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Effect of cold plasma on carotenoid content and instrumental color of carrot juice: a systematic review and meta-analysis

Danyelly Silva AMORIM¹ ⁽ⁱ⁾, Isabelly Silva AMORIM¹ ⁽ⁱ⁾, Renan Campos CHISTÉ² ⁽ⁱ⁾, Fabiano André Narciso FERNANDES³ ⁽ⁱ⁾, Fabrício Batistin ZANATTA⁴ ⁽ⁱ⁾, José TEIXEIRA FILHO^{5,6} ⁽ⁱ⁾, Helena Teixeira GODOY¹ ⁽ⁱ⁾

The effect of cold plasma on carotenoid content and the instrumental color of carrot juice were evaluated. The meta-analyzed data of carrot juice showed that cold plasma resulted in a significantly higher level (95% confidence interval) in a standardized mean difference (SMD) of 4.45 for total carotenoids, for lycopene an SMD of 3.63, for β -carotene an SMD of 0.86, and for lutein an SMD of 0.55, compared with the control. After the extraction of carotenoids from the carrot juice, the highest levels were observed for the following combinations of plasma treatment versus time: total carotenoids and lutein—80 Kv/4 min; β -carotene—70 Kv/3 min; and lycopene—60 Kv/4 min. The instrumental color showed no significant differences between the plasma treatment and control for the coordinates L*, a*, and b*. Therefore, the cold plasma treatment showed a positive effect on the maintenance of carotenoids in carrot juice.

Keywords: cold plasma; non-thermal treatment; carotenoids; carrot juice.

Practical application: Use of cold plasma in carotenoid source matrix.

1 INTRODUCTION

Carrot juice is usually marketed as a functional drink and consists of one of the main carrot-processed products. It is associated with a healthy diet due to its high nutritional value. This juice is rich in dietary fiber, vitamins, minerals, and bioactive compounds such as phenolic compounds and carotenoids (Gouma et al., 2020; Liu et al., 2019). Its carotenoid composition consists of the majority of α -carotene and β -carotene, which are compounds with provitamin A activity. However, lutein and lycopene can also be found in carrot juice prepared with different varieties (Negri Rodríguez et al., 2021; Riganakos et al., 2017).

Carrot juice processing steps comprise sanitizing and peeling carrots, blanching to inhibit enzymatic browning, juice extraction by different techniques, acidification, and thermal processing to ensure inhibition/destruction of pathogenic and spoilage microorganisms (Gouma et al., 2020). Conventional heat treatments (pasteurization and sterilization) are used to inactivate enzymes and microorganisms in order to prolong the shelf life of the juice. However, contact with high temperature can induce undesirable changes in food color, in addition to reducing the content of bioactive compounds (Negri Rodríguez et al., 2021; Riganakos et al., 2017; Szczepańska et al., 2020). Hence, color is the first sensory attribute evaluated in purchase decision-making, followed by the perception of flavor and aroma (Stinco et al., 2019).

Due to the importance of maintaining nutritional and sensory quality in processed food, milder technologies have been studied, such as non-thermal treatments. These technologies combine food safety and retention of nutritional components, as well as a minimal alteration in the sensory attributes of the food. Cold plasma stands out among these emerging milder technologies. The technology consists of a totally or partially ionized gas by different types of energy (electricity, heat, and electromagnetic waves such as radio and microwaves). The chemical species formed by this ionization, such as reactive oxygen species (ROS), reactive nitrogen species (RNS), and ultraviolet (UV) radiation, are considered the main agents of plasma for pathogen inactivation (Basak & Annapure, 2022; Sharma & Singh, 2020). The advantages of cold plasma technology include low operational cost, non-toxic waste, and low-complexity equipment (Chizoba Ekezie et al., 2017; Farber et al., 2019; Zhu et al., 2020).

In recent years, the study of cold plasma effects on carotenoids of plant matrices has been grown (Fernandes et al., 2019; Ranjitha Gracy et al., 2019; Silveira et al., 2019; Umair et al., 2019, 2022). In this context, meta-analysis can be performed to combine studies and improve the accuracy and ability to answer questions that were not answered in primary products and also clarify controversies of the findings about the effect of plasma (Deeks et al., 2019). Thus, this study aimed to conduct a

³Universidade Federal do Ceará, Departamento de Engenharia Química, Fortaleza, Ceará, Brazil.

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¹Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos, Departamento de Ciência de Alimentos e Nutrição, Campinas, São Paulo, Brazil.

²Universidade Federal do Pará, Instituto de Tecnologia, Faculdade de Engenharia de Alimentos, Belém, Pará, Brazil.

⁴Universidade Federal de Santa Maria, Programa de Pós-Graduação em Ciências Odontológicas, Santa Maria, Rio Grande do Sul, Brazil.

⁵Universidade Estadual de Campinas, Faculdade de Engenharia Agronômica, Campinas, São Paulo, Brazil.

⁶Universidade Estadual de Campinas, Instituto de Geociências, Campinas, São Paulo, Brazil.

^{*}Corresponding author: danyellyamorimalimentos@gmail.com

systematic review with a meta-analysis of studies that evaluated the effect of cold plasma on carotenoid content and color, using carrot juice as a case study.

2 MATERIALS AND METHODS

2.1 Search strategies

The following databases were consulted to collect the articles published from 1991 to 2022: PubMed, Web of Science, Science Direct, and Scopus. We used as gray literature the data from studies presented at conferences, lectures, and journals in general. The following search terms were used to identify relevant studies: *cold plasma treatment* AND *carrot*. To identify additional articles, the reference list of included articles was evaluated (Moher et al., 2010; Mousavi Khaneghah et al., 2018).

2.2 Eligibility criteria

After identifying the studies in each database, duplicate articles were excluded with the help of the Mendeley software (v2.67.0). Subsequently, titles and abstracts were read independently by two reviewers (DAS and ISA) for the initial screening of potentially eligible studies. The full texts were read to confirm or not the eligibility of the studies. Inconsistencies or doubts about eligibility were resolved by a third researcher (HTG).

To be eligible for systematic review, the study should have evaluated the effect of cold plasma (intervention) on carotenoid levels (outcome), compared with carrot or its derivatives without cold plasma treatment (control). Articles were excluded when they did not meet these criteria.

2.3 Data extraction

Data extraction was performed by two researchers independently (DAS and ISA). The relevant data were extracted manually, organized, and stored in a spreadsheet (Microsoft Excel[®]). The data extracted from each article were as follows: article citation (study), *n* plasma intervention sample (n-p), the mean concentration of total carotenoids, β -carotene, lycopene, and lutein, L^* , a^* , b^* standard deviation of plasma intervention (sd-p), *n* control intervention sample (n-c), the mean concentration of the control intervention (sd-c), and operational parameter (protocol), as shown in Table 1.

2.4 Meta-analysis

For reasons related to the assumption of clinical-methodological similarity or homogeneity, only studies with similar raw materials and types of sample processing were meta-analyzed. The meta-analysis was evaluated by the random effects model of DerSimonian and Laird (1986). Considering that the parameters for cold plasma application varied between studies, aggregate estimates for carotenoid concentrations were calculated from the SMD and 95% confidence intervals. For interpretation purposes, the magnitude of effect according to

Table 1. Studies included in the systematic review according to methodological and outcome characteristics.

		Cold plasma			Control							
Total carotenoids (µg/mL)												
Study	n-p (grs)	Mean	sd-p	n-c (grs)	Mean	sd-c	Protocol					
Umair et al. (2020)	1,000	10.14	0.13	1,000	9.09	0.11	80 Kv/4 min					
Umair et al. (2022)	1,000	10.23	0.13	1,000	9.97	0.20	70 Kv/3 min					
Umair et al. (2019)	1,000	10.03	0.80	1,000	8.22	0.20	60 Kv/4 min					
		<i>β</i> -C:	arotene (µg/100) mL)								
Umair et al. (2022)	1,000	25.22	0.20	1,000	25.02	0.11	70 Kv/3 min					
Umair et al. (2019)	1,000	24.21	0.27	1,000	24.11	0.10	60 Kv/4 min					
		I	ycopene (µg/m	L)								
Umair et al. (2020)	1,000	1.11	0.12	1,000	0.64	0.14	80 Kv/4 min					
Umair et al. (2022)	1,000	0.91	0.51	1,000	0.69	0.88	70 Kv/3 min					
Umair et al. (2019)	1,000	1.83	0.50	1,000	0.52	0.10	60 Kv/4 min					
			Lutein (µg/mL	.)								
Umair et al. (2020)	1,000	1.53	0.12	1,000	1.41	0.23	80 Kv/4 min					
Umair et al. (2019)	1,000	1.56	0.90	1,000	1.22	0.60	60 Kv/4 min					
			L*									
Umair et al. (2022)	1,000	36.32	0.11	1,000	36.01	0.14	70 Kv/3 min					
Umair et al. (2019)	1,000	33.76	0.33	1,000	35.31	0.24	60 Kv/4 min					
			a*									
Umair et al. (2022)	1,000	18.58	0.20	1,000	18.32	0.12	70 Kv/3 min					
Umair et al. (2019)	1,000	17.80	0.24	1,000	19.53	0.22	60 Kv/4 min					
			b *									
Umair et al. (2022)	1,000	29.05	0.18	1,000	29.28	0.14	70 Kv/3 min					
Umair et al. (2019)	1,000	26.35	0.22	1,000	27.06	0.10	60 Kv/4 min					

n-p: n sampling rate of plasma intervention; sd-p: standard deviation of plasma intervention; n-c: n sampling rate of control intervention; SD-c: standard deviation of the control intervention.

the MDS is evaluated as follows: values from -0.2 to 0.2 (trivial effect or no effect); values from -0.5 to -0.2 or 0.2–0.5 (small effect), -0.8 to -0.5 or 0.5–0.8 (moderate effect), or < -0.8 or > 0.8 (large effect) (Schünemann et al., 2019). For instrumental color, meta-analyses were generated according to the treatment applied. Statistical heterogeneity was evaluated by coefficient I^2 , and defined as low ($I^2 < 25\%$), medium ($25\% < I^2 < 50\%$), and high ($I^2 > 50\%$) (DerSimonian & Laird, 1986). The data were analyzed using the R (version 4.0.3) and RStudio (version 1.4.1106) software, a meta package.

2.5 Publication bias

Publication bias was not determined through the egger statistic or graphic asymmetry Test due to the small number of selected studies that met the eligibility criteria, as a result of the lack of articles in this field of study.

3 RESULTS

The identification phase consisted of searching for studies in the selected databases, in which 977 articles were identified, of which 17 studies were excluded because they were duplicated. During the screening phase, the titles and abstracts were examined, and 955 studies were excluded because they did not use cold plasma processing on carrots or carrot processing products, or because they did not evaluate the carotenoid concentrations of the samples. For the prevalence of the studies, in addition to the carotenoid concentrations, the weight in grams of the samples should be reported, in addition to the standard deviation of the concentrations. In the next phase, the eligibility of the studies was evaluated; among the studies, three applied cold plasma in carrot juice and one in packaged carrots. Thus, carrot juice was selected for meta-analysis by presenting the eligibility criteria and by presenting sufficient studies to compare the results. Finally, the concentration data of total carotenoids, β -carotene, lycopene, lutein, and values for instrumental color were meta-analyzed $(L^*, a^*, and b^*)$ of carrot juice, as shown in Figure 1.

Figure 2 shows the meta-analysis for total carotenoids (Figure 2A), β -carotene (Figure 2B), lycopene (Figure 2C), and lutein (Figure 2D). The application of cold plasma produced a significantly higher effect than the control for all parameters analyzed, as shown by the higher level of these components in the plasma-treated juices. A large effect size (*SMD* > 0.8) was observed for total carotenoids (*SMD* = 4.45), lycopene (*SMD* = 2.51), and β -carotene (*SMD* = 0.86) because for the plasma intervention, there was a higher content of the carotenoids evaluated. The effect on lutein was moderate (*SMD* = 0.55).

As for the instrumental color outcomes (Figure 3), despite the application of cold plasma having slightly reduced the luminosity (L^*) (plasma mean: 35.02, control mean: 35.66), the coordinate a^* (plasma mean: 18.19, control mean: 18.92), and the coordinate b^* (mean plasma: 27.67; mean control: 28.15), it is noteworthy that no significant differences were observed between both treatments for any of the parameters, which shows that cold plasma does not significantly affect (p < 0.05) the color of carrot juice.

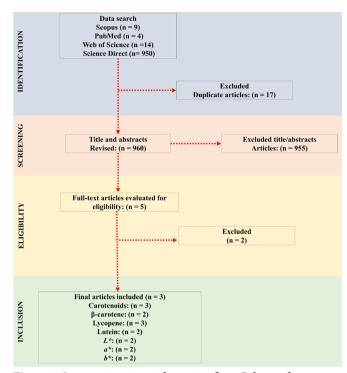
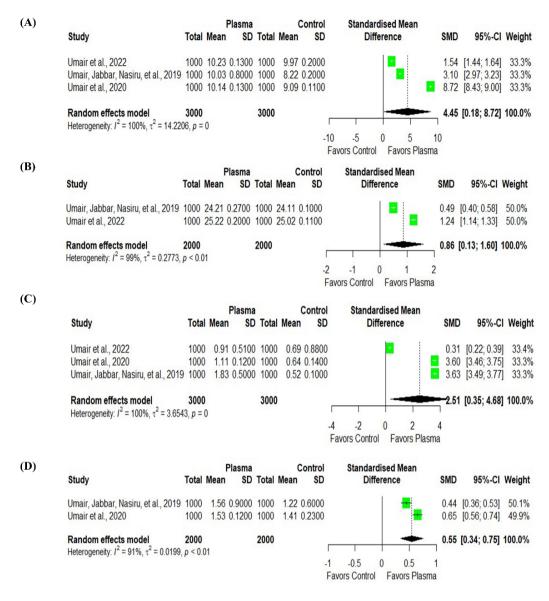


Figure 1. Systematic review information flow. Color preference: colors are only available in the online version.

4 DISCUSSION

This review aimed to determine the effect of non-thermal treatment with cold plasma on carrot juice processing. Cold plasma showed a favorable behavior in the concentration of carotenoids: total carotenoids (SMD = 8.72) > lycopene (SMD = 3.63) > β -carotene (*SMD* = 1.24) > lutein (*SMD* = 0.65), given by the analysis of SMD values that indicate the size of the effect of plasma intervention. For the carotenoids, the protocols that led to the most prominent effects on carrot juices were: 80 Kv/4 min (total carotenoids), 60 Kv/4 min (lycopene), 70 Kv/3 min (β -carotene), and 80 Kv/4 min (lutein). Therefore, higher pigment retention was observed for protocols where the higher voltage was applied. The increased carotenoid level by plasma application is explained by Umair et al. (2020), who proposed that this behavior is probably due to the interaction of reactive species generated in plasma with plant material, influencing the release of carotenoids (da Silva et al., 2017; Yahia et al., 2017). In this case, cold plasma has the ability to break through the cell membranes of chloroplasts, thereby releasing pigments and increasing their content in the extracellular medium. In the untreated carrot juice, some carotenoids were probably contained inside the carrot particles in suspension after preparation. After cold plasma treatment, the carotenoid molecules migrated to the liquid medium more easily, explaining the increase in carotenoid content after treatment.

Our findings corroborate with the results of cold plasma application in acerola juice (Fernandes et al., 2019), guava-flavored whey drink (Silveira et al., 2019), and avocado pulp (Batista et al., 2021). The authors suggested that reactive species can react by breaking the bond between carotenoid molecules and cell



CI: confidence interval; *SMD*: standardized mean difference; l^2 : statistical heterogeneity; *SMD* from -0.2 to 0.2 (trivial or no effect), -0.5 to -0.2 or 0.2–0.5 (small effect), -0.8 to -0.5 or 0.5–0.8 (moderate effect), or < -0.8 or > 0.8 (large effect).

Figure 2. Forest plot of the comparison of pigment content in cold plasma-treated carrot juices and control. (A) Total carotenoids. (B) β -Carotene. (C) Lycopene. (D) Lutein. Color preference: colors are only available in the online version.

membranes, increasing the carotenoid content in the extracellular medium. Also, this behavior can occur by electroporation of cell membranes due to cold plasma treatment.

Carotenoids are part of several *in natura* foods, especially fruits and vegetables, and also in fruit- and vegetable-based products. They are pigments that have important biological functions in human health, such as the precursor action in the synthesis of retinol (vitamin A) attributed to some molecules. It is considered a micronutrient responsible for maintaining the function of vision, immune responses, and reproduction (Meléndez-Martínez, 2019). Thus, carrots are considered a source of phytochemicals such as α - and β -carotene, vitamin E, and

anthocyanins, with the latter being found in purple-colored carrot varieties (Żary-Sikorska et al., 2019). The retention of these phytochemicals during vegetable processing is essential for maintaining the health benefits associated with the intake of these bioactive compounds (Nagraj et al., 2020; Šeregelj et al., 2020).

Regarding the instrumental color, the treatment of carrot juice with cold plasma did not promote a significant reduction in instrumental color values. The color reduction arrangement in carrot juice may be related to the interaction of plasma oxidizing species with the decrease in the number of conjugated double bonds present in the polyene chains of carotenoids (Mahnot

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Study	Total	Mean	SD		Mean		Mean	95%–Cl	Weight
L* plasma Umair et al., 2022 Umair, Jabbar Nasir u, et al., 2019 Random effects model Heterogeneity: $1^2 = 100\%$, $\tau^2 = 0.0027$	1000 2000		0.1100 0.3300		-		36.32 33.76 35.02	[36.31; 36.33] [33.74; 33.78] [32.60; 37.62]	50% 50% 100%
L* control Umair et al., 2022 Umair, Jabbar Nasir u, et al., 2019 Random effects model Heterogeneity: $1^2 = 100\%$, $\tau^2 = 0.0002$	1000 2000		0.1400 0.2400			□ ■ ◆	36.01 35.31 35.66	[36.00; 36.02] [35.30; 35.32] [34.98; 36.35]	50% 50% 100%
a*plasma Umair et al., 2022 Umair, Jabbar Nasir u, et al., 2019 Random effects model Heterogeneity: $1^2 = 100\%$, $\tau^2 = 0.0009$	1000 2000		0.2000 0.2400				18.58 17.80 18.19	[18.57; 18.59] [17.79; 17.81] [17.44; 18.97]	50% 50% 100%
a*control Umair et al., 2022 Umair, Jabbar Nasir u, et al., 2019 Random effects model Heterogeneity: $1^2 = 100\%$, $\tau^2 = 0.0020$	1000 2000		0.1200 0.2200				18.32 19.53 18.92	[18.31; 18.33] [19.52; 19.54] [17.77; 20.14]	50% 50% 100%
b* plasma Umair et al., 2022 Umair, Jabbar Nasir u, et al., 2019 Random effects model Heterogeneity: $1^2 = 100\%$, $\tau^2 = 0.0048$	1000 2000		0.1800 0.2200				29.05 26.35 27.67	[29.04; 29.06] [26.34; 26.36] [25.14; 30.44]	50% 50% 100%
b*control Umair et al., 2022 Umair, Jabbar Nasir u, et al., 2019 Random effects model Heterogeneity: $1^2 = 100\%$, $\tau^2 = 0.0031$	1000 2000		0.1400 0.1000				29.28 27.06 28.15	[29.27; 29.29] [27.05; 27.07] [26.06; 30.41]	50% 50% 100%
			Г 15	5 20	25 30	35 4	0		

CI: confidence interval.

Figure 3. Forest plot of the comparison of instrumental color in cold plasma-treated carrot juices and control. (A) L^* : lightness (+L* = lighter; -L* = darker). (B) a^* : (+a* = red; -a* = green). (C) b^* : (+b* = yellow; -b* = blue). Color preference: colors are only available in the online version.

et al., 2020). In addition, the low doses of UV light present in the plasma can act on the degradation of carotenoids by the formation of singlet oxygen, through biological compounds in the presence of light. Subsequently, singlet oxygen binds to the hydrocarbon chain of carotenoids, leading to their degradation (da Silva et al., 2017; Yahia et al., 2017). Although the cold plasma increased the extraction of the pigments, there was no increase in the value of the coordinates red (a^{*+}) and yellow (b^{*+}). This occurred because the instrumental color may be influenced by the presence of other compounds in the food matrix. In general, high statistical heterogeneity was observed in the estimates, probably arising from the variability of the parameters used in the primary studies.

5 CONCLUSIONS

The results of this study showed that the treatment of carrot juice with cold plasma has a favorable effect on carotenoids, following a trend of retention of pigments at higher voltages applied during processing. Additionally, the cold plasma treatment did not impact instrumental color parameters significantly (p > 0.05). Thus, cold plasma can be an alternative to reduce the undesirable thermal effects on the carotenoid content and color perception of carrot juice. Given the limitation related to the small number of primary studies, more research is needed on the effect of cold plasma on carrot juice carotenoids, as well as on other vegetable matrices.

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