CHAPTER 3

To standardize the process parameters for atmospheric cold plasma (ACP)-assisted processing of orange juice from different cultivars

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3.1 Introduction

Over the past decade, the cold plasma (CP) approach in the food processing sector has gained more importance owing to its significant advantages, such as enzyme inactivation, microbial inactivation, and quality enhancement (Pankaj et al., 2014; Pankaj et al., 2013; Sharma et al., 2018). So far, only a few literatures have demonstrated the implication of CP using different generation plasma sources and gas compositions on orange juices (Andreou et al., 2023; Shi et al., 2011; Souza et al., 2023; Xu et al., 2017). The earlier experiments did not analyse ACP treatment's impact on the quality characteristics of orange (cv. Wakro) juice. Moreover, ACP processing of orange juice of Malta cultivar was also unreported. Standardization of process parameters in juice processing by different techniques plays a pivotal role in ensuring the quality of the final product. Therefore, optimizing the processing conditions of ACP treatment to maximize efficacy is crucial. Response surface methodology (RSM) is an effective statistical tool for optimizing the processing conditions (Kumar et al., 2023). Thus, the current study was aimed to evaluate the effect of ACP treatment on orange juice from two distinct cultivars (Wakro and Malta) and optimize the processing parameters by RSM.

3.2 Materials and Methods

The experimental plan for standardization of process parameters for enzyme inactivation in orange juice by ACP treatment is presented in **Fig. 3.1**.

3.2.1 Raw materials and chemicals

Two different cultivars of oranges (cv. Wakro and Malta) were procured from vendors at the local market in the Tezpur region, Assam, India. Pectin, 2,2-diphenyl-1-picrylhydrazyl, NaCl, $NaHCO_3$ 2,6-dichloroindophenol salt, methanol, NaOH, Folin-Cocteau reagent (FCR), Na_2CO_3, and distilled water were purchased from HiMedia Laboratories Pvt. Ltd., India, Merck Specialities Pvt. Ltd., India, and Sisco Research Laboratories Pvt. Ltd., India.

3.2.2 Juice preparation

The collected oranges were washed, cut into two pieces using a knife, and then squeezed manually by a Dynore stainless steel juicer at the Laboratory, Department of Food Engineering and Technology, Tezpur University, Tezpur, Assam, India. A double layer of muslin cloth was used to remove the fibrous materials from fresh juice. The juice was standardized and maintained at a constant °Brix/acid ratio using a sugar solution conferring to the procedure described by Kumar et al. (2024) before each ACP treatment.

3.2.3 Cold plasma treatment

A batch-type cold plasma system (0–50 kV, 50 Hz, Zeonics Systech, India) consists of a high-voltage electrode, a ground electrode, a pair of dielectric barrier plates (surface area: 1225 cm^2 and thickness: 2.4 cm), glass cover, an air gas inlet and outlet pipe, and a voltage generator. The schematic diagram of the ACP system is shown in **Fig. 3.2**. The distance between the electrodes was fixed at 1.5 cm, while the gap between the juice sample and the upper electrode was 1.4 cm. Atmospheric air was used as a medium for plasma generation. The plasma was generated while the lower electrode was connected to the ground and the upper electrode to the voltage generator. A steady and consistent CP was circulated across the treatment chamber from the dielectric barrier discharge (DBD) plasma source at atmospheric pressure. The variable amount of filtered orange (cv. Wakro and Malta) juice was poured onto a petri plate (diameter: 80 mm, height: 14 mm, thickness: 1 mm) and then treated with varying juice depth, voltage, and treatment duration combinations as per the experimental design.

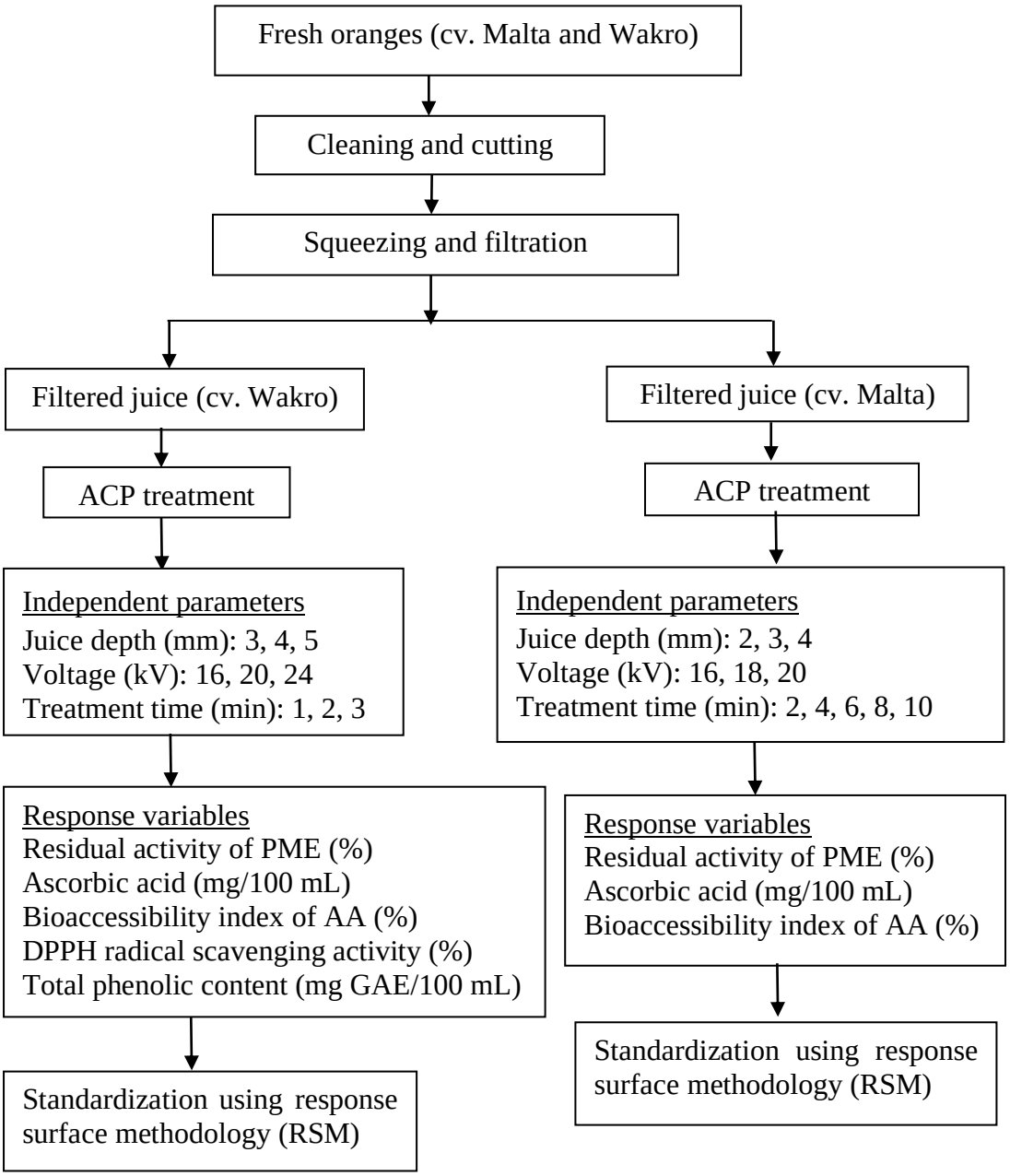


Fig. 3.1 Detailed work plan for objective-1.

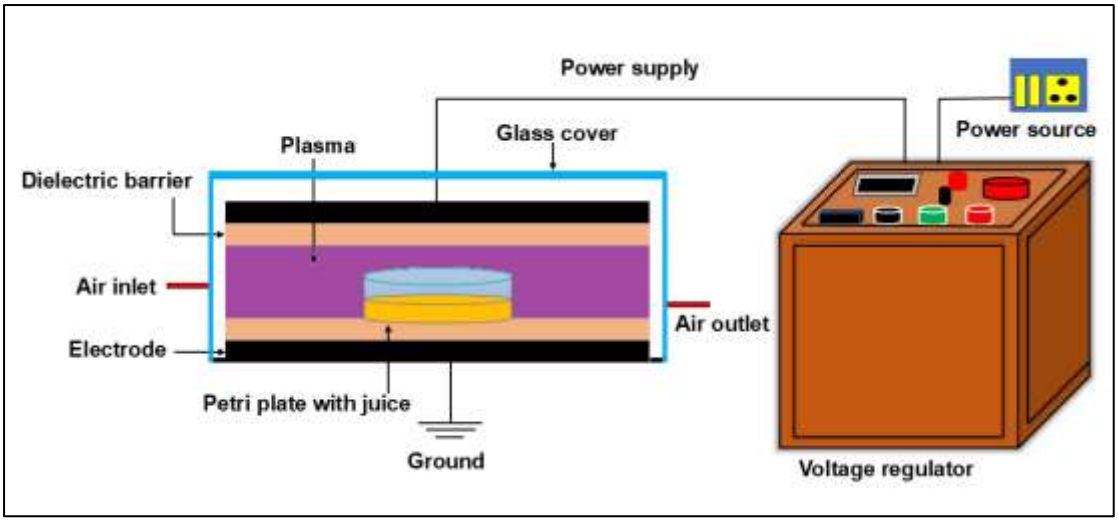


Fig. 3.2 Schematic diagram of DBD-based ACP setup.

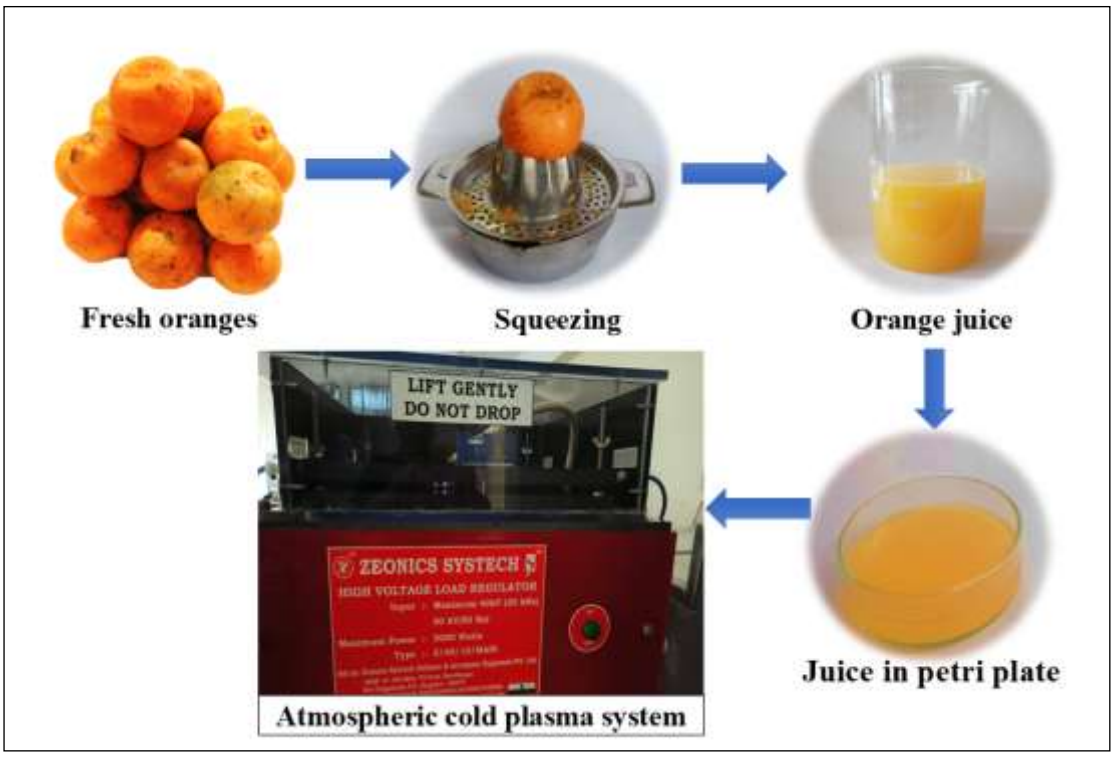


Fig. 3.3 Steps of orange (cv. Wakro) juice preparation and ACP treatment.

3.2.4 Experimental design

The central composite rotatable design (CCRD) was utilized to investigate the influence of voltage (16–24 kV), sample depth (3–5 mm), and treatment duration (1–3 min) on ascorbic acid (AA), residual activity (RA) of pectin methylesterase (PME), bioaccessibility index (BI) of AA, DPPH radical scavenging activity, and total phenolic content (TPC) in orange (cv. Wakro) juice. On the other hand, a full factorial D-optimal experimental design was used to examine the effect of voltage (16–20 kV),

sample depth (2–4 mm), and treatment time (2–10 min) on AA, RA of PME, and BI of AA in orange (cv. Malta) juice. The levels of the independent variables were chosen based on the preliminary trials. Twenty (20) combinations of experimental runs with CCRD for the orange (cv. Wakro) juice and thirty-seven (37) combinations with D-optimal design for the orange (cv Malta) juice was obtained from Design of Expert® (version 13, Stat-Ease Inc., Minneapolis, USA) software. The ACP treatment, with specific levels of independent variables, primarily targeted Wakro orange juice from Arunachal Pradesh in Northeast India. Additionally, the Malta variety was studied at a lower juice depth and voltage range with an extended treatment duration to gather more information on the comparative effects of cold plasma treatment on both orange juice cultivars. The experimental data were fitted to a polynomial model equation (**Eq. 3.1**) for each response to evaluate the effects of process parameters on responses.

$$Y = \beta_0 + \sum \beta_i X_i + \sum \sum \beta_{ij} X_i X_j + \sum \beta_{ii} X_i^2 + \varepsilon \quad (3.1)$$

Where 'Y' is the response variable; β_0, β_i, β_{ii}, and β_{ij} are the constant, linear, quadratic, and interaction coefficients; X_i and X_j are the process variables; ε is the random error, respectively.

Table 3.1 Variables and its levels for ACP processing of orange (cv. Wakro and Malta) juice.

Independent variables	**Code**	**Orange**							
		Wakro			**Malta**				
		Levels			**Levels**				
Juice depth (mm)	A	3	4	5	2	3	4		
Voltage (kV)	B	16	20	24	16	18	20		
Treatment time (min)	C	1	2	3	2	4	6	8	10

3.2.5 Ascorbic acid

The AA content in the orange juice was determined using the method outlined by Ranganna (2001). For titration, a 2,6-dichloroindophenol solution was used as an indicator. A 3% metaphosphoric acid (HPO_3) was used to prepare a standard AA solution with a concentration of 0.1 mg/mL. To prepare the dye solution, 50 mg of 2,6-dichloroindophenol salt was dissolved in 150 mL of water containing 42 mg sodium bicarbonate ($NaHCO_3$), and then the volume was made up to 200 mL. In this method, 10 mL of juice was diluted to 100 mL with 3% HPO_3 and then the mixture was filtrated through Whatman (No. 4) paper. After that, 5 mL of aliquot was titrated with the dye solution until a faint pink color persisted for 15 s, and the consumed

volume was recorded. In short, 5 mL of standard L-ascorbic acid and 5 mL of 3% HPO_3 solution were mixed and titrated for the same. The AA content of the juice sample was found using **Eq. 3.2.**

$$Ascorbic\ acid\ \left(\frac{mg}{100\ mL}\right) = \frac{Titre \times Dye\ factor \times volume\ made\ up\ (mL) \times 100}{Volume\ of\ sample\ (mL) \times aliquot\ of\ extrct(mL)} \quad (3.2)$$

3.2.6 Residual activity of PME

The PME enzyme activity of orange juice was determined using the method of Basak and Ramaswamy (1996). A 50 mL solution containing 0.3 M NaCl and 1% pectin was mixed with 2 mL of juice. The pH of the mixture was then brought to 7.5 using 0.2 N NaOH at a temperature of 30 °C. The reaction mixture was adjusted and titrated with 0.02 N NaOH to maintain a pH of 7.5 for 30 min. During this period, the amount of NaOH consumed was noted down. The PME activity of untreated and ACP-treated juice was calculated using **Eq. 3.3.**

$$PME\ \left(\frac{U}{mL}\right) = \frac{(mL\ of\ NaOH)(N\ of\ NaOH)}{(mL\ of\ juice\ sample) \times (time\ in\ min)} \times 1000 \quad (3.3)$$

The residual activity (RA) of PME and inactivation percentage of PME activity were computed using **Eq. 3.4** and **3.5**.

$$RA\ (\%) = \frac{A_t}{A_0} \times 100 \quad (3.4)$$

$$\text{PME inactivation (\%)} = (100 - RA) \quad (3.5)$$

Where 'A_0' is the initial activity of PME, and 'A_t' is the PME activity after plasma treatment at a time 't.'

3.2.7 Bioaccessibility index of ascorbic acid

Bioaccessibility index (BI) of AA is defined as the amount of AA in the accessible fraction divided by the amount of AA content in the untreated juice (de Castro et al., 2020). The oral phase was not included in the determination of the BI of AA, which was computed only on a percentage basis using the following **Eq. 3.6** (de Castro et al., 2020).

$$Bioaccessibility\ index\ (\%) = \frac{Ascorbic\ acid\ content\ after\ ACP\ treatment}{Ascorbic\ acid\ content\ of\ control} \times 100 \quad (3.6)$$

3.2.8 DPPH radical scavenging activity

The DPPH radical scavenging activity of orange juice was determined according to the procedure described by Islam et al. (2019). For sample extraction, 1 mL of juice was mixed with 1 mL of methanol and water (7:3) and then centrifuged at 5000 rpm for 20 min (R-24, Remi Elektrotechnik Ltd. Pvt., India) (Odriozola-Serrano et al., 2022). A mixed solution of 0.1 mL aliquot of the sample and 1.9 mL methanol was prepared. Then, 2 mL of 0.1 mM DPPH solution in methanol was added, and the reaction mixture was subsequently vortexed and kept in the dark at room temperature for 30 min. The absorbance values of the solution were measured at 517 nm employing a UV-Vis Spectrophotometer (AQUAMATE 8100, Thermo Fisher Scientific, USA). The DPPH radical scavenging activity in juice was calculated from **Eq. 3.7.**

$$DPPH\ radical\ scavanging\ activity\ (\%) = \frac{A_c - A_s}{A_c} \times 100 \quad (3.7)$$

Where 'A_c' and 'A_s' are the absorbance of the control and sample.

3.2.9 Total phenolic content

The TPC of orange juice was determined using the Folin-Cocteau reagent (FCR) method (Illera et al., 2018). For sample extraction, 1 mL of juice was mixed with 9 mL of methanol and water (7:3) and then centrifuged at 5000 rpm for 20 min (R-24, Remi Elektrotechnik Ltd. Pvt., India) (Odriozola-Serrano et al., 2022). A 0.1 mL aliquot was mixed with 2.8 mL of water, followed by 0.1 mL of FCR for the analysis. Subsequently, 2 mL of a 7.5% (w/v) sodium carbonate (Na_2CO_3) solution was added to the test tube. A blank was prepared using distilled water as an aliquot. The absorbance of these reaction mixtures was measured after 60 min of incubation at 750 nm in a UV-Vis spectrophotometer (AQUAMATE 8100, Thermo Fisher Scientific, USA). A calibration curve was prepared with gallic acid as a standard solution. The TPC was expressed in mg gallic acid equivalent (GAE)/100 mL.

3.2.10 Statistical analysis

The results of the experiments were expressed as the mean ± standard deviation (SD) of triplicates. An analysis of variance (ANOVA) was performed to test the significant differences at a 95% confidence level using Design of Expert® (version 13, Stat-Ease Inc., Minneapolis, USA) software.

3.3 Results and Discussion

3.3.1 Response surface modelling

The results of dependent parameters corresponding to each experimental run are presented in **Tables 3.2** and **3.4**. The results of the regression analysis are given in **Tables 3.3** and **3.5**. In regression analysis, a polynomial quadratic model is deemed to be a good fit if its values are $p < 0.05$, lack of fit > 0.05, coefficient of variation (CV) < 10, and coefficient of determination (R^2) > 0.80, respectively (Kumar et al., 2023). The R^2 values for the dependent parameters of orange (cv. Wakro) juice, such as AA, RA of PME, BI of AA, DPPH radical scavenging activity, and TPC, were 0.985, 0.987, 0.985, 0.969, and 0.949, respectively. This data implies that the data fit the regression line well (**Table 3.3**). The model's significance was confirmed by the adjusted-R^2 values of 0.972, 0.975, 0.972, 0.942, and 0.903, respectively, which show that all are suitable for model fitting. The lack of fit for all these five responses was insignificant ($p > 0.05$) with the values of 0.2174 (for AA), 0.1728 (for RA of PME), 0.2174 (for BI of AA), 0.0711 (for DPPH radical scavenging activity), and 0.0611 (for TPC), which implies that the model is adequate for describing the relationship between the corresponding variables. Furthermore, a relatively lower CV value for each response variable with 2.19, 3.32, 2.19, 2.66, and 1.23% indicates higher precision and reliability for predicting the data (**Table 3.3**). On the other hand, the R^2 values for the dependent parameters of orange (cv. Malta) juice, such as AA, RA of PME, and BI of AA, were 0.988, 0.981, and 0.988, respectively. The values of lack of fit values obtained for each response were 0.107 (for AA), 0.091 (for RA of PME), and 0.1066 (for BI of AA), respectively. Nonetheless, the CV for each response was 6.34, 4.13, and 6.34%, respectively, implying good reliability for predicting the data. However, the results of the regression summary suggested that the quadratic model was well performed for analyzing the effect of ACP on both cultivars of orange (cv. Wakro and Malta) juices (**Table 3.5**).

Table 3.2 CCRD design with the values of responses (AA, RA of PME, BI of AA, DPPH radical scavenging activity, and TPC) of (cv. Wakro) juice.

Run	Independent variables			Response variables				
	Juice depth (mm)	Voltage (kV)	Time (min)	AA (mg/100 mL)	RA of PME (%)	BI of AA (%)	DPPH radical scavenging activity (%)	TPC (mg/100 mL)
1	3.4	22.4	2.6	17.07±0.69	27.98±1.73	46.96±1.89	37.65±0.59	48.63±1.35
2	4.6	22.4	1.4	28.59±0.79	49.49±1.75	78.64±2.18	52.04±0.29	49.08±0.45
3	4.0	20.0	2.0	26.20±0.00	50.04±3.54	72.08±0.00	55.03±0.23	50.43±0.90
4	4.0	16.0	2.0	28.96±0.69	58.21±3.06	79.66±1.89	57.85±0.29	45.32±0.52
5	3.0	20.0	2.0	21.28±1.18	44.93±1.77	58.53±3.25	48.01±0.45	49.23±0.69
6	4.6	17.6	2.6	27.95±0.68	42.89±3.06	76.89±1.88	54.96±0.40	48.93±0.94
7	4.0	20.0	3.0	25.01±1.19	29.62±1.77	68.81±3.28	44.98±0.30	50.58±0.94
8	3.4	22.4	1.4	24.22±0.69	44.44±1.75	66.62±1.89	50.25±0.40	50.43±0.45
9	4.0	20.0	2.0	25.61±0.68	48.48±3.03	70.45±1.88	56.05±0.29	50.28±0.52
10	4.0	20.0	1.0	31.10±1.36	59.23±1.77	85.56±3.75	56.76±0.51	51.78±0.45
11	4.0	24.0	2.0	22.23±0.69	34.72±1.77	61.16±1.89	46.60±.19	44.57±0.78
12	4.0	20.0	2.0	26.60±0.69	48.00±1.77	73.17±1.89	55.53±0.11	50.73±0.94
13	5.0	20.0	2.0	29.53±1.18	53.54±1.75	81.22±3.25	57.00±0.11	51.93±0.26
14	4.6	17.6	1.4	31.34±0.69	66.38±1.77	86.21±1.89	59.09±0.40	48.93±0.69
15	4.0	20.0	2.0	25.81±0.69	51.06±1.77	70.99±1.89	54.58±0.30	49.98±0.45
16	4.0	20.0	2.0	25.59±0.68	49.02±3.06	70.39±1.88	56.44±0.11	50.58±0.26
17	4.6	22.4	2.6	25.41±1.38	39.39±3.03	69.90±3.78	42.48±0.60	48.78±0.52
18	3.4	17.6	2.6	24.01±0.68	43.91±1.77	66.06±1.88	49.35±0.88	46.83±0.45
19	3.4	17.6	1.4	29.16±0.68	62.30±1.77	80.21±1.88	56.37±0.19	48.03±0.52
20	4.0	20.0	2.0	26.57±0.68	50.51±2.97	73.08±1.86	54.20±0.00	51.03±0.26

Mean ± SD

Table 3.3 ANOVA results for the effect of ACP treatment on AA, RA of PME, BI of AA, DPPH radical scavenging acidity, and TPC in orange (cv. Wakro) juice.

Source	AA (mg/100 mL)		RA of PME (%)		BI of AA (%)		DPPH (%)		TPC (mg GAE /100 mL)	
	F-value	***p*-value**	**F-value**	***p*-value**	**F-value**	***p*-value**	**F-value**	***p*-value**	**F-value**	***p*-value**
Model	74.1	< 0.0001*	82.02	< 0.0001*	74.1	< 0.0001*	35.18	< 0.0001*	20.68	< 0.0001*
A-Juice depth	239.08	< 0.0001*	33.7	0.0002*	239.08	< 0.0001*	34.39	0.0002*	8	0.0179*
B-Voltage	181.47	< 0.0001*	255.85	< 0.0001*	181.47	< 0.0001*	120.38	< 0.0001*	1.72	0.2192
C-Time	189.28	< 0.0001*	407.69	< 0.0001*	189.28	< 0.0001*	107.31	< 0.0001*	5.63	0.0391*
AB	16.57	0.0022*	8.94	0.0136*	16.57	0.0022*	0.1907	0.6716	5.99	0.0344*
AC	12.51	0.0054*	0.079	0.7844	12.51	0.0054*	2.29	0.1611	2.48	0.1466
BC	1.23	0.2934	11.66	0.0066*	1.23	0.2934	7.85	0.0187*	0.2753	0.6112
A^2	4.46	0.0609	0.0019	0.9661	4.46	0.0609	12.32	0.0056*	0.0009	0.9766
B^2	2.74	0.1286	5.30	0.0442*	2.74	0.1286	14.26	0.0036*	154.19	< 0.0001*
C^2	16.94	0.0021*	16.25	0.0024*	16.94	0.0021*	25.88	0.0005*	1.84	0.2044
Lack of fit	2.10	0.2174	2.46	0.1728	2.10	0.2174	4.18	0.0711	4.54	0.0611
R^2	0.985		0.987		0.985		0.969		0.949	
Adj. R^2	0.972		0.975		0.972		0.942		0.903	
SD	0.57		1.58		1.57		1.39		0.61	
C.V. %	2.19		3.32		2.19		2.66		1.23	

*$p < 0.05$ indicates significant difference

Table 3.4 D-optimal experimental design with the values of responses (AA of RA of PME and BI of AA) of orange (cv. Malta) juice.

Run	Independent variables			Response variables		
	Juice depth (mm)	Voltage (kV)	Time (min)	AA (mg/100 mL)	RA of PME (%)	BI of AA (%)
1	2	16	2	31.43 ± 1.33	84.76 ± 0.66	78.83 ± 3.33
2	4	18	2	33.97 ± 0.66	83.69 ± 1.45	85.20 ± 1.67
3	2	20	8	6.13 ± 0.89	39.14 ± 0.21	15.37 ± 2.22
4	3	16	6	16.87 ± 1.44	78.36 ± 0.36	42.31 ± 3.61
5	4	20	10	9.70 ± 0.00	40.09 ± 4.32	24.33 ± 0.00
6	4	18	6	18.19 ± 0.37	67.62 ± 0.33	45.62 ± 0.93
7	2	18	6	11.73 ± 0.66	55.27 ± 1.84	29.42 ± 1.67
8	2	18	2	30.39 ± 0.66	68.40 ± 0.15	76.22 ± 1.67
9	3	20	10	6.75 ± 1.06	37.86 ± 0.21	16.93 ± 2.66
10	2	16	10	6.90 ± 0.24	67.45 ± 0.79	17.31 ± 3.33
11	3	18	10	7.67 ± 1.33	49.50 ± 0.33	19.24 ± 3.33
12	4	16	6	20.63 ± 1.37	84.10 ± 0.73	51.74 ± 3.45
13	3	16	2	33.73 ± 1.33	88.04 ± 0.92	84.60 ± 3.33
14	2	18	4	20.20 ± 1.27	60.54 ± 2.52	50.66 ± 3.19
15	3	16	4	27.60 ± 0.00	82.19 ± 1.22	69.22 ± 0.00
16	4	18	10	9.94 ± 0.74	55.10 ± 2.51	24.93 ± 1.85
17	2	20	10	4.12 ± 0.00	35.55 ± 0.53	10.33 ± 0.00
18	4	20	8	11.29 ± 1.15	46.08 ± 1.35	28.32 ± 2.89
19	3	20	6	14.56 ± 0.54	46.48 ± 1.62	36.52 ± 1.35
20	4	20	2	31.69 ± 1.12	68.95 ± 0.90	79.48 ± 2.81
21	4	16	8	13.80 ± 0.00	82.79 ± 0.38	34.61 ± 0.00
22	2	20	4	19.17 ± 1.01	45.20 ± 1.01	48.08 ± 2.53
23	3	20	8	9.81 ± 1.06	42.88 ± 1.19	24.60 ± 2.66
24	2	16	4	21.47 ± 1.47	76.07 ± 1.32	53.85 ± 3.68
25	4	18	8	12.40 ± 1.23	60.11 ± 2.52	31.10 ± 3.08
26	4	18	6	17.42 ± 0.55	66.09 ± 1.31	43.69 ± 1.93
27	4	16	4	28.37 ± 1.33	86.95 ± 1.58	71.16 ± 3.33
28	2	18	8	6.95 ± 0.95	49.46 ± 2.54	17.43 ± 2.38
29	4	16	6	21.42 ± 0.00	83.15 ± 1.06	53.72 ± 0.00
30	4	16	10	10.52 ± 0.91	81.20 ± 0.59	26.39 ± 2.55
31	3	18	4	26.83 ± 1.33	66.40 ± 1.55	67.29 ± 3.33
32	2	20	6	10.35 ± 1.22	43.28 ± 4.32	25.96 ± 2.81
33	4	20	4	25.15 ± 1.06	53.17 ± 2.07	63.08 ± 2.66
34	3	16	8	12.27 ± 1.33	76.77 ± 0.88	30.78 ± 3.33
35	3	20	2	30.67 ± 1.06	54.25 ± 1.06	76.93 ± 2.66
36	3	16	6	17.61 ± 1.83	80.60 ± 2.41	44.16 ± 4.60
37	3	18	2	32.20 ± 0.00	77.10 ± 0.70	80.76 ± 0.00

Mean ± SD

Table 3.5 ANOVA results for the effect of ACP treatment on AA, RA of PME, and BI of AA in orange (cv. Malta) juice.

Source	AA (mg/100 mL)		RA of PME (%)		BI of AA (%)	
	F-value	***p*-value**	**F-value**	***p*-value**	**F-value**	***p*-value**
Model	254.88	< 0.0001*	157.84	< 0.0001*	254.94	< 0.0001*
A-Juice depth	131.34	< 0.0001*	92.9	< 0.0001*	131.39	< 0.0001*
B-Voltage	46.96	< 0.0001*	898.75	< 0.0001*	46.97	< 0.0001*
C-Time	1924.49	< 0.0001*	233.24	< 0.0001*	1924.94	< 0.0001*
AB	0.0223	0.8823	2.23	0.1467	0.0221	0.8829
AC	0.0036	0.9523	2.57	0.1208	0.0036	0.9529
BC	2.50	0.1255	2.04	0.1646	2.49	0.1259
A^2	3.67	0.066	0.2861	0.5971	3.67	0.0659
B^2	0.2329	0.6333	2.84	0.1035	0.2343	0.6323
C^2	74.11	< 0.0001*	8.89	0.0060*	74.16	< 0.0001*
Lack of fit	4.92	0.107	5.56	0.091	4.93	0.1066
R^2	0.988		0.981		0.988	
Adj. R^2	0.985		0.975		0.985	
SD	1.15		2.64		2.88	
C.V. %	6.34		4.13		6.34	

*$p < 0.05$ indicates significant difference

3.3.2 Effect of ACP treatment on orange (cv. Wakro) juice

3.3.2.1 Ascorbic acid

The AA content in citrus juice is an essential health benefits bioactive compound that acts as a potent antioxidant, aiding in iron absorption, fighting infections, and indicating the nutritional quality of fruit juices (Chambial et al., 2013). The different thermal and non-thermal processing methods and varieties of oranges impact the AA content in juice, highlighting the importance of optimal nutrition for consumer preference (Tiwari et al., 2008; Souza et al., 2023). The AA content of untreated orange (cv. Wakro) juice was 36.35 ± 1.34 mg/100 mL. According to ANOVA results, the individual terms of juice depth, voltage, and treatment duration significantly impacted the AA content in the juice while exposed to plasma (**Table 3.3**). The interaction terms AB and AC showed a significant effect ($p < 0.05$) on the AA content, whereas BC had an insignificant effect (**Table 3.3**). Among the quadratic terms, only treatment time showed a significant ($p < 0.05$) effect (**Table 3.3**). As shown in **Eq. 3.8**, the negative coefficient of BC (voltage and treatment time) indicating the simultaneous increase of these two variables will degrade the AA content in the juice.

The AA content in juice decreased with a decrease in juice depth and an increase in voltage and treatment time (**Fig. 3.4a** and **3.4b**). The rise in juice depth resulted in minimal effect on the AA content. As illustrated in **Fig. 3.4c**, AA content decreased with increased treatment time and voltage while subjected to ACP treatment. This investigation revealed that the longer treatment time led to a greater reduction in the AA content. The same trend of AA degradation was also observed in orange juice and blueberry juice during CP treatment by Xu et al. (2017) and Hou et al. (2019). The degradation of AA in juice may be due to the oxidative reaction with plasma-generated reactive oxygen species (ROS) and free radicals (Xu et al., 2017; Hosseini et al., 2020). Hosseini et al. (2020) observed a 21% AA loss in CP-treated sour cherry juice. On the other hand, Shi et al. (2011) reported that there was no significant effect on AA in orange juice at 20 s. Silveira et al. (2019) and de Castro et al. (2020) reported that the plasma treatment significantly enhanced the AA content in camu-camu juice and guava-flavored whey beverages.

$$AA\ (\mathrm{mg/100\ mL}) = +26.07 + 2.39\mathrm{A} - 2.09\mathrm{B} - 2.13\mathrm{C} + 0.8235\mathrm{AB} + 0.7156\mathrm{AC} - 0.2243\mathrm{BC} - 0.3182\mathrm{A}^2 - 0.2497\mathrm{B}^2 + 0.6204\mathrm{C}^2 \quad (3.8)$$

3.3.2.2 Residual activity of PME

The PME is an endogenous enzyme, a complex polysaccharide naturally present in plant cell walls. The cloud stability in citrus juice is closely associated with the PME, which influences the juice quality. The loss of cloudiness in orange juice is typically attributed to the PME activity (Basak and Ramaswamy, 1996). Therefore, the inactivation of PME in orange (cv. Wakro) juice through non-thermal ACP treatment is crucial for maintaining juice quality. In this study, the processing parameters of the ACP treatment influenced the PME inactivation. The initial activity of the PME enzyme in orange (cv. Wakro) juice was 0.622 ± 0.03 U/mL. The highest inactivation of PME (72.02%) was achieved at 22.4 kV, 3.4 mm juice depth, and 2.6 min treatment duration. **Table 3.3** shows that the PME inactivation was significantly (p < 0.05) affected by juice depth, voltage, and treatment duration. The interaction terms AB and BC had a significant (p < 0.05) effect on RA of PME, whereas AC showed an insignificant (p > 0.05) effect (**Table 3.3**). On the other hand, quadratic term A positively affected the RA of PME. As observed in **Eq. 3.9**, the coefficient of BC showed the largest, indicating a greater impact on RA of PME. Nonetheless, the

coefficient of AC indicated that the juice depth and treatment time had no severe effect on RA of PME (**Eq. 3.9**).

As illustrated in **Fig. 3.4d** and **3.4e**, the RA of PME significantly decreased with an increase in voltage and treatment duration and a decrease in juice depth. This implies that the influence of ACP depends on the changes in juice depth, resulting in lower depth and more PME inactivation. **Fig. 3.4f** illustrates that the RA of PME in juice is a function of time and voltage; increasing treatment duration and voltage decreases the RA of PME. Andreu et al. (2023) studied the effect of helium (He) gas feed jet source of CP on PME inactivation in orange juice. The authors reported that the PME inactivation increased with voltage and time; they achieved 55–80% inactivation for 2–30 min. In another case, the voltage and time significantly influenced peroxidase (POD) inactivation in kiwifruit juice treated with CP (Kumar et al., 2023). These studies have demonstrated that the CP treatment has significantly inactivated the enzymes in various fruits, including kiwifruit and apple juices (Kumar et al., 2023; Illera et al., 2019). The mechanism involved in PME inactivation is when there is a structural disruption caused by plasma-generated ROS, leading to inactivation (Gan et al., 2021). However, the reactive species and free radicals produced during treatment due to gas ionization are the main substances for inactivation. The enzyme inactivation also depends on the nature of the fruit, varieties, type of fruit, process conditions, and the initial activity of specific enzymes.

$$RA\ of\ PME\ (\%) = +49.50 + 2.49\mathrm{A} - 6.86\mathrm{B} - 8.66\mathrm{C} + 1.68\mathrm{AB} + 0.1575\mathrm{AC} + 1.91\mathrm{BC} + 0.0182\mathrm{A}^2 - 0.9606\mathrm{B}^2 - 1.68\mathrm{C}^2 \quad (3.9)$$

3.3.2.3 Bioaccessibility index of ascorbic acid

The BI of AA in vegetables ranges from 4 to 80%; in fruits, it varies from 2 to 91% (Sánchez-Moreno et al., 2004). According to ANOVA results, the linear terms of the process variables were statistically significant ($p < 0.05$) (**Table 3.3**). As shown in **Table 3.3**, F-value = 239.08 indicates that the juice depth significantly affected the AA BI in juice, followed by time (F-value = 189.28) and voltage (F-value = 181.47). The interaction terms AB and AC showed statistically significant ($p < 0.05$), whereas BC had an insignificant effect ($p > 0.05$) (**Table 3.3**). On the other hand, the quadratic term of treatment time exhibited a significant impact on the BI of AA ($p < 0.05$)

(**Table 3.3**). Moreover, the coefficient values of AB and AC showed a positive effect on BI of AA, whereas the coefficients of AC exhibited a negative impact (**Eq. 3.10**).

As shown in **Fig. 3.4g** and **3.4h**, the BI of AA decreased with the increase in time and voltage and decrease in juice depth. It was observed that an increase in voltage and treatment time significantly affected the BI of AA (**Fig. 3.4i**). This study demonstrated that the BI of AA was significantly influenced by juice depth, voltage, and treatment duration. However, the loss of BI of AA could also be attributed to plasma-generated reactive species (Xu et al., 2017). A similar investigation is correlated with the observations of de Castro et al. (2019); the authors reported the impact of CP on the BI of AA in camu-camu juice.

$$BI\ of\ AA\ (\%) = +71.73 + 6.59\mathrm{A} - 5.74\mathrm{B} - 5.86\mathrm{C} + 2.27\mathrm{AB} + 1.97\mathrm{AC} - 0.6171\mathrm{BC} - 0.8754\mathrm{A}^2 - 0.6869\mathrm{B}^2 + 1.71\mathrm{C}^2 \quad (3.10)$$

3.3.2.4 DPPH radical scavenging activity

The phenolic compounds and AA are the major constituents of fruit juices responsible for the antioxidant activity (Pankaj et al., 2017). These bioactive substances can scavenge free radicals (Aadil et al., 2013). DPPH radical scavenging activity of untreated orange (cv. Wakro) juice was 69.74 ± 0.69%. **Table 3.2** shows the impact of ACP treatment on DPPH radical scavenging activity in orange (cv. Wakro) juice. As shown in **Table 3.3**, the significance test showed that all the linear terms significantly impacted the DPPH radical scavenging activity, whereas only juice depth had a positive impact. The interaction terms AB and AC showed insignificant effects, but the BC significantly ($p < 0.05$) impacted the DPPH radical scavenging activity. All the quadratic terms of juice depth, voltage, and treatment duration significantly ($p < 0.05$) affected the DPPH radical scavenging activity. The negative coefficient of BC implies that the simultaneous increase in voltage and treatment time will reduce the DPPH radical scavenging activity in the juice (**Eq. 3.11**). Besides, the coefficient of AB also indicates the same (**Eq. 3.11**).

With the rise in voltage and decrease in juice depth, the DPPH radical scavenging activity decreased significantly (**Fig. 3.4j**). As shown in **Fig. 3.4l**, the treatment time and voltage rise significantly affected the juice's DPPH radical scavenging activity. The minimum reduction of DPPH radical scavenging activity (59.09 ± 0.40%) was obtained at 4.6 mm juice depth and 17.6 kV voltage for 1.4 min. The investigation

revealed that all the ACP processing parameters significantly influenced the reduction of DPPH radical scavenging activity. As shown in **Table 3.2,** the significant decrease in DPPH radical scavenging activity results correlates with the observations of CP-treated blueberry juice (Hou et al., 2019). A study reported that the DPPH radical scavenging activity decreased to 12.09% in grape juice after high-voltage atmospheric CP treatment at 80 kV for 4 min (Pankaj et al., 2017). DPPH radical scavenging activity reduction in the juice during treatment may be attributed to the higher oxygen concentration in ionized gas and ultraviolet (UV) light (Hou et al., 2019; Kumar et al., 2023). The degradation results of the DPPH radical scavenging activity are mainly influenced by the decrease in AA during DBD treatment. Rodríguez et al. (2017) observed that the DPPH radical scavenging activity is associated with the degradation of AA in CP-treated cashew apple juice. Nevertheless, the other non-thermal methods, such as ozone treatment, also reduced the DPPH radical scavenging activity (Tiwari et al., 2008). In contrast, ultra-sound treatment significantly improved its percentage in orange juice (Guerrouj et al., 2016). On the other hand, Paixão et al. (2019) and de Castro et al. (2020) reported that there is an insignificant effect on DPPH radical scavenging activity in Siriguela and Camu-Camu juice while treated with glow discharge and DBD plasma. Surprisingly, another study documented DPPH radical scavenging activity enhancement in carrot juice after CP treatment (Manzoor et al., 2020). However, the degradation of DPPH radical scavenging activity in fruit juices can be minimized by changing the feed gas and its composition (Xu et al., 2017).

$$DPPH\ radical\ scavenging\ activity\ (\%) = +55.35 + 2.20\mathrm{A} - 4.12\mathrm{B} - 3.89\mathrm{C} - 0.2143\mathrm{AB} + 0.7426\mathrm{AC} - 1.37\mathrm{BC} - 1.28\mathrm{A}^2 - 1.38\mathrm{B}^2 - 1.86\mathrm{C}^2 \quad (3.11)$$

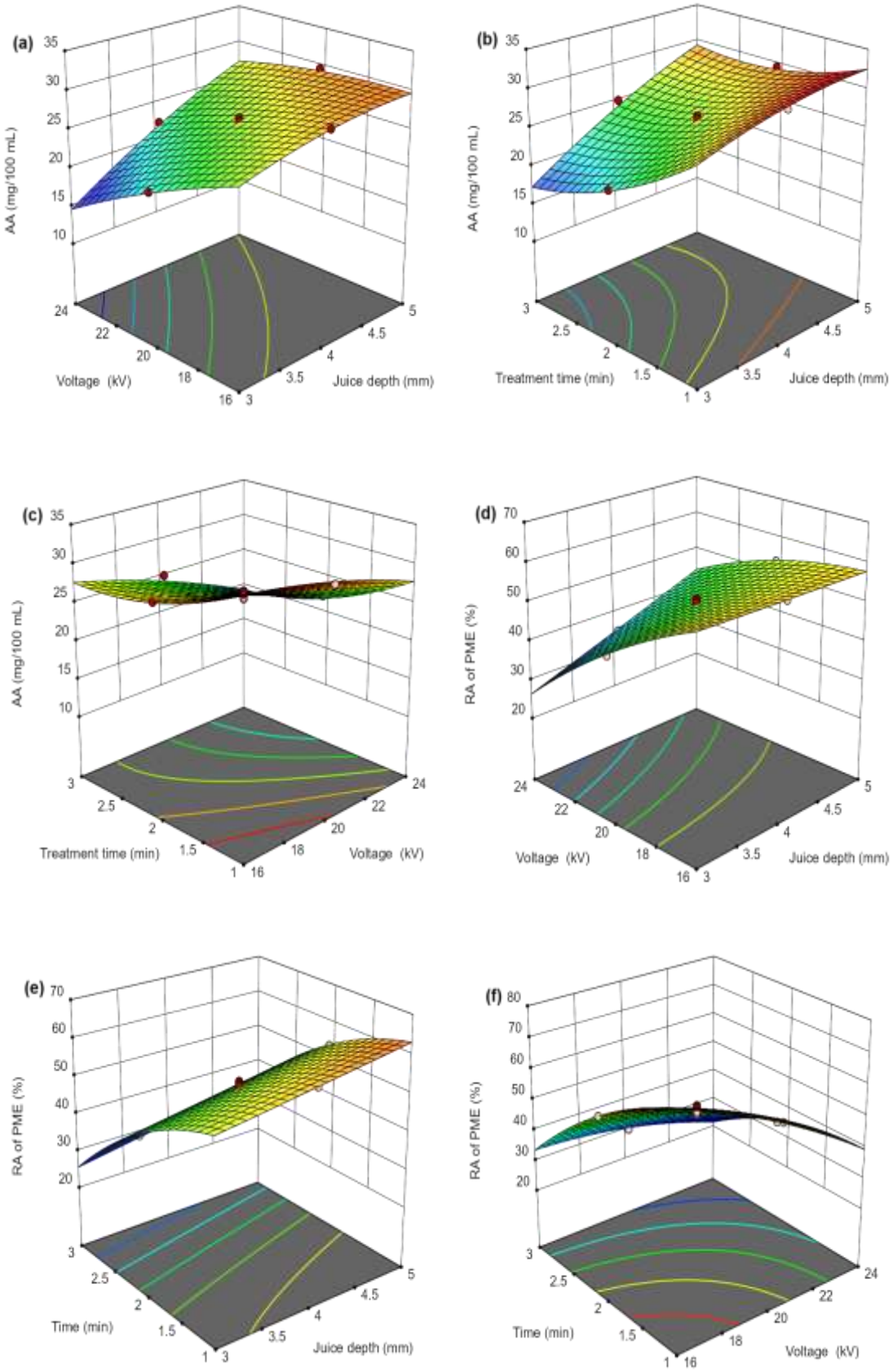
3.3.2.5 Total phenolic content

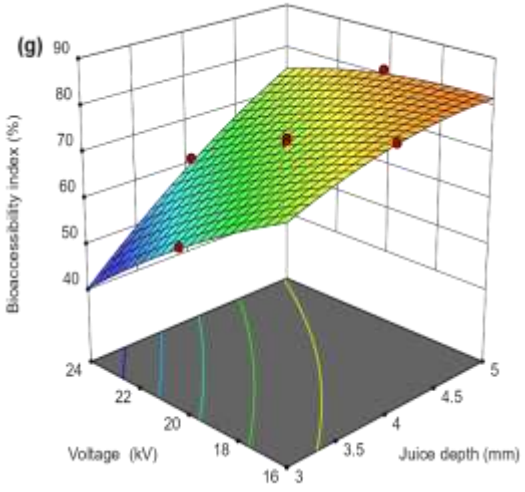
The TPC is a quality indicator of fruit and vegetable juices. It is present in the aqueous phase of the juice and coupled with other molecules (Das et al., 2024). The TPC of orange (cv. Wakro) juice was 46.23 ± 0.69 mg GAE/100 mL. The effect of ACP treatment on TPC in orange (cv. Wakro) juice is shown in **Table 3.2**. The highest amount of TPC (51.93 ± 0.26 mg GAE/100 mL) was obtained in juice after APC treatment (juice depth: 5 mm, voltage: 20 kV, and time: 2 min) (**Table 3.2**). The ANOVA results show that the linear term of juice depth and treatment time had a significant (p < 0.05) effect on TPC, whereas voltage showed insignificant (**Table 3.3**). The interaction terms AB had a significant (p < 0.05) impact on TPC. On the

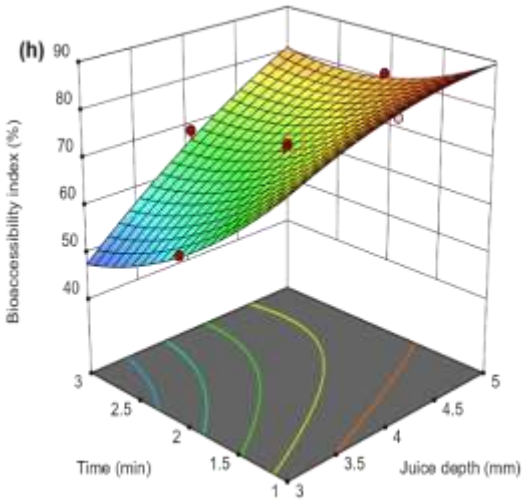
other hand, the quadratic term of juice depth and treatment time had an insignificant (p > 0.05) effect on TPC (**Table 3.3**). In **Eq. 3.12**, the coefficients of AB and AC showed a positive effect, while BC negatively impacted TPC in juice.

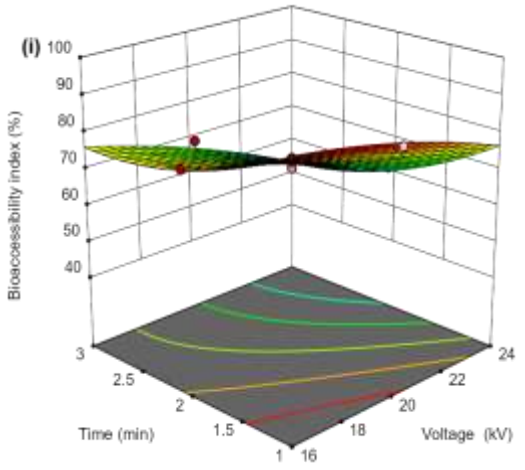
The TPC in juice first increased with an increase in voltage, juice depth, and duration of treatment up to a specific limit, then declined **(Fig. 3.4m** and **3.4o).** This action provides the downward-facing parabola form of the curve. As demonstrated in **Table 3.2,** TPC increased with increasing the juice depth till a specified point as a function of time; further increasing the time, the TPC declined. With a high voltage of 24 kV and a juice depth of 4 mm for 2 min, TPC decreased by 3.59% (**Table 3.2**). However, a considerable rise in TPC was observed up to a specific limit within independent parameters (**Table 3.2**). The study results show that the TPC was increased to 12.33%. The increase in TPC juice may result from the breakdown of cell walls induced by plasma reactive species, where the phenolic compounds are located. It breaks down the cell membranes and promotes the extraction of conjugated phenolic compounds, increasing TPC (Illera et al., 2019). Similar observations were reported in CP-treated kiwifruit juice (Kumar et al., 2023). A significant increase in TPC (14.43%) was also observed in commercial apple juice after CP treatment at 120 s (Dasan and Boyaci, 2018). Nonetheless, other non-thermal ultrasound treatments on orange juice significantly improved the TPC (Guerrouj et al., 2016). However, the increment in TPC during plasma treatment depends on the juice depth, voltage, and treatment time, but extending the voltage intensity and longer treatment time may degrade the TPC in juice samples. Therefore, it is important to consider the optimal conditions of these parameters during plasma treatment to augment the TPC in juice.

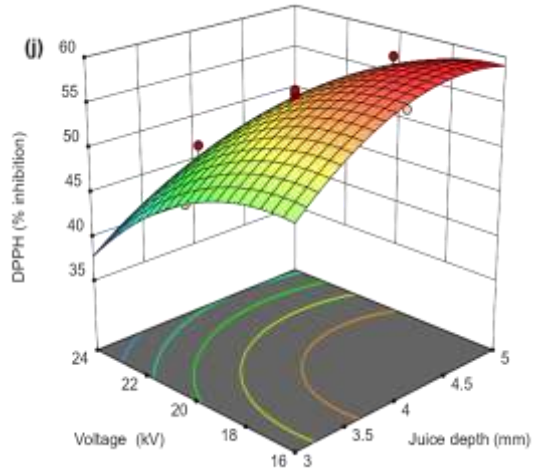
$$\mathrm{TPC\ (mg\ GAE/100\ mL)} = +50.51 + 0.4646\mathrm{A} + 0.2153\mathrm{B} - 0.3896\mathrm{C} - 0.5253\mathrm{AB} + 0.3377\mathrm{AC} - 0.1126\mathrm{BC} + 0.0048\mathrm{A}^2 - 1.99\mathrm{B}^2 + 0.2170\mathrm{C}^2 \quad (3.12)$$

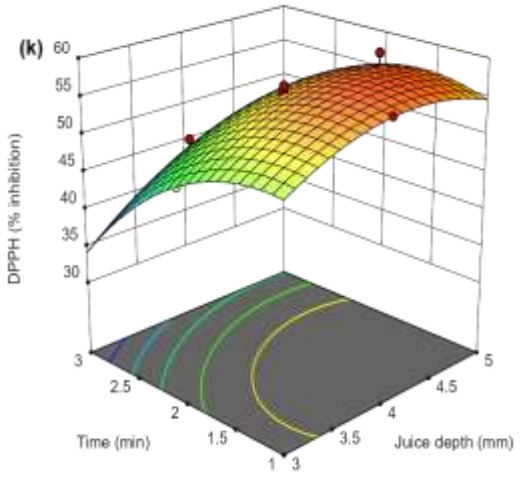

(a)
AA (mg/100 mL)
Voltage (kV)
Juice depth (mm)
(b)
AA (mg/100 mL)
Treatment time (min)
Juice depth (mm)
(c)
AA (mg/100 mL)
Treatment time (min)
Voltage (kV)
(d)
RA of PME (%)
Voltage (kV)
Juice depth (mm)
(e)
RA of PME (%)
Time (min)
Juice depth (mm)
(f)
RA of PME (%)
Time (min)
Voltage (kV)

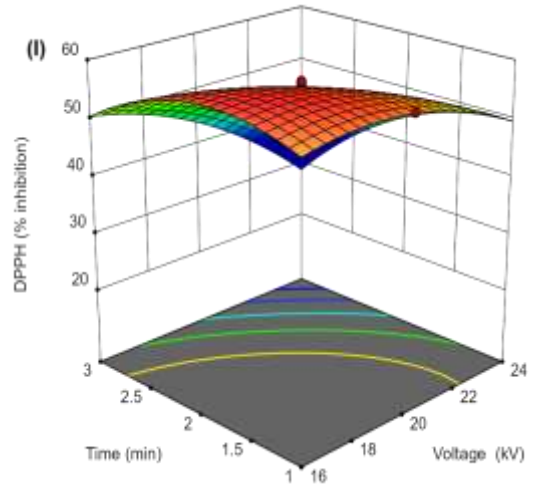

(g)
Bioaccessibility index (%)
90
80
70
60
50
40
24
22
20
18
16
Voltage (kV)
3
3.5
4
4.5
5
Juice depth (mm)


(h)
Bioaccessibility index (%)
90
80
70
60
50
40
3
2.5
2
1.5
1
Time (min)
3
3.5
4
4.5
5
Juice depth (mm)


(i)
Bioaccessibility index (%)
100
90
80
70
60
50
40
3
2.5
2
1.5
1
Time (min)
16
18
20
22
24
Voltage (kV)


(j)
DPPH (% inhibition)
60
55
50
45
40
35
24
22
20
18
16
Voltage (kV)
3
3.5
4
4.5
5
Juice depth (mm)


(k)
DPPH (% inhibition)
60
55
50
45
40
35
30
3
2.5
2
1.5
1
Time (min)
3
3.5
4
4.5
5
Juice depth (mm)


(l)
DPPH (% inhibition)
60
50
40
30
20
3
2.5
2
1.5
1
Time (min)
16
18
20
22
24
Voltage (kV)

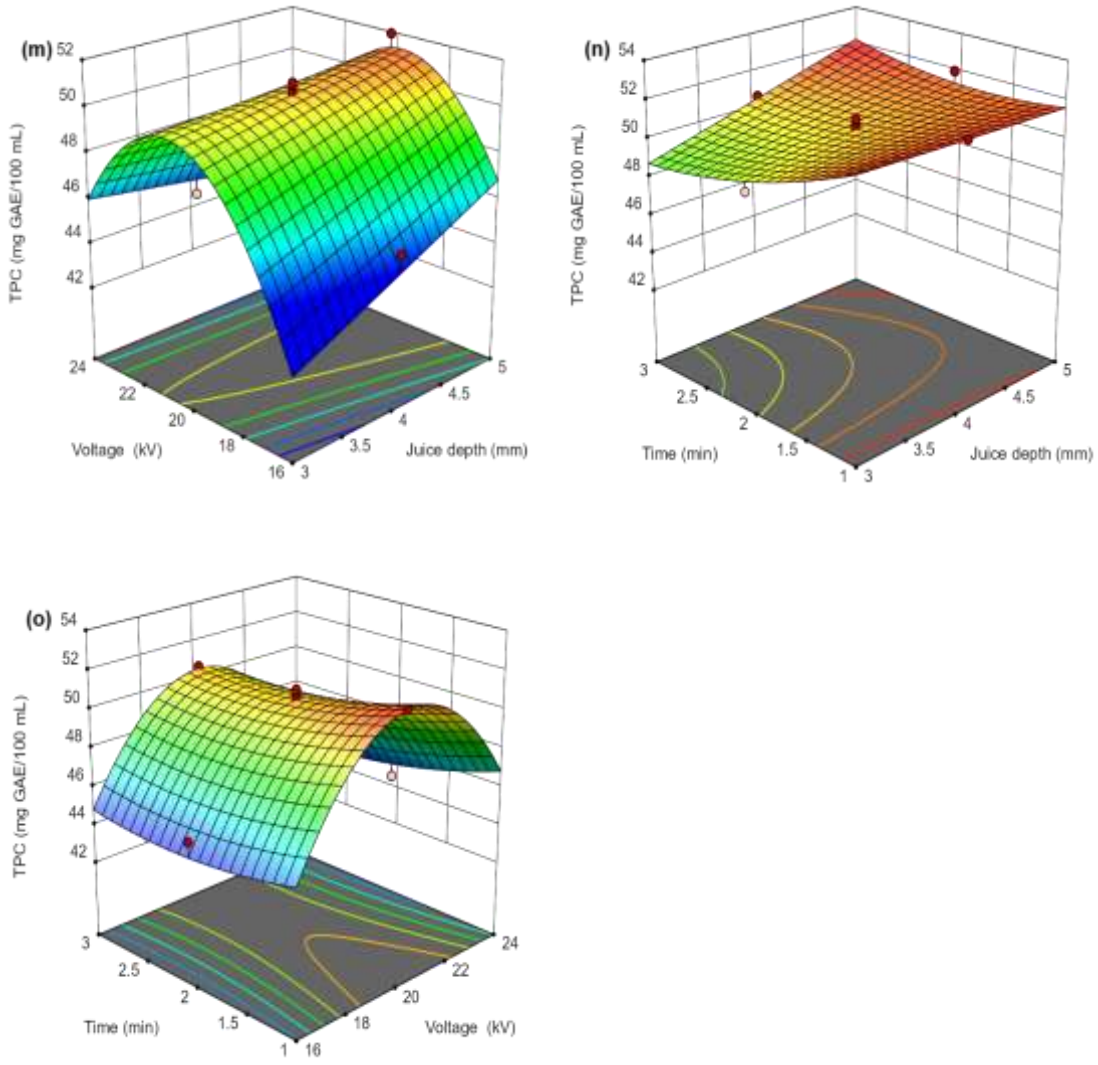


Fig. 3.4 Effect of ACP processing parameters (juice depth, voltage, and treatment time) on the responses (**a–c**: AA, **d–f**: RA of PME, **g–i**: BI of AA, **j–l**: DPPH, and **m–o**: TPC).

3.3.3 Optimization for ACP processing of orange (cv. Wakro) juice and validation

A numerical optimization technique with Design Expert's desirability function was used to optimize the processing conditions of ACP treatment on orange (cv. Wakro) juice experimental data (Kumar et al., 2023). To optimize the conditions, constraint criteria for all independent and response variables were set according to their desired goal and importance in the experiment. The numerical method showed a desirability function value of 0.787. The optimum values were obtained from process and response variables for ACP processing of orange (cv. Wakro) juice. The optimized

ACP treatment conditions for juice were 4.6 mm (juice depth), 19 kV (voltage), and 2.6 min (treatment time). At these conditions, AA, RA of PME, BI of AA, DPPH radical scavenging activity, and TPC were predicted as 28.08 mg/100 mL, 43.80%, 77.25%, 53.92%, 50.80 mg GAE/100 mL, respectively. To validate predicted results, experiments were performed based on optimum process conditions. The experimental values obtained at optimal ACP treatment conditions for AA, RA of PME, BI of AA, DPPH radical scavenging activity, and TPC were 27.41 ± 0.79 mg/100 mL, 42.42 ± 3.03%, 75.41 ± 2.17%, 51.99 ± 0.59 mg/100 mL, 51.33 ± 0.45 mg GAE/100 mL, respectively.

Table 3.6 Optimized ACP process conditions of orange (cv. Wakro) juice obtained from RSM and its validation.

Optimum conditions	Desirability	Parameters	Responses		% Error
			Predicted	Observed	
Juice depth: 4.6 mm		AA (mg/100 mL)	28.08	27.41 ± 0.79	2.44
Voltage: 18.73 ≈ 19		RA of PME (%)	43.80	42.42 ± 3.03	3.25
kV	0.787	BI of AA (%)	77.25	75.41 ± 2.17	2.44
Time: 2.6		DPPH (%)	53.92	51.99 ± 0.59	3.71
min		TPC (mg GAE/100 mL)	50.80	51.33 ± 0.45	1.03

3.3.4 Effect of ACP treatment on orange (cv. Malat) juice

3.3.4.1 Ascorbic acid

The AA content in orange (cv. Malta) juice was 39.87 ± 1.33 mg/100 mL. As shown in **Table 3.5**, the individual linear terms of voltage, sample depth, and treatment time significantly impacted AA. The interaction terms showed an insignificant ($p > 0.05$) effect on the AA (**Table 3.5**). In contrast, the square term of treatment time had a significant ($p < 0.05$) impact, while sample depth and voltage had an insignificant ($p > 0.05$) effect on the AA in juice (**Table 3.5**). It was observed that the voltage and treatment time, followed by sample depth within the range of levels, are associated with reducing the AA in juice. The AA content of juice decreases with increasing treatment time and voltage intensity (**Fig. 3.5c**). The analysis indicates that decreasing sample depth with increasing treatment time reduces the AA content of the juice (**Fig. 3.5b**). On the other hand, increasing the voltage and sample depth showed no severe effect on the AA reduction (**Fig. 3.5a**). The AA loss in juice may be due to free

radicals and the high concentration of ROS generated by ACP (Xu et al., 2017; Tiwari et al., 2008). Xu et al. (2017) reported that plasma reduced the AA content in orange juice by 22% (in air) at 90 kV for 2 min. Another study also reported that the AA content of sugarcane juice was reduced by 6% and 25% while treated with DBD-plasma at 45 kV for 2 min and thermal pasteurization at 90 °C for 5 min (Manzoor et al., 2020). The reduction of AA could be attributed to two pathways: one involving the direct impact of ozone (referred to as the Criegee mechanism) and the other involving excited molecular oxygen and singlet oxygen, following a free radical mechanism (Misra et al., 2015; Kanofsky et al., 1991; Enami et al., 2008).

$$AA\ (\mathrm{mg/100\ mL}) = +16.31 + 2.71\mathrm{A} - 1.63\mathrm{B} - 12.50\mathrm{C} - 0.0456\mathrm{AB} - 0.0213\mathrm{AC} + 0.5588\mathrm{BC} - 0.7894\mathrm{A}^2 + 0.1984\mathrm{B}^2 + 4.02\mathrm{C}^2 \quad (3.13)$$

3.3.4.2 Residual activity of PME

The initial PME activity of orange (cv. Malta) juice was 1.445 ± 0.12 U/mL. This investigation revealed that the PME activity in the juice decreases with increasing voltage and treatment time at varying juice depths. The RA of PME of the Malta orange (cv. Malta) juice was 35.55% with maximum inactivation (64.45%) at 20 kV and 2 mm for 10 min. PME inactivation can be achieved more by increasing voltage intensity and plasma exposure time. A study reported that the PME's inactivation is linked to its structure alterations due to the reactive substances formed in the liquid phase because of ACP treatment (Xu et al., 2017). Other studies also reported that the structural modification of PME is initiated by ROS, which leads to the oxidation of enzymes and their functionality (Henselová et al., 2012; Tappi et al., 2016). According to our results, the linear terms of the sample depth, voltage, and treatment time showed a significant ($p < 0.05$) effect on the RA of PME (**Table 3.5**). The quadratic terms of the sample depth and voltage had an insignificant ($p > 0.05$) effect, whereas treatment time significantly ($p < 0.05$) affected the RA of PME (**Table 3.5**). It was observed that the coefficient of interaction terms AB, AC, and BC showed a negative effect on RA of PME. As shown in **Fig. 3.5f**, the RA of PME decreased with increasing treatment time and voltage intensity. **Fig. 3.5d** and **3.5e** showed that increasing the sample depth had a mild impact on the RA of PME, whereas increasing treatment time and voltage profoundly affected the RA of PME. The inactivation of the PME activity in juice may be due to the rise of reactive species produced by ACP

(Gan et al., 2021). Misra et al. (2015) reported that the reactive species transferred from the gaseous phase into the liquid phase, which inactivates the enzyme.

$$\text{RA of PME } (\%) = +60.19 + 5.24\text{A} - 16.42\text{B} - 10.00\text{C} - 1.05\text{AB} - 1.30\text{AC} - 1.16\text{BC} + 0.5063\text{A}^2 + 1.59\text{B}^2 + 3.20\text{C}^2 \quad (3.14)$$

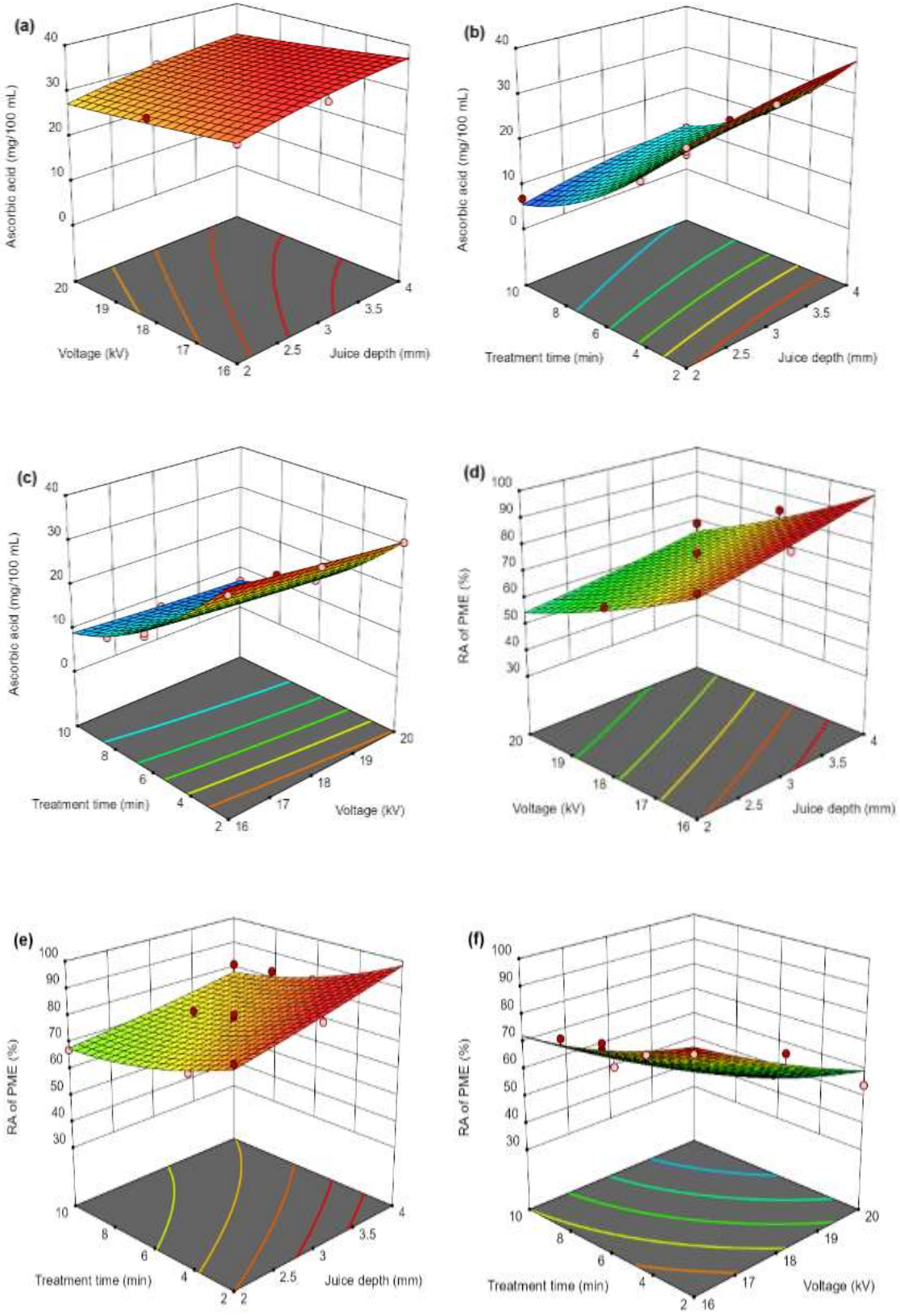
3.3.4.3 Bioaccessibility index of ascorbic acid

The ANOVA results show that all the individual linear terms of the independent parameters were statistically significant ($p < 0.05$). Although the interaction terms showed an insignificant ($p > 0.05$) effect on BI of AA (**Tabel 3.5**), treatment time contributed a significant effect, followed by voltage and sample depth. The square term of treatment time had a significant ($p < 0.05$) impact on the BI of AA (**Table 3.5**). In **Eq. 3.15**, the coefficient of AB and AC showed a negative impact on BI of AA. As illustrated in **Fig. 3.5i**, the BI of AA decreased with increasing plasma exposure time and voltage intensity. Likewise, an increase in treatment duration and a lowering in the juice depth significantly affected the BI of AA (**Fig. 3.5h**). The study results show that the BI of AA decreased with the increase in voltage and treatment time at varying sample depths. The BI of AA is dependent on the AA content in juice. Therefore, the BI of AA reduction is directly associated with the oxidation of AA caused by reactive species and free radicals generated by ACP. However, other novel non-thermal techniques have been employed to improve the bioavailability of AA at different conditions, such as pulsed electric field for orange juice and ultrasound for cashew juice (Fonteles et al., 2016; Tarabová et al., 2021).

$$BI\ of\ AA\ (\%) = +40.90 + 6.81\text{A} - 4.10\text{B} - 31.35\text{C} - 0.1136\text{AB} - 0.0529\text{AC} + 1.40\text{BC} - 1.98\text{A}^2 + 0.4989\text{B}^2 + 10.08\text{C}^2 \quad (3.15)$$

3.3.5 Optimization for ACP processing of (cv. Malta) juice and validation

The numerical optimization showed a desirability of 0.782. The optimum conditions for process variables were 3.3 mm sample depth, 20 kV voltage, and 2 min treatment time, respectively. The corresponding optimized values for the responses were 31.54 mg/100 mL AA, 61.33% RA of PME, and 79.10% BI. It was found that the experimental data were reasonably consistent with the expected results, confirming the derived model's validity. Thus, varying observed mean values of the response parameters (AA of 32.51 ± 1.06 mg/100 mL, RA of PME of 58.53 ± 1.44%, and BI of 81.53 ± 2.66%) could be achieved satisfactorily within a 95% confidence interval.


(a)
Ascorbic acid (mg/100 mL)
Voltage (kV)
Juice depth (mm)
(b)
Ascorbic acid (mg/100 mL)
Treatment time (min)
Juice depth (mm)
(c)
Ascorbic acid (mg/100 mL)
Treatment time (min)
Voltage (kV)
(d)
RA of PME (%)
Voltage (kV)
Juice depth (mm)
(e)
RA of PME (%)
Treatment time (min)
Juice depth (mm)
(f)
RA of PME (%)
Treatment time (min)
Voltage (kV)

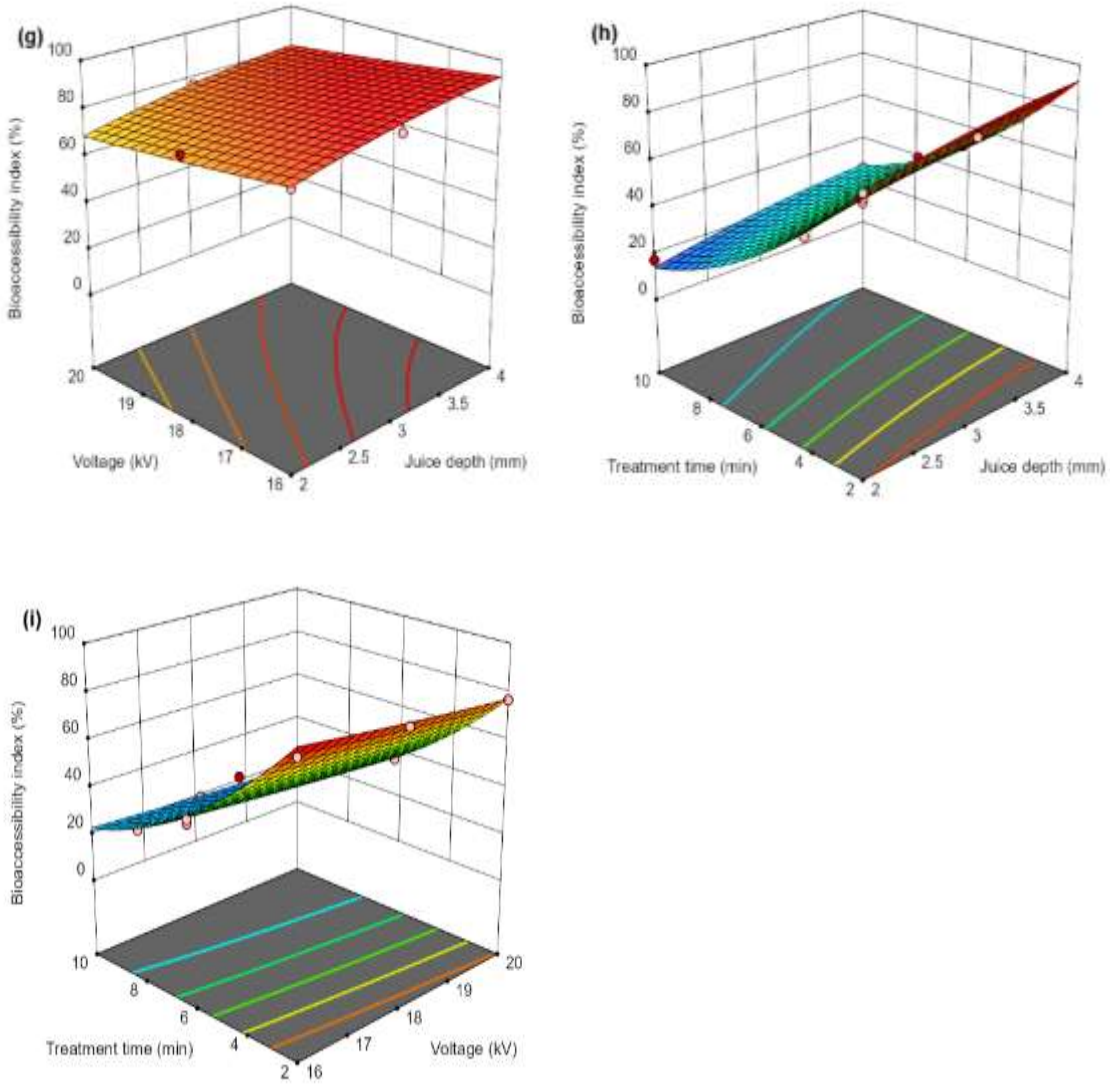


Fig. 3.5 Effect of ACP processing parameters (juice depth, voltage, and treatment time) on the responses (**a–c**: AA, **d–f**: RA of PME, and **g–i**: BI of AA).

Table 3.7 Optimized ACP process conditions of orange (cv. Malta) juice obtained from RSM and its validation.

Optimum conditions	Desirability	Parameters	Responses		% Error
			Predicted	Observed	
Juice depth: 3.29 ≈ 3.3 mm Voltage: 20 kV Time: 2 min	0.782	AA (mg/100 mL)	31.54	32.51 ± 1.06	2.98
		RA of PME (%)	61.33	58.53 ± 1.44	4.78
		BI of AA (%)	79.10	81.53 ± 2.66	2.98

Based on the F-value of ANOVA results from the ACP treatment on both Wakro and Malta orange juices, we can determine the most critical parameters for optimizing the

juice quality, as shown in **Table 3.3** and **3.5**. In identifying the critical process parameters, the F-value results showed that the treatment time and juice depth would yield the best overall results, with some emphasis on voltage depending on the desired quality attribute (**Table 3.3** and **3.5**).

As shown in **Table 3.2** and **3.4**, the effect of batch-type ACP treatment on orange juice of two different cultivars (cv. Wakro and Malta) revealed that PME inactivation was achieved more in Wakro orange juice within a range of 1–3 min treatment time compared to Malta orange juice. On the other hand, the AA degradation was higher in Malta orange juice while the treatment time increased from 2 to 10 min (**Table 3.4**). The AA retention was also significantly influenced by increased juice depth, voltage, and treatment time. However, the differences in independent variable ranges mean the comparison is not equivalent. Still, based on the results and the optimization for each variety, Wakro could be considered more effective in terms of PME inactivation when analyzed under its specific optimized conditions. The study's results suggest that the Wakro orange juice yielded more favourable outcomes under ACP processing conditions than Malta orange juice.

3.4 Conclusion

This study investigated the effect of ACP treatment on the quality attributes of orange (cv. Wakro and Malta) juice and the optimization of process parameters. RSM effectively identified the optimum processing conditions for ACP treatment of orange (cv. Wakro) juice in 3–5 mm juice depth, 16–24 kV voltage, and 1–3 min treatment time. The results showed that the models developed were appropriate for predicting AA, RA of PME, BI of AA, DPPH radical scavenging activity, and TPC of Wakro orange juice within the experimental study. The ACP processing of orange (cv. Wakro) juice at optimum conditions of 4.6 mm juice depth, 19 kV voltage, and 2.6 min treatment time were 28.08 mg/100 mL AA, 43.80% RA of PME, 77.25% BI of AA, 53.92% DPPH radical scavenging activity, and 50.80 mg GAE/100 mL TPC respectively.

Response parameters for ACP processing of orange (cv. Malta) juice were considered mainly AA, RA of PME, and BI, respectively, and optimized by RSM using the numerical optimization technique. The predicted values of AA, RA of PME, and BI were 31.54 mg/100 mL, 61.33%, and 79.10%, respectively, at optimized conditions

(voltage 20 kV, sample depth 3.3 mm, and treatment time 2 min). This methodology has shown good predictability and performance in assessing the effect of ACP treatment in orange (cv. Malta) juice. Treatment time and voltage played a significant role for PME inaction in juice. TPC content of juice was significantly improved within the specific CP process conditions. The present work suggests that DBD plasma source ACP treatment has a potential implication for augmenting the specific bioactive compound and the inactivation of PME activity in citrus fruit juices.

Bibliography

Aadil, R. M., Zeng, X. A., Han, Z., & Sun, D. W. (2013). Effects of ultrasound treatments on quality of grapefruit juice. *Food Chemistry*, *141*(3), 3201-3206.

Andreou, V., Giannoglou, M., Xanthou, M. Z., Passaras, D., Kokkoris, G., Gogolides, E., & Katsaros, G. (2023). Inactivation of pectinmethylesterase in fresh orange juice by cold atmospheric plasma technology: A kinetic study. *Innovative Food Science & Emerging Technologies*, *86*, 103361.

Basak, S., & Ramaswamy, H. S. (1996). Ultra high pressure treatment of orange juice: a kinetic study on inactivation of pectin methyl esterase. *Food Research International*, *29*(7), 601-607.

Chambial, S., Dwivedi, S., Shukla, K. K., John, P. J., & Sharma, P. (2013). Vitamin C in disease prevention and cure: an overview. *Indian Journal of Clinical Biochemistry*, *28*, 314-328.

Das, P. S., Das, P., Nayak, P. K., Islary, A., & Krishnan Kesavan, R. (2024). Process optimization of thermosonicated modhusuleng (*polygonum microcephalum*) leaf juice for quality enhancement using response surface methodology. *Measurement: Food*, *15*, 100181.

Dasan, B. G., & Boyaci, I. H. (2018). Effect of cold atmospheric plasma on inactivation of *Escherichia coli* and physicochemical properties of apple, orange, tomato juices, and sour cherry nectar. *Food and Bioprocess Technology*, *11*, 334-343.

de Castro, D. R. G., Mar, J. M., da Silva, L. S., da Silva, K. A., Sanches, E. A., de Araújo Bezerra, J., Rodrigues, S. Fernandes, F. A. N. & Campelo, P. H. (2020). Dielectric barrier atmospheric cold plasma applied on camu-camu juice processing: Effect of the excitation frequency. *Food Research International*, *131*, 109044.

Enami, S., Hoffmann, M. R., & Colussi, A. J. (2008). Acidity enhances the formation of a persistent ozonide at aqueous ascorbate/ozone gas interfaces. *Proceedings of the National Academy of Sciences*, *105*(21), 7365-7369.

Fonteles, T. V., Leite, A. K. F., Silva, A. R. A., Carneiro, A. P. G., de Castro Miguel, E., Cavada, B. S., Fernandes, F. A. N., & Rodrigues, S. (2016). Ultrasound

processing to enhance drying of cashew apple bagasse puree: Influence on antioxidant properties and in vitro bioaccessibility of bioactive compounds. *Ultrasonics Sonochemistry*, *31*, 237-249.

Gan, Z., Feng, X., Hou, Y., Sun, A., & Wang, R. (2021). Cold plasma jet with dielectric barrier configuration: Investigating its effect on the cell membrane of E. coli and S. cerevisiae and its impact on the quality of chokeberry juice. *LWT-Food Science and Technology*, *136*, 110223.

Guerrouj, K., Sánchez-Rubio, M., Taboada-Rodríguez, A., Cava-Roda, R. M., & Marín-Iniesta, F. (2016). Sonication at mild temperatures enhances bioactive compounds and microbiological quality of orange juice. *Food and Bioproducts Processing*, *99*, 20-28.

Henselová, M., Slováková, Ľ., Martinka, M., & Zahoranová, A. (2012). Growth, anatomy and enzyme activity changes in maize roots induced by treatment of seeds with low-temperature plasma. *Biologia*, *67*, 490-497.

Hosseini, S. M., Rostami, S., Hosseinzadeh Samani, B., & Lorigooini, Z. (2020). The effect of atmospheric pressure cold plasma on the inactivation of Escherichia coli in sour cherry juice and its qualitative properties. *Food Science & Nutrition*, *8*(2), 870-883.

Hou, Y., Wang, R., Gan, Z., Shao, T., Zhang, X., He, M., & Sun, A. (2019). Effect of cold plasma on blueberry juice quality. *Food Chemistry*, *290*, 79-86.

Illera, A. E., Chaple, S., Sanz, M. T., Ng, S., Lu, P., Jones, J., Carey, E., & Bourke, P. (2019). Effect of cold plasma on polyphenol oxidase inactivation in cloudy apple juice and on the quality parameters of the juice during storage. *Food Chemistry: X*, *3*, 100049.

Illera, A. E., Sanz, M. T., Beltrán, S., Melgosa, R., Solaesa, A. G., & Ruiz, M. O. (2018). Evaluation of HPCD batch treatments on enzyme inactivation kinetics and selected quality characteristics of cloudy juice from Golden delicious apples. Journal *of Food Engineering*, *221*, 141-150.

Islam, S., Purkayastha, M. D., Saikia, S., & Tamuly, S. (2019). Augmenting the yield of polyphenols and its antioxidant activity from fresh tea leaves of Assam by response surface approach. *The Pharm Innovation Journal*, *8*(6), 560-566.

Kanofsky, J. R., & Sima, P. (1991). Singlet oxygen production from the reactions of ozone with biological molecules. *Journal of Biological Chemistry*, *266*(14), 9039-9042.

Kumar, A., Kumar, M., Mahboob, M. R., & Srivastava, B. (2024). Influence of °Brix/Acid, and flow rate of pineapple juice and electric field strength on the performance of continuous ohmic heating system. *Journal of Food Science and Technology*, *61*(6), 1188-1200.

Kumar, S., Pipliya, S., & Srivastav, P. P. (2023). Effect of cold plasma processing on physicochemical and nutritional quality attributes of kiwifruit juice. *Journal of Food Science*, *88*(4), 1533-1552.

Manzoor, M. F., Ahmad, N., Ahmed, Z., Siddique, R., Mehmood, A., Usman, M., & Zeng, X. A. (2020). Effect of dielectric barrier discharge plasma, ultra-sonication, and thermal processing on the rheological and functional properties of sugarcane juice. *Journal of Food Science*, *85*(11), 3823-3832.

Misra, N. N., S. K. Pankaj, J. M. Frias, K. M. Keener, & P. J. Cullen. (2015). The effects of nonthermal plasma on chemical quality of strawberries. *Postharvest Biology and Technology*, *110*, 197-202.

Odriozola-Serrano, I., Bellí, G., Puigpinós, J., Herrero, E., & Martín-Belloso, O. (2022). Screening the antioxidant activity of thermal or non-thermally treated fruit juices by in vitro and in vivo assays. *Beverages*, *8*(2), 36.

Paixão, L. M., Fonteles, T. V., Oliveira, V. S., Fernandes, F. A., & Rodrigues, S. (2019). Cold plasma effects on functional compounds of siriguela juice. *Food and Bioprocess Technology*, *12*, 110-121.

Pankaj, S. K., Bueno-Ferrer, C., Misra, N. N., Milosavljević, V., O'Donnell, C. P., Bourke, P., Keener, K. M., & Cullen, P. J. (2014). Applications of cold plasma technology in food packaging. *Trends in Food Science & Technology*, *35*(1), 5-17.

Pankaj, S. K., Misra, N. N., & Cullen, P. J. (2013). Kinetics of tomato peroxidase inactivation by atmospheric pressure cold plasma based on dielectric barrier discharge. *Innovative Food Science & Emerging Technologies*, *19*, 153-157.

Pankaj, S. K., Wan, Z., Colonna, W., & Keener, K. M. (2017). Effect of high voltage atmospheric cold plasma on white grape juice quality. *Journal of the Science of Food and Agriculture*, *97*(12), 4016-4021.

Ranganna, S. (2001). *Handbook of Analysis and Quality Control for Fruit and Vegetable Products*. Tata McGraw-Hill Education.

Rodríguez, Ó., Gomes, W. F., Rodrigues, S., & Fernandes, F. A. (2017). Effect of indirect cold plasma treatment on cashew apple juice (*Anacardium occidentale* L.). *LWT-Food Science and Technology*, *84*, 457-463.

Sánchez-Moreno, C., Cano, M. P., de Ancos, B., Plaza, L., Olmedilla, B., Granado, F., Elez-Martínez, P., Martín-Belloso, O., & Martín, A. (2004). Pulsed electric fields–processed orange juice consumption increases plasma vitamin C and decreases F2-isoprostanes in healthy humans. *The Journal of Nutritional Biochemistry*, *15*(10), 601-607.

Sharma, R. R., Reddy, S. V. R., & Sethi, S. (2018). Cold plasma technology for surface disinfection of fruits and vegetables. In *Postharvest Disinfection of Fruits and Vegetables* (pp. 197-209). Academic Press.

Shi, X. M., Zhang, G. J., Wu, X. L., Li, Y. X., Ma, Y., & Shao, X. J. (2011). Effect of low-temperature plasma on microorganism inactivation and quality of freshly squeezed orange juice. *IEEE Transactions on Plasma Science*, 39(7), 1591-1597.

Silveira, M. R., Coutinho, N. M., Esmerino, E. A., Moraes, J., Fernandes, L. M., Pimentel, T. C., Freitas, M. Q., Silva, M. C., Raices, R. S. L., Senaka Ranadheera, C., Borges, F. O., Neto, R. P. C., Tavares, M. I. B., Fernandes, F. A. N., Fonteles, T. V., Nazzaro, F., Rodrigues, S. & Cruz, A. G. (2019). Guava-flavored whey beverage processed by cold plasma technology: Bioactive compounds, fatty acid profile and volatile compounds. *Food Chemistry*, *279*, 120-127.

Souza, D. V. S., Melo, M. F., Ambrósio, M. M. Q., Alves, C., Melo, N. J. A., Costa, L. L., & Morais, P. L. D. (2023). Effect of plasma and heat treatments on orange juice quality. *Brazilian Journal of Biology*, *83*, e272709.

Tappi, S., Gozzi, G., Vannini, L., Berardinelli, A., Romani, S., Ragni, L., & Rocculi, P. (2016). Cold plasma treatment for fresh-cut melon stabilization. *Innovative Food Science & Emerging Technologies*, *33*, 225-233.

Tarabová, B., Tampieri, F., Maran, E., Marotta, E., Ostrihoňová, A., Krewing, M., & Machala, Z. (2021). Chemical and antimicrobial effects of air non-thermal plasma processing of fresh apple juice with focus on safety aspects. *Foods*, *10*(9), 2055.

Tiwari, B. K., Muthukumarappan, K., O'Donnell, C. P., & Cullen, P. J. (2008). Kinetics of freshly squeezed orange juice quality changes during ozone processing. *Journal of Agricultural and Food Chemistry*, *56*(15), 6416-6422.

Xu, L., Garner, A. L., Tao, B., & Keener, K. M. (2017). Microbial inactivation and quality changes in orange juice treated by high voltage atmospheric cold plasma. *Food and Bioprocess Technology*, *10*, 1778-1791.