



## Estimation of ascorbic acid reduction in heated simulated fruit juice systems using predictive model equations



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### ABSTRACT

Predictive models for the estimation of residual ascorbic acid (rAA) and ascorbic acid percent change (%AA<sub>r</sub>) in heat-treated simulated fruit juices were established. The tested ranges of predictor product properties (pH and initial AA level) were based on initial surveys conducted involving processed and freshly squeezed juices, while those of predictor process parameters (heating temperature and treatment time) were based on thermal inactivation of *Escherichia coli* O157:H7 modeled earlier in our lab. Results showed that both rAA and %AA<sub>r</sub> data sets had highly significant ( $P < 0.0001$ ) fit in a linear and quadratic model, respectively. Temperature, time, and initial AA were found significant predictors of rAA. On the other hand, temperature, time, initial AA, temperature<sup>2</sup> and initial AA<sup>2</sup> were found to be significant predictors of %AA<sub>r</sub>. Predictive performance indices were within acceptable values, confirming model utility in decision making for a more comprehensive control of juice nutritional functional quality and safety.

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### 1. Introduction

The World Health Organization (WHO) reported that low fruit and vegetable intake is among the major risk factors for global mortality. Hence together with the Food and Agriculture Organization, the WHO recommended the consumption of 5 servings of fruits and vegetables for the prevention of chronic illnesses, as well as for the prevention and alleviation of several micronutrient deficiencies, particularly in less developed countries (WHO, 2003). Fruit and vegetable consumption in developing economies may however be hampered by challenges on convenience, availability, and accessibility. Fruit juice processing may address these gaps. In recent years, the popularity of the fruit juice industry has grown rapidly due to the convenience these commodities offer, and the increasing number of studies that report the nutritional and health benefits of fruit juice consumption (Echeverria & Lopez, 2014; Falguera & Ibarz, 2014). In comparison to fresh fruits, juices are more easily consumed, stored and transported (Yekeler, Ozyurek, &

Tamer, 2013). Furthermore, fruit juices are reported to contain significant amounts of vitamins, minerals, and other essential nutrients that contribute to the functional property of the food product (Sethi, Sethi, Deka, & Meena, 2005).

Among the nutrients in fruit juices, ascorbic acid is being given attention due to the health benefits it provides consumers. Also called Vitamin C, it acts as a natural antioxidant that prevents damage of macromolecules in the body due to free radicals, reactive oxygen species, and nitrogen species (Sikorska-Wisniewska & Szumera, 2007; de Lorgeril & Salen, 2006). Ascorbic acid intake has been associated with the reduction of risks of developing diseases such as cancer, cardiovascular disease, age-related macular degeneration, cataract, common cold, and asthma (Higdon & Drake, 2012; de Lorgeril & Salen, 2006). The antioxidant activity of ascorbic acid also protects iron in the body from oxidation to increase its intestinal absorption (Sikorska-Wisniewska & Szumera, 2007). Moreover, ascorbic acid is found to be essential in the synthesis of collagen for efficient wound healing and growth of bones, teeth, ligaments, and tendons, in the synthesis of neurotransmitters and carnitine, for the prevention of scurvy, and in cholesterol metabolism (Higdon & Drake, 2012). In food products, ascorbic acid also acts as an antioxidant that inhibits enzymatic browning and other quality changes brought about by oxidative reactions (Branen

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& Davidson, 1997).

However, despite these health benefits one may obtain from fruit juice consumption, non-pasteurized fruit juices have also been reported to become vectors of *Escherichia coli* O157:H7 and *Salmonella enterica* infections (Vojdani, Beuchat, & Tauxe, 2008). Spoilage-causing microorganisms can similarly contaminate the juice products and mediate physicochemical changes, alongside other deteriorative mechanisms mediated by intrinsic biochemical components of the fruits (Bates, Morris, & Crandall, 2001; Echeverria & Lopez, 2014). These challenges on the safety and quality of fruit juices may be addressed by subjecting the fruit juice to processing technologies. Heating is a traditional, cheap, yet very effective means of processing fruit juices (Buchanan & Edelson, 1999; Mak, Ingham, & Ingham, 2001). This food processing technology involves the exposure of the food sample at a temperature range above ambient for a designated period of time to inactivate enzymes, toxins, and microorganisms for shelf life stability and safety (Awuah, Ramaswamy, & Economides, 2007; Chauhan & Unni, 2015).

Subjecting raw materials to heating however results in sensory quality changes and in deterioration of heat-labile nutrients in processed juices, which include ascorbic acid and other phenolic substances that similarly confer functionality to the food product (Deliza, Rosenthal, Abadio, Silva, & Castillo, 2005; De Paepe et al., 2014; Shakuntala Manay & Shadaksharaswamy, 2001; Vegara, Marti, Mena, Saura, & Valero, 2013). Furthermore, the efficacy of heating against microorganisms was demonstrated to be dependent on intrinsic food properties, extrinsic process parameters, implicit microbial characteristics, and their interactions. Such effects have been previously demonstrated in earlier works conducted by Gabriel, Barrios, and Azanza (2008) and Gabriel (2012), both of which established predictive models that help estimate the inactivation rates of disease-causing microorganisms in fruit juices from product- and process-related variables.

This study similarly explored the possibility of establishing predictive models that can be used to estimate the changes in the nutritive value of heat-treated fruit juices, particularly ascorbic acid, from common physicochemical properties such as pH and initial ascorbic acid concentration; and process variables, namely process temperature and time. The predictive model is meant to be used alongside the previously validated model for the inactivation rates of *E. coli* O157:H7 in simulated fruit juices (Gabriel, 2012). The predictive model may be used to guide food processors in establishing thermal process schedules for heat-pasteurized fruit juices that optimize ascorbic acid retention without compromising safety from disease causing microorganisms, helping the industry address the consumer demand paradox for safe food products with optimal freshness and nutritional benefits. Providing processors with such a model will also aid them in their ascorbic acid supplementation/fortification efforts.

## 2. Materials and methods

### 2.1. Design of the experiment

A Rotatable Central Composite Design of experiment (CCRD) was employed to determine the combinations of the product (pH and initial ascorbic acid, AA concentration) and process (temperature and time) variables investigated in this study. The experimental design consisted of 16 factorial combinations of high and low settings, 8 axial combinations of very low/very high and intermediate settings, and 12-time replicated center point combinations where all factors were set to intermediate values. In the CCRD, the ranges for the variable settings of pH and initial AA concentration were based on the reported physicochemical

characteristics of fresh and processed citrus juice products in the study of Gabriel et al. (2015). Furthermore, the temperature and time ranges were based on the previously established decimal reduction times (D-values) of *E. coli* O157:H7 in simulated fruit juices at varying heating temperatures (Gabriel, 2012). Table 1 summarizes the CCRD combinations generated using the Stat-Ease Design-Expert version 7.0.0, trial version (Stat-Ease Inc., Minneapolis, Minnesota, USA).

### 2.2. Simulated fruit juice preparation

Using the generated CCRD combinations of the tested variables, the different simulated fruit juice (SFJ) systems were formulated. The pH of specific volumes of boiled deionized distilled (dd) water were first adjusted using 8.0 M HCl (RCI Labscan, Bangkok, Thailand) or 8.0 M NaOH (RCI Labscan, Bangkok, Thailand). Fifty milliliters of the solutions with adjusted pH were then transferred to identical sterile amber bottles covered with aluminum foil, and set aside for no longer than 1 h prior to experimentation.

### 2.3. SFJ heating

Heat treatment of the different SFJ systems was performed by placing the sample solutions in a water bath (PolyScience, Illinois, USA) that was set to heating temperature that would allow the SFJ to reach and maintain the temperature pre-determined in the CCRD. To ensure that the assigned temperature is reached, a thermometer was placed in the cold point of the SFJ. Upon reaching the

**Table 1**  
CCRD variable combinations of simulated fruit juice systems.

Experimental runs	Variable combinations			
	Temp (°C)	Time (min)	pH	Initial AA (mg/L)
1	59.5	15.4	2.75	400
2	84.5	15.4	2.75	400
3	59.5	45.1	2.75	400
4	84.5	45.1	2.75	400
5	59.5	15.4	4.25	400
6	84.5	15.4	4.25	400
7	59.5	45.1	4.25	400
8	84.5	45.1	4.25	400
9	59.5	15.4	2.75	800
10	84.5	15.4	2.75	800
11	59.5	45.1	2.75	800
12	84.5	45.1	2.75	800
13	59.5	15.4	4.25	800
14	84.5	15.4	4.25	800
15	59.5	45.1	4.25	800
16	84.5	45.1	4.25	800
17	47.0	30.3	3.5	600
18	97.0	30.3	3.5	600
19	72.0	0.50	3.5	600
20	72.0	60.0	3.5	600
21	72.0	30.3	2.0	600
22	72.0	30.3	5.0	600
23	72.0	30.3	3.5	200
24	72.0	30.3	3.5	1000
25	72.0	30.3	3.5	600
26	72.0	30.3	3.5	600
27	72.0	30.3	3.5	600
28	72.0	30.3	3.5	600
29	72.0	30.3	3.5	600
30	72.0	30.3	3.5	600
31	72.0	30.3	3.5	600
32	72.0	30.3	3.5	600
33	72.0	30.3	3.5	600
34	72.0	30.3	3.5	600
35	72.0	30.3	3.5	600
36	72.0	30.3	3.5	600

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