Development of Antioxidant Gummy Jelly Candy Supplemented with *Psidium guajava* Leaf Extract

Ratchanee Charoen*
Department of Innovation and Product Development Technology, Faculty of Agro-Industry, King Mongkut's University of Technology North Bangkok, Prachinburi Campus, Prachin Buri, Thailand

Wanticha Savedboworn, Samart Phuditcharnchnakun and Thanakit Khuntaweetap
Department of Agro-Industry Technology and Management, Faculty of Agro-Industry, King Mongkut's University of Technology North Bangkok, Prachinburi Campus, Prachin Buri, Thailand

* Corresponding author. E-mail: ch_ratchanee@yahoo.com
Received: 15 August 2014; Accepted: 4 February 2015; Published online: 24 February 2015

Abstract
The aim of this research was to develop gummy jelly candy supplemented with the crude extract of Psidium guajava Linn. leaf. Effect of drying temperature (50, 60 and 70°C) on the total antioxidant activity (TAA) of the obtained crude extracts was investigated using the DPPH method at various pH conditions (4.0, 6.5 and 8.5). Results showed that the crude extract obtained from the drying temperature of 50°C and testing condition at pH 4.0 had the highest TAA value. However, there was interaction between drying temperature and pH conditions, thus the treatment at 50°C, pH 6.5 and 70°C, pH 4.0 was not significant different.The relationships between temperature and pH on TAA were explained using the multiple regression (R^2=0.987). The crude extracts caused changes in texture properties of gummy jelly indicated by decreases in gumminess and chewiness of the products. Result was consistent with the results obtained from sensory evaluation (9-points hedonic scale) that sensory texture (springiness and toughness) and color scores decreased. This study suggested that the extraction of Psidium guajava Linn. leaf has the potential to be used as antioxidant supplements and it can be used in gummy jelly products.

Keywords: *Crude extract, Gummy jelly, Psidium guajava*

1 Introduction

Customer attitudes and behaviors have moved towards health foods because they have more concerns on increasing environmental stresses such as pollution and toxic substances in the environment. Environmental pollutants and lifestyle factors induce oxidative stress leading to many long term health complications in human such as diabetes, cancer and cardiovascular disease. Formation of free radicals in the bloodstream may lead to the formation of oxidized low-density lipoprotein (oxidized LDL), which is one of the critical factors in atherogenesis [1,2]. To inactivate free radicals, a variety of antioxidants such as vitamin C, vitamin E, selenium, beta-carotene, vitamin A, and plant polyphenolic compounds have been identified as antioxidant supplements that can be obtained from the consumption of fruits and vegetables. Moreover, it was noted that many polyphenolic compounds in herbal extracts exhibited high antioxidant activities [3]. A variety of antioxidant mechanisms of plant polyphenolic compounds have been reported [4].

Please cite this article as: R. Charoen, W. Savedboworn, S. Phuditcharnchnakun, and T. Khuntaweetap, "Development of Antioxidant Gummy Jelly Candy Supplemented with *Psidium guajava* Leaf Extract," *KMUTNB Int J Appl Sci Technol*, Vol. 8, No. 2, pp. 145-151, Apr.-June 2015, http://dx.doi.org/10.14416/j.ijast.2015.02.002

Estimating antioxidant activities of plant polyphenolics could be performed using various types of stable free radicals such as DPPH radical (DPPH•, 2, 2-diphenyl-2-picrylhydrazyl radical) and ABTS radial cation (ABTS•$^+$, 2, 2-azinobis (3-ethylbenzothiozline-6-sulphonate). Gordon (2001) suggested that among many antioxidant assays conducted in polar solvents e.g. methanol, DPPH radical scavenging assay has been frequently used due to simplicity of the method and stability of DPPH radicals [5,6]. A decrease in DPPH radicals is indicated by the decreased absorbance at 515 nm [7], since the DPPH radical is reduced by antioxidants to DPPH-H, which is a non-radical product. So, color of the reaction is changed from purple to yellow [7,8]. Because of different preferences of customers for herbs, fruits and vegetables, their daily intake of antioxidants may vary considerably. *Psidium guajava* Linn. from Ban Dong Bang Herbal Community, Prachinburi province is one of the most popular herbal. The bioactive compounds in *Psidium guajava* Linn. are phenols, flavones, saponins glycosides, alkaloids, steroidal glycosides etc [9] which can be act as the natural antioxidant by inactivating lipid free radical [3]. Thus, the idea of adding natural plant extracts into a candy product have been developed. Gummy jelly candies, represent approximately 50% of candy market value [10], are made with a base of gelatin. Customers prefer gummy jelly candies due to their unique texture and chewability. In this research, ethanolic extracts of *Psidium guajava* leaf have been added into gummy jelly candies. Effects of processing parameters (drying temperature and pH) on antioxidant, texture and sensory properties of the products treated with the extracts were investigated.

2 Materials and Methods

2.1 *Sample preparation*

Fresh samples of *Psidium guajava* leaves were obtained locally from Ban Dong Bang Herbal Community, Prachinburi province, Thailand. The leaves were washed with clear water before being cut into smaller pieces.

2.2 *Effect of drying temperatures and pH conditions on antioxidant activity*

The leaves were dried in a tray dryer using varying temperatures of 50, 60, and 70 (±1)°C. for 10-12 hours until the water activity value, a_w was lower than 0.6. The dried leaves were then packaged in a polypropylene (PP) bags until further analysis. For the study on pH conditions, the extract solutions were adjusted the pH values to 4.0, 6.5 and 8.5 (±0.1) by 0.1N HCl and 0.5N NaoH. Then, the extract solutions were determined for DPPH radical scavenging activity.

2.3 *Extraction procedure*

Five grams of dried leaves were soaked in 100 ml of 80% ethanol (v/v). The mixture was shaken at 80 rpm for 12 hours at room temperature. The leaf residues were removed using filtration with Whatman No.4 filter paper. The residues were washed three times and the volume of the mixture was adjusted to 250 ml with 80% ethanol [11,12].

2.4 *DPPH radical scavenging assay*

Antioxidant activity of the extracts was determined using DPPH radical scavenging assay according to the method described by Brand-William et al. [6,7]. All leaf extracts were diluted to obtain a final concentration of 8g/l using methanol. Nine hundred fifty microliters of freshly prepared DPPH methanolic solution (the absorbance at 515 nm = 0.6-0.7) was mixed with 50 µl of the extracts solution. The absorbance at 515 nm was recorded at 0 and 15 min. A calibration curve of ascorbic acid was used ($y = 0.0148+0.0867$, $R^2 = 0.922$). The antioxidant activity of the extract was expressed as micromoles per milliliter of extract solution. For evaluation of antioxidant activity of gummy jelly product, antioxidant activity was expressed in terms of total antioxidant activity (DPPH method). Three grams of jelly sample was prepared in 30 ml of warm water:ethanol (9:1 v/v) and heat for 50°C for 2 min according the method described by Herrera et. al, with some modification [13,14]. Evaluated the radical scavenging (DPPH method) and reported as µmole/ml of jelly solution.

2.5 *Gummy jelly candy preparation*

Gummy jelly was prepared using a standard gummy jelly candy formulation described by Garcia (2000) with some modification [10]. The dried leaf sample at

50°C was extracted according to previously described method. The extract solution was further concentrated using a vacuum evaporator (55°C and 200 mbar for 15 min). The total soluble solid of crude extract was 21.3±1.7 after removed ethanol. The composition of gummy jelly was shown in Table 1. Different levels of the concentrated crude extract were additionally mixed into the standard gummy jelly recipe (additional levels at 10.0, 13.3 and 16.7 ml into the 100 gram recipe of the gummy jelly candy).

For the cooking procedure, glucose syrup, sugar and water were mixed and heated in a brass pan. Gelatin solution was prepared by dissolving the gelatin with warm water (70°C). Then, the gelatin solution was added into the previously prepared glucose syrup solution and kept mixing to prevent the mixture from burning. When the temperature of the mixture was raised to 116°C, citric acid was added and mixed. The brix value of the mixture was adjusted to 68-75% by adding a portion of water. Before adding the different levels of the crude extracts, the temperature of the mixture was then decreased to 63, 68 and 73°C to investigate influence of cooking temperatures on the final product. Some air bubbles were removed before the mixture was poured into a mold. The ethanol residue from the crude extracts was expected to be removed by evaporation during the process.

Table 1: The composition of gummy jelly candy with the addition of the crude extracts*

Composition	Weight (g)
Gelatin 240 bloom	8.0
Water	24.5
Sugar	33.0
Glucose syrup 40DE	31.5
Citric acid solution 50%	3.0
Total	100.0

* The concentrated crude extracts of 10, 13.3 and 16.7 ml were additionally added into the main composition.

2.6 *Texture analysis and color measurement*

Texture properties of gummy jelly were evaluated using texture analyzer (TA-TX plus model). The experiments conducted with texture profile analysis (TPA) mode using a 0.5p/SW probe and test speed at 1 mm/s. Cohesiveness, Springiness, gumminess and chewiness of the products were reported. Color characteristics of products were determined using a Hunter Lab colorflex 4510, (AEKCHAI, USA). The color was evaluated by means of CIE Lab color components (L*; lightness, a*; redness and b*; yellowness).

2.7 *Total titratable acidity (TA) and total solid content*

Total titratable acidity of gummy jelly products was determined as described by Woo [15]. Citric acid was used as a standard compound. Total solid content of gummy jelly products was determined as the method described by Garcia [10].

2.8 *Sensory evaluation*

Sensory evaluation of the products was performed using 50 untrained panels using a preference test with 9-point hedonic scale (1=dislike extremely, 9= like extremely) to determine color, springiness, toughness and overall liking of products.

2.9 *Statistical analysis*

This research was conducted using 3 × 3 factorial design in completely randomized design (CRD). All experiments were performed in triplicate. Comparisons of the means were performed using Duncan's multiple range tests by SPSS version 18.0. Multiple regression was used to explain the effects of drying temperature and pH conditions on antioxidant activity. The mathematical model was used as the following equation (1).

$$Y_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 x_2 + \beta_4 x_1^2 + \beta_5 x_2^2 \quad (1)$$

Where Y_i represents total antioxidant activity; X_1 represents drying temperature and X_2 represents pH conditions.

3 Results and Discussion

3.1 *Effects of drying temperature and pH on antioxidant activity*

Antioxidant activity of *Psidium guajava* extracts may be influenced by drying temperatures and acidic-basic testing conditions of the extracts. As illustrated in Table 2, results showed that the crude extract obtained from the drying temperature of 50°C exhibited higher total antioxidant activity than the crude extracts obtained from drying at 60 and 70°C. Increases in pH value of the extracts resulted in decreases in total antioxidant activity in all drying treatments. The extract exhibited the highest antioxidant activity at pH 4.0 compared to other pH conditions (6.5 and 8.5) [16,17]. Overall, drying the leaves at 50°C was the optimal drying condition for further experiments on effect of the crude extract on physicochemical properties of gummy jelly products.

Table 2: Total antioxidant activity of *Psidium guajava* extract solutions

pH	TAA (µmole/ml of extract solution)		
	50°C	60°C	70°C
4.0	94.44 ± 0.51[a]	78.71 ± 0.65[e]	92.90 ± 0.36[a]
6.5	92.94 ± 0.98[a]	78.49 ± 0.36[e]	89.71 ± 0.40[b]
8.5	86.33 ± 1.91[c]	71.73 ± 1.21[f]	84.53 ± 0.86[d]

Means (±SD) with different superscript letters (a-f) indicate significant differences ($P < 0.05$).

Multiple regression analysis was performed to create a mathematical model to predict the relationship between drying temperature and pH on the total antioxidant activity. The model was shown as the following equation (2).

$$Y = 561.70 - 16.51X_1 - 4.66X_2 + 0.003X_1X_2 + 0.14X_1^2 - 0.57X_2^2 \quad (2)$$

$R^2 = 0.985$

Where Y represents total antioxidant activity, β represents regression coefficient, X_1 represents drying temperature and X_2 represents pH value.

For the equation, the R squared value suggests that model explains approximately 98.5% for total variance. Total antioxidant activity showed negative relationships with increases in drying temperature (X_1) and pH values (X_2).

It was suggested that H-atom transfer (HAT) and sequential proton –loss-electron-transfer (SPLET) are the two main antioxidant mechanisms playing important roles in polar media such as methanol [18,19]. In addition, it was reported that the ability of antioxidants to donate an electron and/or H-atoms was strongly influenced by types of solvents and pH conditions [20,21]. Under polar protic solvents e.g. methanol, it was proposed that obtained DPPH scavenging activity data may be overestimated due to rate constants of dissociated forms of phenolic antioxidant towards DPPH• with SPLET are very fast [22]. Under different pH conditions, antioxidants can undergo partial ionization in ionizing solvents. Mukai and coworkers [21] reported that rate constants of the reaction of flavonoids and vitamin C towards a radical were pH-dependent reaction [21]. They suggested that the rate constants increase with increasing pH values.

In contrast to our results, the crude extract of *Psidium guajava* leaves exhibited higher antioxidant activity at acidic condition (pH 4.0) than alkaline condition (pH 8.5). However, results suggested that overall antioxidant activity may be independent with rate constants of antioxidants in the reaction. Adjusting pH of the crude extract to alkaline condition prior to react with DPPH• may cause instability of antioxidants before the test.

3.2 *Effects of processing parameters on physicochemical properties of gummy jelly candy*

During the preparation of gummy jelly candies, processing temperatures of the product mixtures were varied at 63, 68 and 73°C, and the different levels of the crude extracts were added into the gummy jelly candies. Some physicochemical properties of the gummy jelly candies were determined as shown in Table 3 and 4.

As illustrated in Table 3, the addition of the crude extract into the gummy jelly candy have a tendency to increase total solid content, total acidity and total antioxidant activity (% radical scavenging activity), but there were no impacts on the water activity of the products. Adding the extract beyond 13.3 ml, no significant differences in total solid content, total acidity and the antioxidant activity were observed ($P > 0.05$). Moreover, increasing process temperatures showed no significant effects on these parameters. However, texture properties and color characteristics of the gummy jelly candy were strongly influenced by process temperatures and the levels of the extract as shown in Table 4. Adding the crude extract decreased gumminess and chewiness of the product, but had no effects on their cohesiveness and springiness. Increasing the process temperatures from 63 to 73°C had no significant impacts on texture properties ($P > 0.05$). It was suggested that acid hydrolysis of gelatin contributed to decrease the gel strength of gelatin [13]. In this research, the crude extract could increase total acidity of the gummy jelly candy. It could explain decreases in gumminess and chewiness of the product.

Increasing the levels of the crude extract showed a tendency to decrease lightness (L*) and yellowness (b*). However, the results were not statistically significant ($P > 0.05$). Except for redness (a*), adding the crude extract at the highest level (16.7 ml) decreased a* value significantly ($P < 0.05$), indicating the direct effect of the crude extract color on the gummy jelly candy.

Table 3: Physicochemical properties of gummy jelly candy influenced by process temperatures and the extract levels

Treatments	Process conditions		Physicochemical properties			
	Temperature (°C)	Crude extract (ml/100g gummy candy)	Total soluble solid (%)	Total acidity (g of citric acid/100g sample)	TAA (µmole/ml of extract solution)	a_w ns
1	63	10.0	64.0±2.8b	2.78±0.1b	86.14±2.6a	0.79±0.01
2	63	13.3	73.9±4.2a	3.50±0.1a	91.85±1.2b	0.77±0.01
3	63	16.7	75.0±3.5a	3.59±0.1a	91.20±3.1b	0.79±0.02
4	68	10.0	62.0±3.5b	2.85±0.1b	84.10±1.7a	0.77±0.04
5	68	13.3	74.0±4.5a	3.59±0.1a	90.93±1.3b	0.75±0.03
6	68	16.7	74.0±1.3a	3.59±0.0a	90.47±2.1b	0.79±0.03
7	73	10.0	68.0±1.0ab	3.06±0.0ab	86.39±2.4a	0.77±0.03
8	73	13.3	74.0±1.9a	3.57±0.2a	89.50±3.1ab	0.76±0.03
9	73	16.7	74.0±1.1a	3.60±0.1a	91.81±2.2b	0.77±0.03

Means (±SD) with different superscript letters in the same column (a-b) indicate significant differences ($P < 0.05$). The superscript "ns" indicates no significant differences among the means in the same column.

Table 4: Physical properties of gummy jelly cadies treated with different process temperatures and levels of the crude extract

Treatments	Physical properties							
	Gumminess (N)	Chewiness (Nmm)	Cohesiveness ns	Springiness (mm) ns	a_w ns	L*	a*	b* ns
1	1,273±340b	1,226±341b	0.90±0.1a	0.97±0.0a	0.79±0.0	31.14±7.9b	-0.07±0.6a	2.97±1.7
2	861±152a	864±151a	0.90±0.1a	0.97±0.0a	0.77±0.0	28.78±8.0ab	-0.26±0.3a	2.91±1.3
3	773±108a	779±141a	0.98±0.1b	0.97±0.0a	0.79±0.0	28.93±7.6ab	-0.60±0.5b	2.63±2.1
4	1,168±325ab	1,670±332b	0.92±0.0a	0.96±0.0a	0.77±0.0	29.95±7.7ab	-0.21±0.6ab	2.81±2.7
5	1,039±334a	1,007±322ab	0.94±0.0ab	0.97±0.0a	0.75±0.0	27.85±7.9ab	-0.36±0.3ab	2.61±0.7
6	870±137a	1,043±305ab	0.94±0.0ab	0.98±0.0a	0.79±0.0	28.96±7.1ab	-0.44±0.3ab	2.54±3.1
7	1,071±342ab	886±311a	0.94±0.0ab	0.96±0.0a	0.77±0.0	29.09±2.9ab	-0.09±0.2a	2.73±1.5
8	923±310a	924±291ab	0.94±0.0ab	1.00±0.0b	0.76±0.0	28.65±4.7ab	-0.10±0.4a	2.50±1.7
9	994±320a	701±208a	0.94±0.0ab	0.99±0.0a	0.77±0.0	26.18±6.17a	-0.72±0.5b	2.51±1.8

Means (±SD) with different superscript letters in the same column (a-b) indicate significant differences ($P < 0.05$).The superscript "ns" indicates no significant differences among the means in the same column.

3.3 *Sensory evaluation of gummy jelly candy*

Sensory evaluations of gummy jelly candies treated with the different process temperatures and levels of the crude extract were performed on 4 different attributes such as springiness, toughness, color and overall liking using 9-point hedonic scale (Table 5). Results showed the tendency of adding more the crude extract could result in decreases in all the attributes. It was noted that using the crude extract at the lowest level in the gummy jelly candy was recommended. Among the test attributes, color attribute of the gummy jelly candy with the crude extract was received the average scores lower than 6.0, indicated that this aspect should be improved in the future study.

Table 5: Sensory analysis of gummy jelly candies treated with different process temperatures and levels of the crude extract

Treatments	Preference tests			
	Springiness	Toughness	Color	Overall liking
1	6.5±1.8a	6.1±0.8a	5.9±1.2a	6.9±1.5a
2	6.8±1.2a	5.9±1.2ab	5.3±1.5a	5.5±1.8ab
3	5.3±1.5ab	4.9±0.9b	4.9±1.5c	5.9±1.3ab
4	6.5±1.5a	7.3±1.3a	6.3±1.3a	7.0±1.4a
5	6.4±1.5a	5.1±1.3ab	6.4±1.4a	6.1±1.7a
6	4.7±1.3b	4.6±1.3c	5.4±1.4ab	5.1±1.8b
7	6.0±1.0a	6.8±1.5a	4.5±1.3c	6.5±1.1a
8	6.4±1.6a	5.5±1.9ab	6.1±1.7a	6.0±1.5a
9	5.0±1.1a	5.3±1.0ab	5.0±1.1ab	4.5±1.3b

Means (±SD) with different superscript letters in the same column (a-b) indicate significant differences ($P < 0.05$)

4. Conclusions

To obtain the highest antioxidant activity of the crude extract, drying temperature of 50°C and pH 4.0-6.5 was recommended. Due to some limitations of the crude extract impacting on texture and color of the product, the crude extract was recommended to use at the lowest level (10 ml) in this research.

Acknowledgements

The authors express their sincere appreciation to Faculty of Agro-industry, King Mongkut's University of Technology North Bangkok, Prachinburi campus for the financial support.

References

[1] R.R. Ross Watson and V.R. Preedy, *Bioactive foods in promoting health*, 1st ed., London, UK: Academic Press in an imprint of Elsevier, 2010, pp. 285-298.

[2] A.M. Papas, "Beyond Tocopherol: The role of the other tocopherols and ocotrienols," in *Phytochemicals in nutrition and health*, edited by M.S. Meskin, W.R. Bidlack, A.J. Davies and S.T. Omaye, New York: CRC Press, 2002.

[3] J. Pokorny and S. Schmidt, "Natural antioxidant functionality during food processing," in *Antioxidants in food practical applications*, edited by J. Pokorny, N. Yanishlieva and M. Gordon, New York: Woodhead Publishing, 2001.

[4] S.K. Kondo, N.M. Tsuda, and J. Ueda, "Antioxidative activity of apple skin or flesh extracts associated with fruit development on selected apple cultivars," *Scientia Horticulturae*, vol. 96, pp. 177-185, 2002.

[5] M. H. Gordon, "Measuring antioxidant activity," in *Antioxidants in food practical applications*, edited by J. Pokorny, N. Yanishlieva and M. Gordon, New York: Woodhead Publishing, 2001.

[6] P. Molyneux, "The use of the stable free radical diphenylpicry-hydrazyl (DPPH) for extimating antioxidant activity," *Journal science and Technology*, vol. 26, pp. 211-219, 2004.

[7] W. Brand-Williams, M.E. Cuvelier, and C. Berset, "Use of free radical method to evaluate antioxidant activity," *Lebensmittle Wissenschaft Technologie,* vol. 28, pp. 25-30, 1995.

[8] S.T. Chang, J.H. Wu, S.Y. Wang, P.L. Kang, N.S. Yang, and L.F. Shyur, "Antioxidant activity of extracts form Acacia confuse Bark and Hertwood," *Journal of Agricultural and Food Chemistry*, vol. 49, pp. 3420-3424, 2001.

[9] R. Manikandan, A.V. Anand, and G.D. Muthumani, "Phytochemical and in vitro anti-diabetic activity of methanolic extract of *Psidium guajava* leaves," *International Journal of Current Microbiology and Applied Sciences*, vol. 2(2), pp. 15-19, 2013.

[10] T. Garcia, "Analysis and gelatin-based confections," *The manufactoring confectioner*, vol. 80(6), pp. 93-101, 2000.

[11] A. Chanwitheesuk, N. Rakariyatham, and A. Teerawutgulrag, "Screening of antioxidants

from some Thai vegetables and herbs," *Chiang Mai Journal Science*, vol. 29, pp. 1-5, 2006.

[12] A. J-Escrig, M. Rincon, and F. S-Calixto, "Guave fruit (*Psidium guajava L.*) as a new source of antioxidant dietary fibers," *Journal of Agricultural and Food Chemistry*, vol. 49, pp. 5489-5493, 2001.

[13] M. Herrera, A. Herrera, and A. Arino, "Estimation of dietary intake of ochratoxin A from liquorice confectionery," *Food and Chemical Toxicology*, vol. 47, pp. 2002-2006, 2009.

[14] A. Periche, A. Heredia, I. Escriche, A. Andres, and M.L. Castello, "Optical mechanical and sensory properties of based-isomaltulose gummy confections," *Food Bioscience*, vol. 7, pp. 37-44, 2014.

[15] A. Woo, "Use of organic acids in confectionery," *The manufactoring confectioner*, vol. 78(8), pp. 63-70, 1996.

[16] N. Ruenroengklin, J. Zhong, X. Duan, B. Yang, Y. Jianrong, and Y. Jiang, "Effects of various temperatures and pH values on the extraction yield of phenolics from Litchi fruit pericarp tissue and the antioxidant activity of the extracted anthocyanins," *International Journal of Molecular Sciences*, vol. 9(7), pp. 1333-1341, 2008.

[17] B. Ozcelix, J.H. Lee, and D.B. Min, "Effects of light, Oxygen, and pH on the absorbance of 2,2-diphenyl-1-picrylhydrazyl," *Journal of Food Sciece*, vol. 68(2), pp. 487-490, 2003.

[18] H.Y. Zhang and H.F. Ji, "How vitamin E scavenger DPPH radicals in polar protic media," *New Journal of Chemistry*, vol. 30(4), pp. 503-504, 2006.

[19] M. Musialik and G. Litwinieko, "Scavenging of DPPH• radicals by vitamin E is accelerated by Its partial ionization: The role of sequential proton loss electron transfer," *Organic Letters*, vol. 7(22), pp. 4951-4954, 2005.

[20] S. Mitani, A. Ouchi, E. Watanabe, Y. Kanesaki, S.I. Nagaoka, and K. Mukai, "Stopped-flow kinetic study of the aroxyl radical-scavenging action of catechins and vitamin C in ethanol and micellar solutions," *Journal of Agricultural and Food Chemistry*, vol. 56(12), pp. 4406-4417, 2008.

[21] K. Mukai, S. Mitani, K. Ohara, and S. Nagaoka, "Structure-activity relationship of the tocopherol-regeneration reaction by catechins," *Free radical biology & medicine*, vol. 38(9), pp. 1243-1256, 2005.

[22] G. Litwinienko and K.U. Ingold, "Abnormal solvent effects on hydrogen atom abstractions. 1. The reactions of phenols with 2,2-diphenyl-1-picrylhydrazyl (dpph(center dot)) in alcohols," *Journal of Organic Chemistry*, vol. 68(9), pp. 3433-3438, 2003.