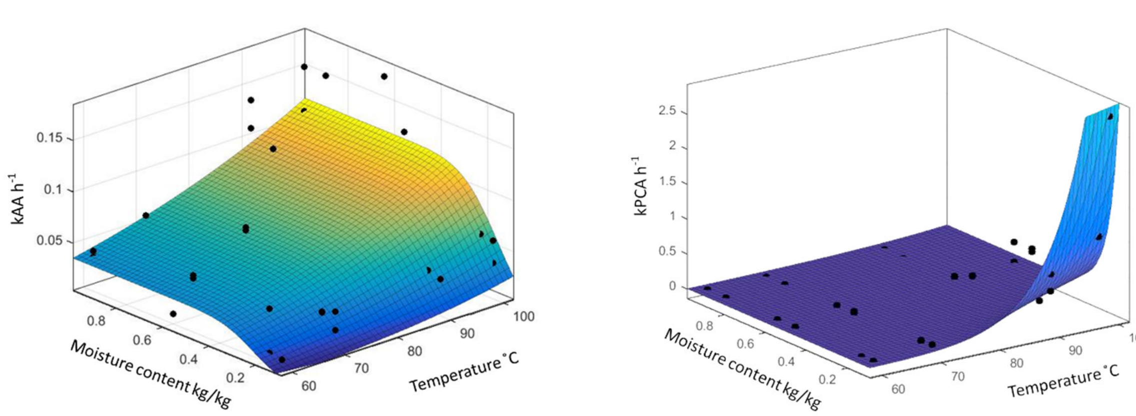
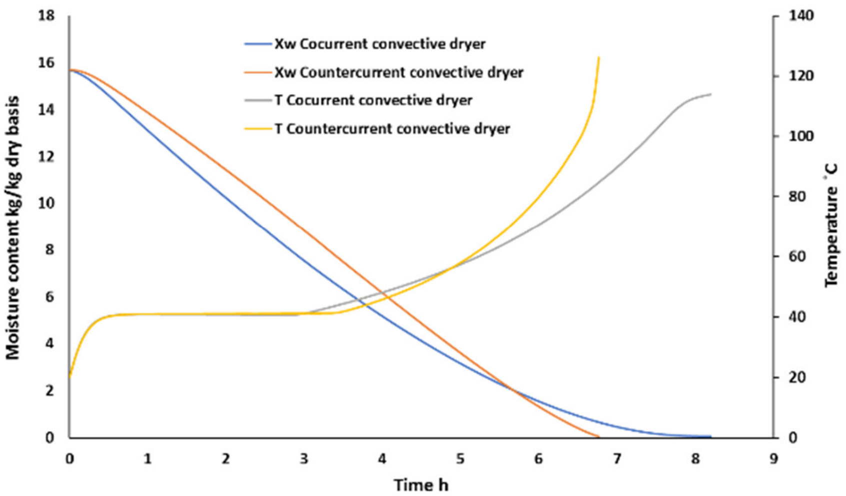


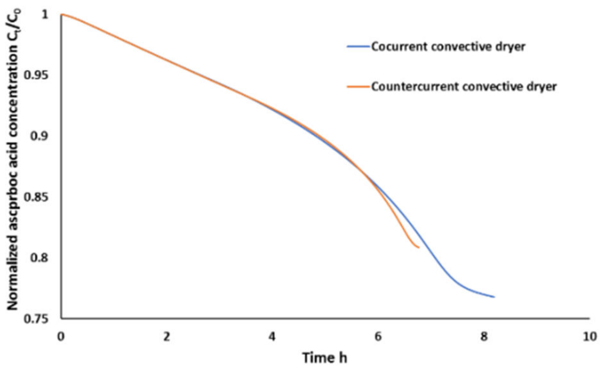
Figure 3 Reaction rates of: (A) Ascorbic acid degradation and (B) PCA formation. The black dots represent the reaction rates from experimental data and the grid surfaces represent the model predictions.

The developed kinetic models are used to evaluate the effect of air drying of a tomato product on quality via the degradation of ascorbic acid and formation of PCA being important taste markers. A heat and mass transport model for a co- and countercurrent air dryer was used to calculate the product temperature and moisture content of the product during drying [27]. In this case, a tomato-based product with an initial moisture content of 15.7 kg/kg dry basis (0.94 kg/kg wet basis) is dried to a final moisture content of 0.05 kg/kg dry basis. In both dryer configurations, the inlet air temperature is 150 ˚C. The simulated product temperature and moisture content profiles are presented in Figure 4 (A). The temperature of the product is first heated up to the web bulb temperature and keeps constant during the initial constant rate drying period. When the moisture content is lower than the critical moisture content of 7.85 kg/kg dry basis [35], the process enters the falling rate drying period and the product temperature gradually increases to the air temperature.

(A)



(B)



(C)

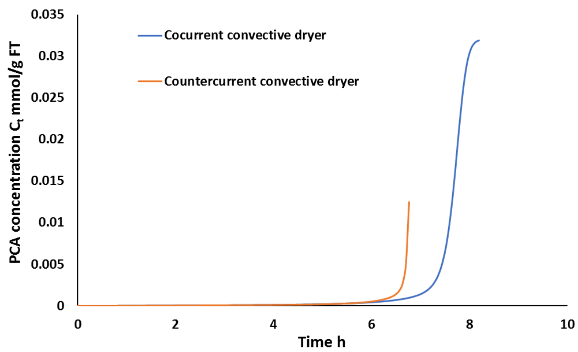


Figure 4 (A) Simulations of moisture content and temperature profiles of the tomato-based product during cocurrent and countercurrent convective drying. (B) Ascorbic acid degradation and (B) PCA formation during convective air drying.

Subsequently, based on Figure 4 (A), the degradation and formation of the two organic acids were calculated. In Figure 4 (B) and Figure 4 (C) the concentrations of ascorbic acid and PCA are shown during the drying process. 23% of the ascorbic acid degrades after cocurrent drying, while 19% degrades after countercurrent drying. Only at the end of the process there is a difference between both drying strategies, which is explained by the higher temperature at the end of the process during countercurrent drying. Due to the higher temperature, the degradation in the countercurrent dryer is faster. However, the residual ascorbic acid content is still higher as the total time for countercurrent drying is shorter. For PCA it can be observed that the differences occur also primarily at the end of the process. The PCA formation (0.03 mmol/g FT) after cocurrent drying is much larger than after countercurrent drying (0.01 mmol/g FT).

Conclusions

Thermal processing and drying affect the level of organic acids in tomato-based products with a consequent impact on perceived freshness. Ascorbic acid degrades while pyroglutamic acid (PCA) forms during thermal processing. Both the degradation reaction of ascorbic acid and formation reaction of PCA are found dependent on the temperature and the moisture content. The reactions were described with a first order kinetics. An extended Arrhenius model was used to describe the dependence of the kinetics on both moisture content and temperature. Parameters of the kinetic models were estimated with data from isothermal heating experiments at different moisture contents. At lower moisture contents the degradation rate of ascorbic acid decreases, while the formation rate of PCA is enhanced, which could be related to the different mechanisms of both reactions. Finally, a convective air drying model was used to evaluate the effect of drying on presence of both acids. Significant degradation of ascorbic acid and formation of PCA was calculated, depending on the mode of operation (co- or counter current drying) chosen. Of course, other choices, such as product thickness and temperature of the drying air, also will have an effect on the outcome of the model predictions. Ultimately, the models developed in this study can assist in evaluation and optimisation of drying processes of tomato products on perceived freshness.

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