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Improving the Nutritional and Antioxidant Properties of Broccoli Florets using Vacuum Impregnation with Ascorbic Acid and Calcium Chloride Solution

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ABSTRACT: Broccoli is highly susceptible to degradation and spoilage, challenging the food industry to preserve its nutritional and sensory quality. This study we aim to address the challenge by investigating the effect of vacuum impregnation on the bioactive compounds and quality attributes of broccoli florets. The florets were impregnated with a solution of ascorbic acid and calcium chloride at different levels of vacuum pressure (20, 40, and 60 kPa); and the total phenolic content (TPC), total flavonoid content (TFC), total ascorbic acid content (AAC), total soluble solids (TSS), water activity (aw), Total chlorophyll content (TCC) and carotenoid content (CC), and antioxidant activity (AA) of treated as well as untreated samples were determined. The TPC, TFC, AAC, TCC, CC, and AA of vacuum impregnated broccoli florets increased significantly (p ≤0.05). At 0.6 bar vacuum impregnation, a significant increase in biochemical compounds was observed. The rise in TPC, TFC, and AAC could be attributed to stress caused by vacuum pressure and impregnation solution, which may have stimulated the phenylpropanoid pathways involved in these compounds' biosynthesis. The osmotic effect of the impregnation solution could explain the increase in TSS and water activity.

Keywords: Vacuum impregnation, calcium chloride, ascorbic acid, biochemical.

INTRODUCTION

Broccoli (*Brassica oleracea*) is a cruciferous vegetable known for its high nutritional value, disease-fighting properties, and culinary versatility. It is high in fiber, vitamins (A, C, K), minerals (calcium, iron, and potassium), and phytochemicals (carotenoids, flavonoids, and glucosinolates), all of which have been linked to a lower risk of chronic diseases like cancer, cardiovascular disease, and diabetes (Li *et al*., 2022). Emerging clinical evidence has shown that broccoli may offer a protective effect against severe symptoms of COVID-19 (Bousquet *et al*., 2021), highlighting its potential as a functional food with health benefits.

Broccoli's perishability, rapid moisture loss, and susceptibility to microbial spoilage and enzymatic browning, on the other hand, compromise its quality and shelf life. These factors can lead to decreased sensory attributes like color, texture, flavor, and nutritional content, resulting in lower consumer acceptance and economic losses for growers, processors, and retailers. To address these issues, various preservation techniques for broccoli have been developed to increase its shelf life while maintaining its quality and nutritional value. Over the years, broccoli has been subjected to a variety of physical preservation techniques, including modified atmosphere storage, film bagging with materials such as polyethylene (PE) or polylactic acid (PLA), exposure to high electrostatic fields, electrostatic atomized water treatment, light irradiation with ultraviolet-B (UV-B), UV-C, low-intensity light-emitting diodes (LEDs), or fluorescent lighting, and temperature control methods such as drying, controlled freezing (Luo *et al.,* 2019a; Luo *et al.,* 2020).

Despite various physical preservation techniques, broccoli's short shelf-life still needs to be improved. Vacuum impregnation is a promising method for infusing beneficial substances into fruits and vegetables to extend their shelf life. It has been reported that vacuum impregnation of broccoli florets with ascorbic acid and calcium chloride improves nutritional quality and extends shelf-life (Li *et al.,* 2022). VI entails using a vacuum to remove air from plant tissue, infusing a solution or liquid into the tissue while under vacuum pressure. VI has several advantages, including preserving the tissue's natural structure and composition, preserving nutritional and bioactive components, reducing water loss and microbial growth, and enhancing texture, color, and flavor. VI can be used for various applications, including incorporating bioactive compounds, nutrient fortification, salt or sugar content reduction, off-flavor removal, and shelf-life enhancement (Roa *et al*., 2001; Radziejewska *et al.,* 2014). Calcium chloride is essential in plant physiological processes, helping to reduce water loss through respiration and transpiration, resulting in longer shelf life and improved firmness. Furthermore, calcium treatment of plant tissue can delay ripening, yellowness, aging, decay, and maintain chlorophyll content, resulting in improved nutritional value (Martin *et al.,* 2007; Vicente *et al.,* 2007; Romero *et al*., 2022). Ascorbic acid, also known as vitamin C, is a necessary nutrient that aids in various bodily processes. It is a potent antioxidant that protects cells from free radical damage. *Ascorbic acid* is a naturally occurring organic compound found in various fruits and vegetables that is frequently used to extend the shelf life of harvested produce. Before storing, use ascorbic acid as a dip or spray solution to help reduce decay, discoloration, and flavor loss. Furthermore, ascorbic acid has been shown to reduce the activity of specific enzymes that cause fruit and vegetable spoilage (Radziejewska *et al.,* 2014; Suttirak and Manurakchinakorn 2010).

The effects of ascorbic acid and calcium chloride on the quality and durability of VI-treated broccoli need to be better comprehended. More research is required to enhance the conditions for their use. This study aims to aim at how VI with ascorbic acid and calcium chloride affects broccoli floret quality. During the VI process, we will vary the concentration and exposure time of the impregnation solutions and evaluate the biochemical characteristics of the impregnated samples over time. The findings of this study will provide essential insights into the potential use of VI in conjunction with ascorbic acid and calcium chloride as a novel preservation method for broccoli and other vegetables.

MATERIAL AND METHOD

A. Material Selection

Broccoli florets (F1 hybrid) were collected from the Farm Section of ICAR-Central Institute of Agricultural Engineering in Bhopal, India, during the winter season of 2022. The bunches were harvested and carefully cut into florets with a sharp knife before being washed under running tap water.

B. Vacuum impregnation

The broccoli florets were impregnated with a solution containing 1% ascorbic acid and calcium chloride in the laboratory scale vaccum impregnation system developed by the authors. The broccolisamples were subjected to varying levels of vacuum pressure (20, 40, and 60kPa) for 7 minutes, followed by a 15-minute restoration time. The purpose of the study was to see how different vacuum pressures affected the treated broccoli florets.

C. Determination of bio-chemical parameters of Broccoli florets

The Folin-Ciocalteu reagent was used to measure Total phenolic content, following a modified procedure by Singleton *et al.* (1999). Ascorbic acid content (AsA) was determined using a spectrophotometric assay with Folin-Ciocalteu reagent (FCR), following the method described by Jagota and Dani (1982). Total flavonoid content was quantified using the aluminum chloride procedure by Zhishen *et al.* (1999). Chlorophyll content was determined using the method described by Albanese *et al.* (2007), and the total chlorophyll content was calculated as mg/g fresh weight. Carotenoid content was estimated using the method described by Seely *et al.* (1972), and the total carotenoid content was calculated as mg/g fresh weight. To determine the antioxidant activity of the samples, the spectrophotometric method described by Brand-Williams *et al.* (1995) was used. Water activity was measured using a water activity meter (WA-160A, Meter group Inc. USA) at a temperature of 25±1°C, with stable readings noted after 5 minutes. The analysis was carried out in three replicates. The TSS of Broccoli florets was measured at a temperature of 20°C using a handheld pocket refractometer (PAL-1, ATAGO, Japan). For each parameter, three replicates of each sample were examined.

The estimation of chlorophyll content was calculated by using the following formulas:

Total chlorophyll (mg/g) =((20.2*A645) +(8.02*A663)) *V/(1000*w)

Where, A645 and A663 are the absorbance's of samples at wavelengths of 645nm and 663nm, respectively.

V-final volume of supernatant; w-weight of sample (g)

The DPPH scavenging activity was assessed by measuring the reduction in absorbance, and the percent inhibition was calculated using the following equation:

$$\% \text{ Antioxidant activity(AA)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Where Control OD is the absorbance of the control, and Sample OD is the absorbance of the sample.

D. Statistical Analysis

ANOVA and Tukey's multiple comparison test were used to determine the statistical significance of the treatments, with a significance level of $p \leq 0.05$. The Statistical Package for Social Science (SPSS) version 26.0 was used to analyse the data. For the statistical analysis, univariate analyses of variance (UNI-ANOVA) in the general linear model were used.

RESULT AND DISCUSSION

The effect of vacuum pressure on the biochemical properties of broccoli florets was investigated by impregnating the florets with a solution containing ascorbic acid and calcium chloride at different vacuum pressures (20, 40, and 60kPa) (Fig. 1). The results showed a significant increase ($p < 0.05$) in the total phenolic content (TPC), flavonoid content (TFC), ascorbic acid content (AAC), total soluble solids (TSS), water activity (Aw), total chlorophyll content (TCC), carotenoid content (CC), and antioxidant activity (AA%) of the broccoli florets after vacuum impregnation (Table 1). Untreated broccoli florets contained 115.48 mg GAE/100g total phenolic content, 111.38 mg/100g FW ascorbic acid content, 59.10% antioxidant activity, 7.94 mg/100g flavonoid content. The total soluble solids (TSS), water activity, total chlorophyll content, and carotenoid content were 7.89˚Brix, 0.83, 0.40 mg/g, and 2.38 mg/g, respectively.

The increase in vacuum pressure during vacuum impregnation increased biochemical compound uptake into the porous material. Because the increased vacuum pressure creates a more significant pressure gradient between the absorbent material and the impregnating solution, more of the solution is driven into the material's pores (Mujica-Paz *et al.,* 2003).

The current study focused on how to vacuum impregnation at various pressures (20, 40, and 60 kPa) affected broccoli florets' biochemical and physicochemical properties. The total phenolic content (TPC), total flavonoid content (TFC), and total ascorbic acid content (AAC) of broccoli florets increased significantly (p 0.05) after impregnation. At 60 kPa pressure, a significant increase in these biochemical compounds was observed.

The observed increase in TPC, TFC, and AAC could be attributed to stress caused by the vacuum pressure and impregnation solution, which may have stimulated the phenylpropanoid pathways involved in these compounds' biosynthesis (Barzegar *et al.,* 2018). Furthermore, after vacuum impregnation, the increase in TSS and Aw could be explained by the osmotic effect of the impregnation solution, which increased solute concentration while decreasing floret water potential, resulting in water uptake from the surroundings (Dandjouma *et al.,* 2004).

Furthermore, after vacuum impregnation, broccoli florets' chlorophyll, and carotenoid content increased significantly. This increase in chlorophyll and carotenoid content could be attributed to ascorbic acid's protective effect against oxidative degradation of these pigments, which are required for photosynthesis and contribute to the nutritional value of the florets. Finally, after vacuum impregnation, the antioxidant activity of broccoli florets increased. This increase in antioxidant activity could be attributed to the rise in antioxidant-rich TPC, TFC, AAC, chlorophyll, and carotenoid content Mansouri *et al.* (2005).

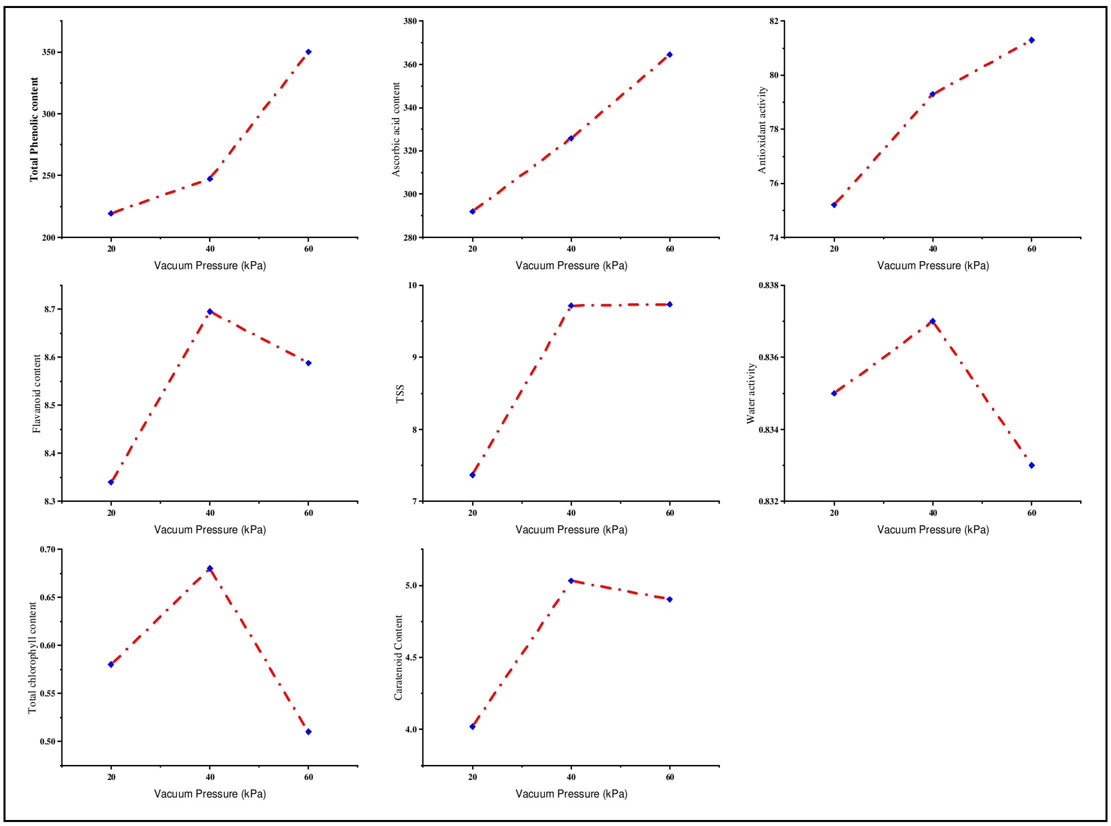

Fig. 1. Effect of vacuum pressure on biochemical properties of broccoli florets.

Table 1: Biochemical properties of control and treated broccoli florets.

VP (kPa)	TPC (mg GAE/100g)	AsA (mg/100g)	AA (%)	FC (mg/100g)	TSS (˚Brix)	aw	TCC (mg/g)	CC (mg/g)
0 (untreated)	115.48^{a}	111.38^{a}	59.10^{a}	7.94^{a}	7.89^{a}	0.83^{a}	0.40^{a}	2.38^{a}
20	219.31^{b}	292.01^{b}	75.20^{b}	8.34^{b}	7.37^{b}	0.84^{a}	0.58^{b}	4.02^{b}
40	247.4^{c}	325.84^{c}	79.34^{c}	8.69^{c}	9.72^{c}	0.84^{a}	0.68^{c}	5.03^{c}
60	350.21^{d}	364.49^{d}	81.30^{d}	8.59^{d}	9.73^{c}	0.83^{a}	0.51^{d}	4.902^{c}
Model F-value	4297.45*	2561.09*	976.037*	43.02*	227.81*	1.066**	189.57*	198.10*

Means followed by the different lower-case letters on the same column differ significantly by the Tukey test ($p \leq 0.05$). Values are mean of 3 replications. Means with the same letter are not significantly different in a column at 95% confidence interval. *Significant terms; **non-significant terms

CONCLUSIONS

In conclusion, vacuum impregnation with ascorbic acid and calcium chloride solution can enhance broccoli florets' bioactive compounds and antioxidant activity. The treatment resulted in significant increases in total phenolic content, flavonoid content, ascorbic acid content, chlorophyll and carotenoid content, and antioxidant activity. These improvements may have been induced by the stress created by the vacuum pressure and impregnation solution, which stimulated the phenylpropanoid pathways involved in the biosynthesis of these compounds. Therefore, vacuum impregnation can be a promising technique for improving the nutritional value and shelf life of broccoli and other fruits and vegetables. The effects of ascorbic acid and calcium chloride on the quality and durability of VI-treated broccoli need to be better comprehended. More research is required to enhance the conditions for their use.

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