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Effects of sodium alginate and calcium on quality attributes of *Shepherd's purse* under ultrasound impregnation during blanching processing

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Abstract

This study aimed to improve the quality of blanched *Shepherd's purse*. Before blanching, *Shepherd's purse* was impregnated, combined with ultrasonic treatment (US) (0, 5, 15 min) and different impregnators (calcium chloride (Ca), glucose (Gl), sodium alginate (Al)) to investigate the effects on the quality of blanched *Shepherd's purse*. The contents of ascorbic acid, phenolic compounds and calcium were determined after blanching for 1 min, and the color, structure and volatile substances were analyzed. It was found that ultrasonic assisted sodium alginate impregnation for 15 min and calcium chloride treatment (Al + Ca + US15) produced very favorable effects on the quality characteristics of blanched *Shepherd's purse*. Specifically, the ascorbic acid content of Al + Ca + US15 was 1.57 folds higher than that of the control group (CK), and the gallic acid and rutin content increased by 35.2% and 24.7%, respectively, compared to the CK. Additionally, the calcium content in Al + Ca + US15 was 1.27 folds higher than that in the CK. Furthermore, compared with CK, Al + Ca + US15 treatment of blanched *Shepherd's purse* ΔE value was significantly reduced by 49.1%. Microstructure analysis revealed no significant difference in the internal cell space after Al + Ca + US15 treatment, and the cell morphology remained normal. In addition, the volatile data indicated that the impregnation treatment decreased the loss of volatiles after blanching. Overall, the treatment of ultrasound assisted impregnation has been found to be bio-protective for leaf vegetables when exposed to heat.

Keywords Ultrasound, Impregnation, Blanching, *Shepherd's purse*, Bioactive components, Quality

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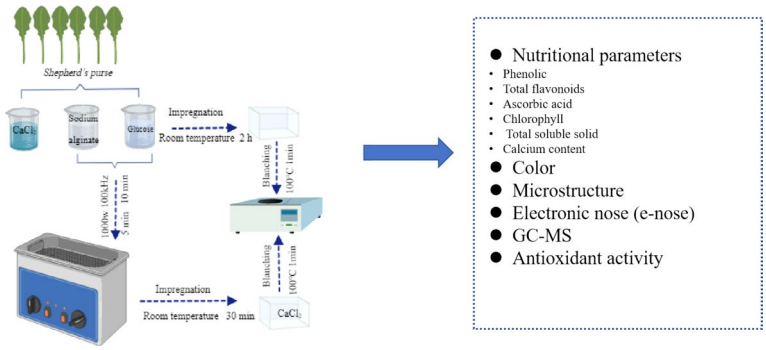
Graphical Abstract



Introduction

Shepherd's purse (Capsella bursa-pastoris (L.) Medik.) is a wild vegetable widely distributed throughout the world. *Shepherd's purse* is rich in health-promoting nutrients such as proteins, polysaccharides, alkaloids, fiber, and minerals. Fresh leaves of *Shepherd's purse* are edible and widely used in China as a traditional herbal medicine and culinary ingredient. In the food industry, *Shepherd's purse* is often processed into quick-frozen or stuffed products in order to improve the convenience of transportation and extend the shelf life of the product (Peng et al., 2019). Blanching is a common pretreatment method for *Shepherd's purse* products.

Blanching can soften tissues, improve permeability, enhance heat and mass transfer (Tabibian et al., 2020), and increase the rate of water diffusion (L.-Z. Deng et al., 2018). At the same time, conventional blanching has been used to inactivate bacteria and enzyme activity, which can blunt peroxidase activity, and inhibit tissue browning and undesirable flavors caused by enzymatic reactions, which is beneficial for maintaining color and high quality of vegetables. However, high-temperature thermal treatment affects the concentration of heat-sensitive bioactive compounds such as polyphenols, vitamins, carotenoids, and flavonoids, as well as the organoleptic properties of vegetables (Medina-Meza et al., 2015). In addition, the quality deterioration caused by unsuitable blanching is related to the color and texture changes of vegetables during thermal processing. To some extent, the quality of vegetable products, such as color, texture, and nutritional value, can be improved by blanching combined with salt and sugar impregnation processing. The use of $CaCl_2$ as impregnating agent can significantly improve the quality of cress, and when the concentration of $CaCl_2$ in the soaking solution increases to 0.5%, the hardness index of cress no longer increased, the texture deteriorated, there was a clear sense of fiber and the astringent, sensory quality was reduced, controlling the $CaCl_2$ concentration to 0.4% can significantly improve the crispness of cress after blanching (Ye et al., 2013). It was demonstrated that following the impregnation of carrot with alginate and maltose, the structural characteristics exhibited by the tissue were enhanced. This suggests that polysaccharide treatment may exert a protective effect on the tissue structure of blanched carrots (Neri et al., 2014).

Sodium alginate is a natural macromolecular polysaccharide that is a by-product of the extraction of iodine and mannitol from kelp or sargassum of the brown algae, its molecule is composed of linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) linked by a (1→4) bond. It comprises a significant amount of -COO-, which can demonstrate polyanionic behavior in aqueous solutions (Fan et al., 2021). It possesses a certain level of adhesion, excellent gel properties, and film-forming abilities, and is extensively utilized as a material for edible membranes (Chen et al., 2021). During the processing, it can enter the cell space, enhance the cell construction, and create a gel with Ca^{2+}, resembling an "egg carton", to fill the matrix, resulting in better preservation of vegetable cells. The preparation of a compound preservative with sodium alginate and calcium chloride has been demonstrated to markedly enhance the quality of instant-type flavouring undaria pinnatifida (Fan et al., 2013). Furthermore, the application of a treatment comprising 0.4% calcium chloride and 3% sodium alginate has been shown to exert a more favourable impact on the crispness and colour of soaked red chilies (Zhang et al., 2013). In addition, the incorporation of calcium ions into noodles containing sodium alginate was found to reduce water absorption by the noodles during cooking, improved the noodle

texture, as shown by the measurement of hardness, and chewability (Wang et al., 2023b). However, the use of sodium alginate combined with calcium salt impregnation in pre-blanching treatment has not been reported, and the protective effect of sodium alginate combined with calcium salt impregnation on vegetable tissues is expected.

Ultrasound (US) is a type of sound wave that exceeds the upper threshold of human hearing. According to its different ultrasonic frequencies, US can be divided into high-frequency, low-energy (100 kHz-1 MHz) detection US for quality non-destructive testing and low-frequency, high-energy (20–100 kHz) power US for mass and heat transfer. High-energy ultrasonic waves carry a large amount of energy and their propagation in the medium usually results in physical and chemical effects such as cavitation, mechanical effects, thermal effects, and so on, which lead to changes in the properties of the material. Recently, US has been used in pretreatment processes because it can change the structure of the raw material (increasing porosity and loosening tissues) and facilitate the introduction of the impregnating solution. Dominik used US-assisted vacuum impregnation of low-porosity potatoes as raw material and found that US intensified the flow of the impregnating solution and increased the ascorbic acid content of potato tissues (Mierzwa et al., 2023). In the work of Yılmaz, apple tissues were enriched with calcium and phenolic by US-enhanced (96–198 W, 35 kHz) vacuum impregnation. It was found that the application of US during impregnation increased the calcium, total phenolic, flavonoid, and anthocyanin contents, and improved the antioxidant activity of the apple as well (Yılmaz & Ersus Bilek, 2018). Thus, the use of US-assisted impregnation with sodium alginate and calcium salts to improve the protective effect on vegetable tissues and to reduce tissue destruction and loss of bioactive compounds caused by high-temperature blanching can be further investigated.

In this work, we evaluated the effect of US-assisted impregnation pretreatments on the quality parameters of *Shepherd's purses* after blanching treatment. US -assisted impregnation of glucose and calcium salts as a control for us-assisted impregnation of sodium alginate and calcium salts. *Shepherd's purse* was analyzed for ascorbic acid content, phenol compounds content, calcium, and soluble solid chlorophyll content. Further, the volatile flavor changes of *Shepherd's purse* after US-assisted impregnation were analyzed by electronic nose and GC-MS. Finally, the effect of US-assisted impregnation treatments on the antioxidant activity of *Shepherd's purse* was analyzed.

Materials and methods

Materials

Fresh *Shepherd's purses* were purchased from the Xiao Ling Wei market center in Nanjing, China. *Shepherd's purses* of uniform size with no mechanical damage were selected as experimental samples. The *Shepherd's purse* leaves were broken off from the root of the vegetables, some consistent size of the leaves, rinsed under the running water, and then drained water. Subsequently, *Shepherd's purses* were cut into leaves and petioles at the joint of leaves and petioles, the leaves of which were used in the next test.

US-assisted impregnation pretreatment

Shepherd's purse samples were individually impregnated in 0.5% w/w calcium chloride (Ca), 0.5% w/w sodium alginate (Al), and 3% w/w glucose (Gl). The ratio of raw material to impregnation solution was maintained at 1:20(w/v).

Two different methods of impregnation were applied to the samples. The first method involved impregnating the samples at room temperature for 2 h. At the same time, the samples were impregnated in the equivalent volume of ultra-pure water treatment group as a blank control (CK). The second experimental treatment was as follows: *Shepherd's purse* samples were impregnated in 0.5% w/w Al, 3% w/w Gl, respectively, and the ultrasonic bath (KQ-S1000VDE, Kun shan Ultrasonic Instruments Co., Ltd., Suzhou, China) with ultrasonic power of 1000 W at an ultrasonic frequency of 100 kHz was used for 5 min (US5) and 15 min (US15) at room temperature. Then, the sample was washed with pure water to remove the surface residual liquid and impregnated in the same volume of 0.5%(w/w) Ca for 30 min.

After the impregnation treatment, with or without US the samples were removed from the impregnation medium. They were immediately rinsed with distilled water for 30s to remove any excess impregnation solution. Next, the water on the surface of the samples was absorbed with clean paper towel. Finally, the samples were dispensed for the next treatment.

Blanching treatment

After pretreatment, *Shepherd's purse* samples were blanched in boiling water (1:30, w/v) for 1 min. Subsequently, the samples were cooled promptly with cold water to prevent excessive blanching and wiped off moisture with kitchen papers for analysis.

Extraction and quantification of bioactive compounds

Determination of ascorbic acid

The ascorbic acid content of *Shepherd's purse* samples after blanching was determined by HPLC (Agilent 1260,

Agilent Technologies Inc., California, USA). Slight modifications based on this method (Kim et al., 2021). The detailed steps were as follows: a 2.5 g sample was homogenized within 25 mL 0.25% metaphosphate pre-cooling at 4 ℃ in advance, followed by centrifugation at 9000 rpm for 5 min, and filtered through a 0.45 μm filter.

The chromatographic separation was achieved using ZORBAX 300SB-C18 (250 mm × 4.6 mm × 5 μm) column and set at 25 ℃ with a mobile phase of 0.03 mol/L phosphoric acid (0.8 mL/min). Data were calculated according to a pre-established standard curve, and the result was expressed as mg/100 g DW.

Determination of phenolic compounds

A 0.5 g sample was homogenized with 5 mL methanol and was extracted for 30 min in the ultrasonic water bath (40 kHz,250 W) (KH-500DE, Kun shan Ultrasonic Instruments Co., Ltd., Suzhou, China) Then it was centrifuged at 4 ℃ followed by centrifugation at 10,000 rpm for 30 min, then the clear supernatant was filtered into the liquid phase vials to be analyzed by HPLC (Agilent 1260, Agilent Technologies Inc., California, USA). Extracts were separated on an Inertsil ODS column (4.6 mm×250 mm, 3.5 nm) with a diode array detector. The column temperature was 25 °C and the injection volume was 20 μL.

A 0.5 g sample was homogenised with 5 mL of methanol and extracted in an ultrasonic water bath (40 kHz, 250 W) (KH-500DE, Kunshan Ultrasonic Instrument Co., LTD., Suzhou, China) for 30 min Then it was centrifuged at 10,000 rpm at 4℃ for 30 min, and the clear supernatant was filtered into a liquid phase vial. HPLC (Agilent 1260, Agilent Technologies Inc., California, USA) was used for analysis.

To obtain the standard curve, four compounds (gallic acid, protocatechuic acid, chlorogenic acid, rutin) were mixed with methanol to prepare a reserve solution of 10 mg/L, and the reserve solution was diluted. The concentration below the first point of the analytical curve where the chromatographic peak was identified was taken as the limit of detection (LOD) of 0.005 mg/L, and according to the same recommendations, the lowest concentration value of the analytical curve was taken as the limit of quantification (LOQ) of 0.01 mg/L. The resulting curve was then fitted linearly and the R^2 values for gallic acid, protocatechuic acid, chlorogenic acid and rutin were 0.995, 0.992, 0.999 and 0.992 respectively.

All analyses were performed in triplicate and the identity of the analyte was confirmed by comparing the retention time and chromatographic peak curve of the sample with the retention time and chromatographic peak distribution of the analytical standard.

Determination of chlorophyll

The chlorophyll content of blanched leaves was determined by a spectrophotometer. In short, 0.2 g of the sample was ground with 95% ethanol until the tissue turned white left for 3–5 min, and then diluted with extraction solution to 25 mL ready for determination. The whole experiment was carried out under dark conditions.

The absorbance of the mixture was measured at 649 and 665 nm respectively (EP0CH, BioTek Instruments, Inc.,Vermont, USA), and the chlorophyll content was calculated by the following formula:

$$C_a = 13.95D_{665} - 6.88D_{649} \quad (1)$$

$$C_b = 24.96D_{649} - 7.32D_{665} \quad (2)$$

$$\mathrm{CT} = C_a + C_b \quad (3)$$

Among them, C_a, C_b, and CT were the content of chlorophyll a, chlorophyll b, and total chlorophyll separately.

Determination of total soluble solid

The blanched leaves of *Shepherd's purse* with dry, were used for the determination of total soluble solids (TSS). The samples were ground into homogenates and then determined by an Abbe refractometer (PAL-1, Atago Co., Ltd., Tokyo, Japan).

Determination of calcium content

Determination of calcium content in blanched *Shepherd's purse* by an atomic absorption spectrophotometry described by Xu (Xu et al., 2015). The method was as follows: an appropriate amount of *Shepherd's purse* sample was homogenized, and soluble calcium was extracted with 1 mol/L NaCl solution, the supernatant was collected, and at the same time, the volume was fixed to 100 mL. The sample liquid(5 mL)was then placed in the crucible heated on a low heat and carbonized until smokeless. Residual char was transferred to the muffle furnace and ashed at 550 ℃ for 3–4 h to white and grey. It was then cooled out and transferred with 50% HNO_3 to the calibration tube, after that the volume was adjusted to 25 mL with water. Lastly, the sample liquid was diluted properly, and a certain volume of lanthanum solution was added to ensure the concentration of the final diluent was 1 g/L before the test. The absorbance at 422.7 nm was determined by flame atomic absorption spectrometer (PinAAcle 900T, Perkinelmer, Inc., Massachusetts, USA) after mixing it well.

Color measurement

Color determination of *Shepherd's purse* blanched was performed after removing excess moisture with paper towels. The color parameters of the blanched samples were measured using a colorimeter (CM-700d1, Konica minolta, inc.,Tokyo, Japan).

The leaves of the *Shepherd's purse* were spread and selected 5 positions randomly that avoid the veins for the color measure. The final result was the average of the total color difference of the five positions.

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (4)$$

In the formula, L^*, a^*, and b^* represented lightness, redness/greenness, and yellowness/blueness, respectively L_0^*, a_0^*, and b_0^* were the values of fresh, untreated samples.

Shepherd's purse microstructure

The treated *Shepherd's purse* samples were taken, and the material was cut into 1 cm^2 blades with a razor blade, placed in 10 mL round bottom centrifuge tubes, and fixed by adding the appropriate amount of FAA fixative to make paraffin sections. The slices were dewaxed to water and rinsed with distilled water. They were impregnated in an aqueous periodic acid solution, oxidized for 10 min, rinsed with running water for 10 min, and changed twice with distilled water, and then, impregnated in Schiff's reagent for 30 min, rinsed with running water for 5 min. The specimen was subsequently stained with hematoxylin for 30 s, followed by washing, differentiation with aqueous hydrochloric acid, and rinsing, countering with ammonia, final rinsed with running water. Ethanol gradient dehydration was performed, and the sections were sealed with neutral gum after transparency. The magnification was set as 40 ×, three slides were observed for each treatment group (NIKON ECLIPSE E100, Nikon Corporation, Tokyo, Japan).

Electronic nose (e-nose) analysis

The PEN3 type electronic nose (HT2000H, Arisense, Inc., Mecklenburg, Germany) was used for the electronic nose analysis of volatiles, and the electronic nose includes 10 metal oxidation sensors, gas flow control, and analytical control such as that, in which 10 sensors have different sensitivities to different types of volatile compounds. The response flavor substances corresponding to the electronic sensors are described as follows: W1C is sensitive to aromatics, W5S is flexible to nitrogen oxides, W3C is selected to hydrides, W5C is dynamic to alkanes and aromatics, W1W is sensitized to inorganic sulfides, pyrazines, and terpenes, W2S is for alcohols, aldehydes and ketones, W2W is for aromatic and organic sulfides, and W3S is for methane.

The electronic nose method for the analysis of *Shepherd's purse* was modified (Liu et al., 2023). *Shepherd's purse* was cut into pieces, then placed in a headspace bottle. It was then sealed with a lid, and enriched for 1 h at room temperature, after which the electronic nose probe was inserted into the upper end of the bottle for the analysis of the flavor. The charm samples were determined three times in parallel.

The parameters of the electronic nose were set as follows: the test time was 80 s the sensing cleaning time was 80 s, the internal flow control was 100 mL/min, and the sensor was stabilized after 1 s.

GC-MS analysis

The volatile components of the *Shepherd's purse* were extracted and separated by headspace solid-phase micro-extraction (SPME) (Zhao et al., 2023). Added 5 g of sample to 20 mL of distilled water tissue grinder to grind to homogenize. In addition, after homogenizing, 5 mL of solution was transferred to a 15 mL headspace bottle for extraction, and 2 μL of 40 μg/mL n-Nonane was added as the internal standard, 1.6 g of sodium chloride was added as well, which was to quantitative analysis by gas chromatogram-plain (7890 A, Agilent Technologies Inc., California, USA).

The CAR/ PDMS/DVB solid-phase micro-extraction needle (50/30 μL divinyl benzene carboxene-poly (dimethylsiloxane), Supelco, Bellefonte, PA, USA) was inserted into the headspace of the sample vial. The samples were allowed to reach thermal equilibrium at 50 °C for 15 min before headspace extraction lasting 40 min. Subsequently, the needle used for the extraction was removed, inserted into the GC-MS injector and desorbed at 250 °C for 5 min.

Antioxidant activity

The supernatant of 2.4.2 was also used to evaluate $ABTS^+$ and FRAR radical scavenging ability based on the followed methods. For the $ABTS^+$ assay, ABTS was dissolved in acetic acid buffer (20 mM, pH 4.5) to obtain $ABTS^+$ storage liquid (7 mM). The $ABTS^+$ storage liquid was mixed with potassium persulfate solution (pH 4.5, 2.45 mmol/L) in equal volume and reacted for 12–16 h in the dark that mixture was called $ABTS^+$ working liquid. The $ABTS^+$ working liquid was diluted appropriately before use to ensure that the absorption value was about 0.700 ± 0.002. During the determination, 20 μL sample solution was added to 180 μL working solution, and the light absorption value was

measured at 734 nm. For the FRAP assay, the 50 μL sample extraction solution was added to 150 μL TPTZ working solution (composed of 0.3 M pH 3.6 acetic acid solution, 10 mM TPTZ solution, and 20 mM $FeCl_3$ solution at a ratio of 10:1:1), mixed and placed at 37 ℃ for 10 min, the light absorption value of the mixture was measured at 593 nm. The antioxidant capacity was expressed by ascorbic acid equivalent.

Statistical analysis

Three experiments were performed in parallel. IBM SPSS Statistics 25 (SPSS software, US) was used to analyze the mean value and standard deviation of the experimental data, and Origin 2019 b (Origin software, US) was used to draw graphs. The experimental data were compared by one-way ANOVA, followed by Tukey's multiple comparisons. Where $P<0.05$ was considered statistically significant.

Results and discussion

Effect of US- assisted impregnation on nutritional parameters

Blanching is a common pretreatment for fruits and vegetables that plays a vital role in ascertaining the final product's quality. Ascorbic acid is an indispensable marker for assessing the nutritional excellence of fruits and vegetables (Wang et al., 2020). Ascorbic acid loss was greatest in the group without impregnation treatment, which retained only 1.95 mg/100 g. The reduction in the content of ascorbic acid is attributed to the disruption of plant tissues during blanching, leading to the leakage of ascorbic acid from the cells into the blanching water. US-assisted impregnation significantly increased the contents of ascorbic acid after blanching, with the highest ascorbic acid content in Shepherd's purse treated with Al + Ca + US15. The level of ascorbic acid in the Al + Ca + US15 treated group was 1.57 folds higher than in the CK. As seen in Table 1, gallic acid and rutin were the major phenolic compounds of *Shepherd's purse*. Blanching significantly reduced the content of phenolic components in vegetables. After blanching, the content of gallic acid, protocatechuic acid, chlorogenic acid and rutin in *Shepherd's purse* were only 4.06, 2.12, 3.87 and 7.99 mg/g respectively. Heat treatment can break down the cellular structure. This breakdown can lead to the release of phenolic components, thereby decreasing the content of phenolic components after heat treatment (Rizzo et al., 2016). The content of phenolic compounds in Al + Ca + US15 treatment group was the highest, and the contents of gallic acid, protocatechuic acid, chlorogenic acid and rutin was significantly increased by 35.2%, 92.5%, 40.8% and 24.7% compared to the CK. The content of chlorophyll in different impregnation treatments was showed in Table 1. The chlorophyll content of the CK was 5.19 mg/g. Different impregnation treatments significantly ($P<0.05$) increased the chlorophyll content of *Shepherd's purse*. The highest chlorophyll content of 8.07 mg/g was recorded in the Al treated group, which significantly increased by 55.5% ($P<0.05$) compared to the CK. The effects of different impregnation treatments on TSS of *Shepherd's purse* can be seen in Table 1 Hot blanching likely caused the greatest loss of soluble material due to the destruction of tissue cells. Blanched samples without impregnation had the lowest TSS content of 1.25 °Brix. After impregnation, the TSS of the samples changed significantly. In particular, the TSS in the Gl treatment group was significantly increased, and the content was 1.92 °Brix, which was higher than that in the Al treatment group. However, there was no significant difference in the TSS content of the group treated with Al, with or without US. The coating properties of sodium alginate restrict the gain of solids during impregnation. Reducing sugars, due to their smaller molecular weights and greater solubility in water, which can more easily penetrate *Shepherd's purse* tissues. This may be due to the adhesive properties of sodium alginate. In addition, the impregnation

Table 1 Effect of US-assisted impregnation on nutritional parameters

Treatment	Ascorbic acid mg/100 g DW	Phenolic compounds mg/g				Chlorophyll mg/g	Soluble Solids °Brix	Calcium mg/100 g
		Gallic acid	Protocatechuic acid	Chlorogenic acid	Rutin			
CK	1.96 ± 0.01^{f}	4.06 ± 0.09^{f}	2.12 ± 0.01^{e}	3.87 ± 0.03^{e}	7.99 ± 0.06^{e}	5.19 ± 0.13^{e}	1.25 ± 0.05^{d}	10.71 ± 0.26^{e}
Ca	3.40 ± 0.03^{d}	5.04 ± 0.08^{d}	3.52 ± 0.11^{c}	4.60 ± 0.04^{c}	8.85 ± 0.18^{c}	5.68 ± 0.18^{d}	1.47 ± 0.14^{bc}	15.27 ± 0.35^{a}
Gl	2.92 ± 0.26^{e}	5.31 ± 0.06^{b}	2.69 ± 0.01^{d}	5.09 ± 0.05^{b}	9.42 ± 0.06^{b}	6.07 ± 0.23^{d}	1.92 ± 0.18^{a}	12.21 ± 0.10^{c}
Gl + Ca + US5	4.83 ± 0.00^{b}	5.37 ± 0.01^{b}	2.91 ± 0.05^{d}	5.19 ± 0.12^{b}	9.89 ± 0.13^{a}	6.57 ± 0.12^{c}	1.92 ± 0.15^{a}	12.24 ± 0.45^{c}
Gl + Ca + US15	4.39 ± 0.01^{c}	3.74 ± 0.01^{g}	2.60 ± 0.03^{d}	3.68 ± 0.11^{f}	7.32 ± 0.07^{f}	5.21 ± 0.22^{e}	1.57 ± 0.14^{b}	7.67 ± 0.11^{f}
Al	4.40 ± 0.08^{c}	4.63 ± 0.02^{e}	3.71 ± 0.05^{b}	3.80 ± 0.03^{ef}	8.35 ± 0.19^{d}	8.07 ± 0.36^{a}	1.45 ± 0.10^{bc}	12.21 ± 0.08^{c}
Al + Ca + US5	4.90 ± 0.03^{ab}	5.16 ± 0.01^{c}	4.01 ± 0.04^{a}	4.35 ± 0.04^{d}	9.45 ± 0.05^{b}	5.71 ± 0.12^{d}	1.30 ± 0.06^{cd}	11.68 ± 0.07^{d}
Al + Ca + US15	5.04 ± 0.09^{a}	5.49 ± 0.01^{a}	4.08 ± 0.05^{a}	5.45 ± 0.05^{a}	9.96 ± 0.06^{a}	6.62 ± 0.19^{c}	1.45 ± 0.18^{bc}	13.63 ± 0.16^{b}

Data points with different letters in the same column were significantly different at $P<0.05$

efficiency depends on many factors, such as impregnation method, treatment time, voltage, penetrant type, food structure and solute concentration (Ma et al., 2021).

After blanching, there is a significant enhancement in the levels of heat-sensitive components such as polyphenols, flavonoids, and chlorophyll in *Shepherd's purse*. This increase is likely attributed to the protective effects on *Shepherd's purse* tissues resulting from the impregnation of calcium chloride, glucose, and sodium alginate into cells. Treatment of fresh cut taro with 2% sodium alginate significantly inhibited the browning of fresh cut taro and prolonged the freshness of fresh cut taro (Wang et al., 2016). Alginate is commonly employed as a cryoprotectant to mitigate the destruction of cell structure that arises from ice crystal formation (Santarelli et al., 2021). In this study, impregnation of *Shepherd's purse* tissues with sodium alginate may have protected the heat-sensitive components by forming a film on their surface to reduce the damage caused by blanching. Moreover, studies have shown that after $CaCl_2$ impregnation, Ca^{2+} in the chlorophyll replaces Mg^{2+} to form a more stable chlorophyll, thereby reducing chlorophyll loss during blanching (He et al., 2005). Therefore, in this study, the increase in chlorophyll content in *Shepherd's purse* after calcium salt impregnation may be attributed to the substitution of Mg^{2+} by Ca^{2+}, enhancing the stability of chlorophyll during the blanching process.

It is worth noting that different impregnation treatments showed significant variations in the protective effects on three heat-sensitive components. US-assisted impregnation treatment exhibited the strongest protective effect on ascorbic acid, followed by phenolic, and flavonoids, and chlorophyll demonstrated the least protection. This is mainly attributed to the distinct stability and forms of these three heat-sensitive components in vegetables. Ascorbic acid primarily exists in the cellular fluid of plants. After impregnation with calcium chloride, glucose, and sodium alginate, Ca^{2+} cross-linked with sodium alginate, forming an eggshell-like structure that strengthened the cell framework. Simultaneously, glucose filled the gaps within the framework. These two actions synergistically protected and stabilized ascorbic acid, which has the least stability. Polyphenols and flavonoids are mainly found in the cytoplasm, vesicles, and some specific cell structures, while they may interact with cell wall polysaccharides and proteins. Chlorophylls are relatively stable in chloroplasts but can be changed by some external factors. It was demonstrated (Radziejewska-Kubzdela et al., 2014) that sugars significantly enhanced the stability of phenolic and color during processing. The blueberry and strawberry jams impregnated at sucrose concentrations of 1.65 M and 1.46 M, and soaking times of 242 min and 219 min, respectively, exhibited the highest retention of anthocyanin and total phenolic (Barraza-Jáuregui et al., 2017). At the same time, the total phenolic content of dried apple slices also can be increased by adopting synergistic treatment that used calcium lactate and sucrose as osmotic mediators along with vacuum (VC) and US treatments (Wang et al., 2023a). The application of fructose, calcium chloride, and ascorbic acid to the impregnation of nectarines can reduce enzymatic browning caused by phenolic oxidation during processing. This is achieved by increasing the phenolic content in freeze-dried peach slices (Blanda et al., 2008). In a similar study, Dehsheikh treated bananas with carboxymethyl cellulose (CMC) coating and found that varying the concentration of CMC reduced the loss of total phenolic content during drying, which has been attributed to the protective effect of coatings during the drying process (Nadery Dehsheikh & Taghian Dinani., 2019). It should be noted that, in contrast to the current findings, the preservation of ascorbic acid in dried chili peppers was lower when sucrose and sodium chloride were used as osmotic medium, with only 17.5% retention observed (Silva et al., 2012). In this study, calcium chloride, glucose, and sodium alginate impregnation were able to protect vegetable tissues from polyphenols, flavonoids, and chlorophyll loss during blanching process, and US-assisted impregnation promoted this protective effect.

Effect of US-assisted impregnation on calcium content

Calcium impregnation has the potential to enhance the nutritional value of processed fruit and vegetable products as a source of this important mineral. In addition, calcium interacts with plant cell wall components to form a calcium pectinate network that reinforces the cellular structure of fruit and vegetables, making it an effective fortifier for products. Using the property that Ca^{2+} can be solubilised by sodium chloride, the study investigated the effect of different impregnation treatments on the calcium content of blanched *Shepherd's purse*. As can be seen from Table 1, the calcium content of *Shepherd's purse* after blanching was 10.71 mg/100 g. Furthermore, in the Al group that underwent US treatment, the calcium content increased with longer treatment time. The highest calcium content of 13.63 mg/100 g was observed in the Al + Ca + US15 treated group, representing a significant increase of 27.3% compared to the CK. In contrast, the Gl group showed a significant decrease in calcium content with increasing US time, the lowest calcium content was found in the Gl + Ca + US15 group, which was only 7.67 mg/100 g. However, in the group without US treatment, no significant effect was observed on the calcium content in the Al and Gl groups. This may be related to the application of US to improve the mass

transfer of the impregnation, as well as the gel properties of sodium alginate. The cavitation effect induced by acoustic waves is considered to be an important cause of altered cell permeability. In the cavitation phenomenon, the collapse of bubbles contributes to the formation of intercellular space in plant tissues. US can accelerate the formation of cell microchannels and promote the mass transfer of sodium alginate through the cavitation effect. In the presence of Ca^{2+}, $H^{+}$and Na^{+} are easily replaced by Ca^{2+}, and together with G residues to generate a stable "eggshell structure", this three-dimensional network of calcium alginate formed by the three-dimensional structure of thermal irreversibility (Cao et al., 2020), which can resist the blanching process of high temperature on the physical destruction of the cellular tissue. This is an objective statement that explains how treatment with Al + Ca + US15 increased the high retention of the heat-sensitive components of Shepherd's purse after blanching.

Effect of US-assisted impregnation on color

Color are significant indicator for assessing the quality of green vegetables and their processed products during treatment. Various degrees of enzymatic and non-enzymatic reactions, changes in the structure of starch and proteins, and browning caused by the melted and caramelization reactions during processing and storage affect the color of the products. The effects of different impregnation treatments on the color of blanched *Shepherd's purse* were shown in Table 2. A higher value for L^* indicates a lighter *Shepherd's purse*, while a lower value for ΔE indicates that blanched *Shepherd's purses* were more similar in color to fresh samples. In comparison with the fresh *Shepherd's purse*, the L^*, a^*, and b^* values of the blanched *Shepherd's purse* samples showed different changes. In particular, the control group had the lowest L^* value, the highest ΔE value, and the worst color quality. The Al + Ca + Ul15 treatment had the highest L^* value and the lowest ΔE value, resulting in the best color quality of the blanched *Shepherd's purse.* Compared with the control group, the L^* value of the Al + Ca + US15 treatment group increased by 10.6%, and the ΔE value decreased by 49.1% ($P < 0.05$). Hot blanching treatment can effectively remove oxygen in the tissue of *Shepherd's purse* and prevent chlorophyll from oxidation, but high-temperature blanching will break the biofilm structure of plant cells, resulting in the inactivation of lipoproteins, and at the same time make a large number of intercellular acid release, which can combine with the chlorophyll-protein complex, promoting chlorophyll degradation. Furthermore, heating would trigger the isomerization of chlorophyll and could result in the formation of yellowish-brown pyropheophorbide. After the impregnation treatment, various impregnants filled the spaces within the tissues, effectively reducing tissue collapse caused by heat treatment. This also contributed to alleviate the thermal degradation of chlorophyll, with the most significant results being achieved with the use of Al + Ca + US15. These findings were consistent with Czaikoski's study, which showed that the addition of sucrose can better protect the desired green color of canned vegetable-type soybeans (Czaikoski et al., 2013).

Effect of US-assisted impregnation on microstructure

The microstructure of blanched *Shepherd's purse* samples was shown in Fig. 1. Fresh *Shepherd's purse* cells display normal morphology, with clear cell boundaries, neat arrangement, uniform size, strong intercellular adhesion, and no observable gaps. However, after blanching, the cells and inter-organizational connections underwent structural changes, resulting in the loss of the original shape. The application of several impregnation treatments had a protective effect on the cellular tissues of the blanched *Shepherd's purse.* Notably, Al + Ca + US15 treatment resulted in no discernible distinctions in the internal cell space and preserved normal cell morphology. Therefore, this treatment closely replicated the

Table 2 Effect of different impregnation treatments on the color of blanched *Shepherd's purse*

Treatment	L*	a*	b*	ΔE
CK	43.49 ± 0.80^{b}	-15.07 ± 0.67^{ab}	34.64 ± 3.45^{abc}	7.65 ± 0.56^{a}
Ca	43.87 ± 1.64^{b}	-14.84 ± 1.08^{a}	32.05 ± 3.25^{bc}	6.93 ± 0.34^{a}
Gl	44.01 ± 0.65^{b}	-14.62 ± 1.06^{ab}	31.77 ± 1.98^{bc}	6.26 ± 0.75^{ab}
Gl + Ca + US5	49.12 ± 1.06^{a}	-15.61 ± 0.55^{bcd}	33.48 ± 2.45^{abc}	4.54 ± 1.07^{bc}
Gl + Ca + US15	46.76 ± 1.06^{a}	-13.55 ± 0.56^{a}	30.08 ± 0.99^{c}	3.92 ± 0.93^{c}
Al	47.68 ± 1.49^{a}	-16.28 ± 0.89^{c}	37.54 ± 2.11^{a}	7.35 ± 1.84^{a}
Al + Ca + US5	48.64 ± 2.11^{a}	-16.82 ± 0.31^{d}	35.45 ± 1.84^{ab}	6.45 ± 0.31^{a}
Al + Ca + US15	48.09 ± 1.16^{a}	-16.21 ± 0.64^{bc}	31.90 ± 1.12^{bc}	3.90 ± 0.28^{c}

L*, a*, and b* represented lightness, redness/greenness, and yellowness/blueness, respectively. Values in the same column with different letters were significantly different at $P < 0.05$

Table 3 Identification of volatile substances in blanched *Shepherd's purse*

Serial Number	RetentionTime(min)	Flavor substances	CK (ng/g)	Ca (ng/g)	Gl (ng/g)	Gl + Ca + US5 (ng/g)	Gl + Ca + US15 (ng/g)	Al (ng/g)	Al + Ca + US5 (ng/g)	Al + Ca + US15 (ng/g)
		Alkanes	2.89	14.53	11	24.03	25.50	1.12	0.77	10.41
1	0.78	n-Hexane	-	5.98	-	11.79	25.50	-	-	9.93
2	3.82	Trichloromethane	0.90	-	-	-	-	-	-	-
3	6.40	Methane, bromodichloro-	1.15	-	-	-	-	0.42	0.77	0.48
4	8.63	Tridecane	-	-	-	2.55	-	-	-	-
5	8.83	Methane, dibromochloro-	0.47	-	-	-	-	0.26	-	-
6	9.54	Tritetracontane	-	-	-	2.83	-	-	-	-
7	10.20	Tetradecane	-	-	4.64	-	-	-	-	-
8	11.49	Heptacosane	-	0.72	-	-	-	-	-	-
9	11.55	Pentadecane	0.37	2.19	3.13	-	-	-	-	-
10	12.83	Hexadecane	-	5.64	3.23	6.86	-	0.44	-	-
		Olefins	7.60	2.44	-	4.53	3.45	4.03	-	7.65
11	6.99	D-Limonene	-	-	-	4.53	3.45	-	-	-
12	14.70	Azulene	7.60	-	-	-	-	4.03	-	7.65
13	15.92	Benzocycloheptatriene	-	2.44	-	-	-	-	-	-
		Aldehydes	0.36	3.74	-	-	-	6.96	0.55	1.37
14	2.69	Pentanal	-	-	-	-	-	3.30	-	-
15	4.82	Hexanal	-	-	-	-	-	2.49	-	-
16	7.45	2-Hexenal, (E)-	-	-	4.55	6.88	-	0.37	1.20	-
17	10.22	Nonanal	-	-	-	-	-	-	0.55	1.37
18	11.18	2,4-Heptadienal, (E, E)-	0.36	1.55	-	-	-	0.26	-	-
19	12.05	Benzaldehyde	-	2.19	-	-	-	0.54	-	-
		Alcohols	13.46	9.70	37.62	49.94	3.45	19.18	8.94	26.02
20	8.05	1-Pentanol	13.46	9.70	33.07	43.06	3.45	13.58	7.74	26.02
21	11.53	1-Hexanol, 2-ethyl-	-	-	4.55	6.88	-	1.24	1.20	
22	12.39	1-Octanol	-	-	-	-	-	0.84	-	-
23	17.48	Pentanol	-	-	-	-	-	3.52	-	-
		Ethers	0.71	40.13	142.29	84.85	99.78	1.35	0.79	2.42
24	4.43	Disulfide, dimethyl	0.71	40.13	142.29	84.85	99.78	1.35	0.79	2.42
		Esters	1.92	-	-	9.66	-	-	-	-
25	2.27	Arsenous acid, tris(trimethylsilyl) ester	1.92	-	-	9.66	-	-	-	-
		Ketones	0.50	1.71	10.87	-	-	0.37	0.27	-
26	9.35	5-Hepten-2-one, 6-methyl-	0.50	-	9.00	-	-	-	-	-
27	16.76	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	-	1.71	1.87	-	-	0.37	0.27	-
		Acids	-	-	-	-	-	0.35	-	-

Table 3 (continued)

Serial Number	RetentionTime(min)	Flavor substances	CK (ng/g)	Ca (ng/g)	Gl (ng/g)	Gl + Ca + US5 (ng/g)	Gl + Ca + US15 (ng/g)	Al (ng/g)	Al + Ca + US5 (ng/g)	Al + Ca + US15 (ng/g)
28	12.85	Nonahexacontanoic acid	-	-	-	-	-	0.30	-	-
29	20.24	4-Aminocinnamic acid	-	-	-	-	-	0.05	-	-
		Aromatic compounds	11.22	51.86	79.36	88.64	109.15	27.81	18.75	33.41
30	3.92	Toluene	5.16	25.63	52.17	53.23	46.01	17.04	12.18	24.58
31	14.69	Naphthalene	3.69	22.90	16.64	20.47	53.77	7.31	4.25	5.42
32	15.92	1,3-Dimethylnaphthalene	2.37	2.44	4.61	8.48	4.95	1.68	1.76	1.98
33	17.07	Naphthalene, 2,7-dimethyl-	-	1.16	-	1.62	-	-	-	-
34	17.07	Naphthalene, 2-methyl-	-	-	1.03	-	-	-	-	-
35	17.34	Biphenyl	-	-	-	-	-	0.26	-	0.43
36	17.48	Phenol	-	-	4.91	4.84	2.21	1.52	0.56	1.00
37	20.27	Phenol, 2,4-bis(1,1-dimethylethyl)-	-	-	-	-	2.21	-	-	-
		Others	2.64	25.03	-	-	-	1.35	-	2.47
38	1.47	Benzo[h]quinoline, 2,4-dimethyl-	-	25.03	-	-	-	-	-	1.95
39	9.26	Pentanoic acid, 2-methyl-, anhydride	-	-	-	-	-	0.55	-	-
40	11.69	Pyrazine, 2-methoxy-3-(1-methylpropyl)-	0.27	-	-	-	-	0.56	-	-

Table 4 Effect of US-assisted impregnation on antioxidant activity

	ABTS(mg AA/g)	FRAP(mg AA/g)
CK	3.01 ± 0.14^{d}	0.18 ± 0.02^{f}
Ca	3.95 ± 0.17^{c}	0.31 ± 0.02^{b}
Gl	3.33 ± 0.38^{d}	0.21 ± 0.00^{e}
Gl + Ca + US5	4.63 ± 0.22^{ab}	0.25 ± 0.03^{c}
Gl + Ca + US15	4.16 ± 0.30^{b}	0.23 ± 0.03^{d}
Al	4.92 ± 0.02^{a}	0.40 ± 0.01^{a}
Al + Ca + US5	5.04 ± 0.06^{a}	0.23 ± 0.02^{d}
Al + Ca + US15	4.18 ± 0.33^{b}	0.28 ± 0.01^{b}

Values in the same column with different letters were significantly different at $P < 0.05$

tissue structure of the fresh samples. In the Gl-impregnated groups, extension of ultrasound time resulted in an amorphous shift in cell morphology. Blanching causes membrane rupture and cell wall damage, and in US-assisted impregnation samples, the intercellular spaces were filled with impregnation solution and the cell structure was protected during blanching.

Effect of US-assisted impregnation on volatile flavor

The radar graph characterizes diverse volatiles detected by the electronic nose sensor. The baseline is a circle with a response value of 1 (Yakubu et al., 2023). Figure 2 presents the flavor analysis of the blanched *Shepherd's purse* with the electronic nose. It indicates that sensor response

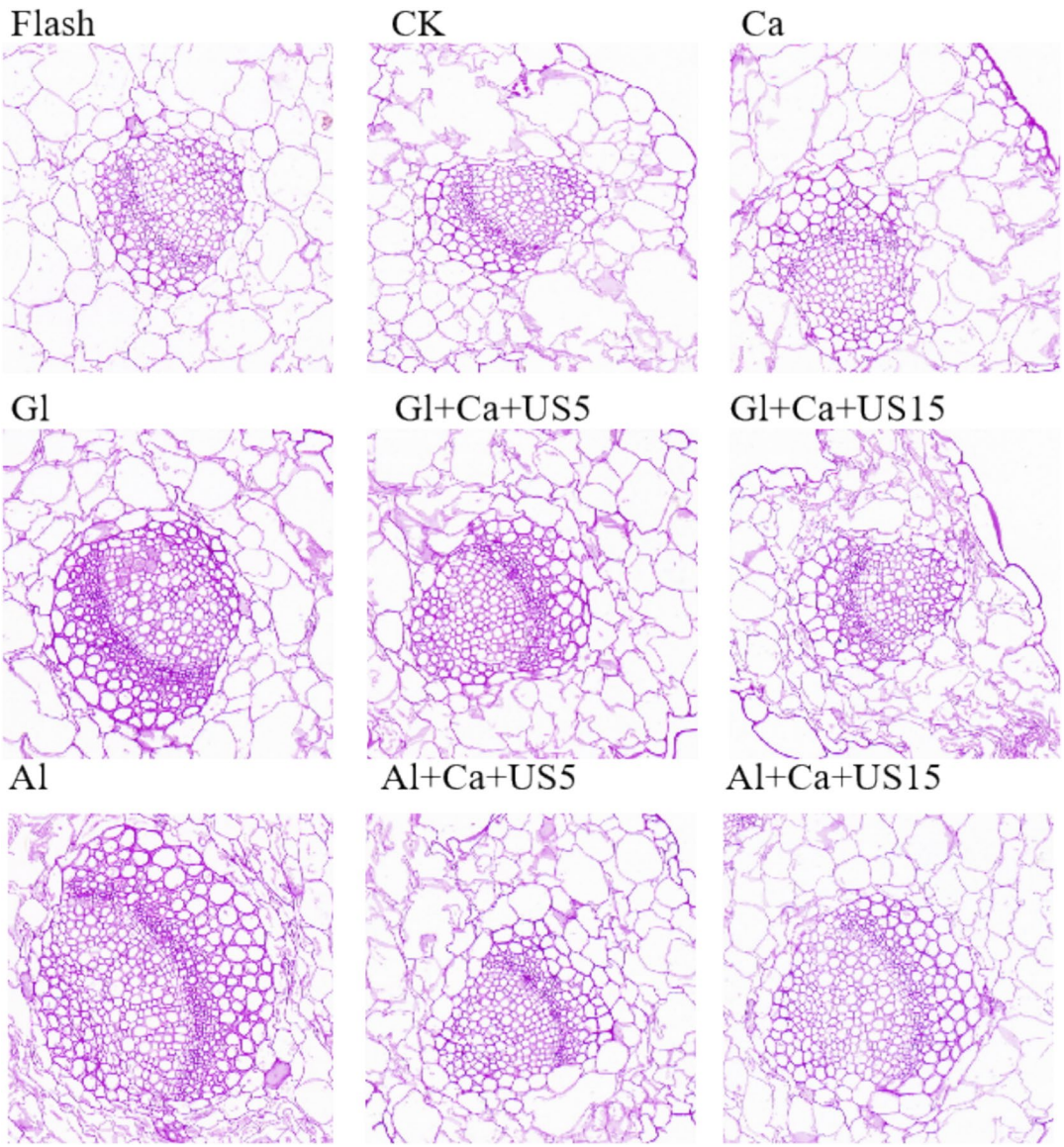


Fig. 1 Microstructure of *Shepherd's purse* cell wall

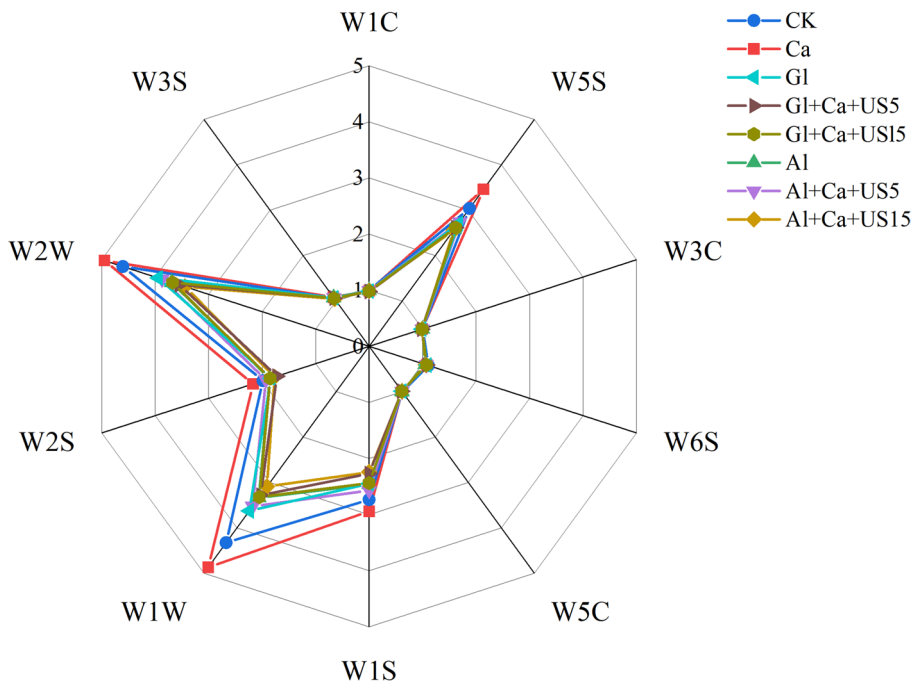


Fig. 2 Radar chart of an electronic nose for blanched *Shepherd's purse*

values for W1C, W3C, W5C, W3S, and W5S were small and not statistically significant ($P > 0.05$). The results indicate that there is a significant presence of inorganic sulfides, aromatic components, organic sulfides, methyl groups, alcohols, ethers, aldehydes, ketones, and nitrogen oxides in the blanched Shepherd's purse as suggested by the large and significant different sensor response values for W1W, W2W, W1S, W2S, and W5S ($P < 0.05$). All the different impregnation treatments exhibited significant differences from the untreated samples.

The principal component analysis (PCA) plot was created to reduce the dimensionality of the electronic nose's output data. It also serves to position samples with similar properties close to the numerical axis. This approach has been successfully applied in studies conducted by Aghili (Aghili et al., 2022), and Gu (Gu et al., 2019). The electronic nose method was employed to collect data on Shepherd's purse samples after blanching and dipping treatments. The contribution rates of the first principal component and the second principal component were 50.0% and 28.8%, respectively. The sum of the two contribution rates was 78.8%, almost reaching 80%. This indicates that the electronic nose method gathered data that better reflects the original information of the *Shepherd's purse* samples. This is depicted in Fig. 3. The variance in the area of *Shepherd's purse* samples among varied impregnation treatments suggests that the flavor of bleached *Shepherd's purse* alters distinctly under different treatment conditions.

Calcium ions interact with pectin on the plant cell wall to increase cell structure hardness, which helps to maintain product characteristics. The addition of calcium salts strengthens vegetable tissues. However, the blanching process can result in sulphurized odors, which affect the product's sensory characteristics. This study aims to investigate this issue. After impregnation with calcium chloride, the signal response values of the aprotic sulfide and mailed organic sulfide were higher. Conversely, the signal values were lower when treated with sodium alginate synergistic calcium chloride and glucose. This suggests that synchro-impregnation treatment is potentially more effective in reducing unpleasant odors.

As can be seen from Table 3, forty-two compounds were extracted and identified in the blanched *Shepherd's purse*. These volatile compounds were categorized into eleven groups, consisting of ten alkanes, three olefins, six aldehydes, four alcohols, one ether, one ester, two ketones, two acids, eight aromatic compounds, and five other compounds. These 11 volatile substances displayed both similarities and significant differences in under various treatment conditions. Four types of volatile compounds were identified in all eight treatments on *Shepherd's purse*, including alkanes, alcohols, ethers, and aromatic compounds. Toluene and naphthalene were identified as representative substances with the highest content of aromatic compounds, followed by ether volatile compounds, with an average of 46.54 ng/g and

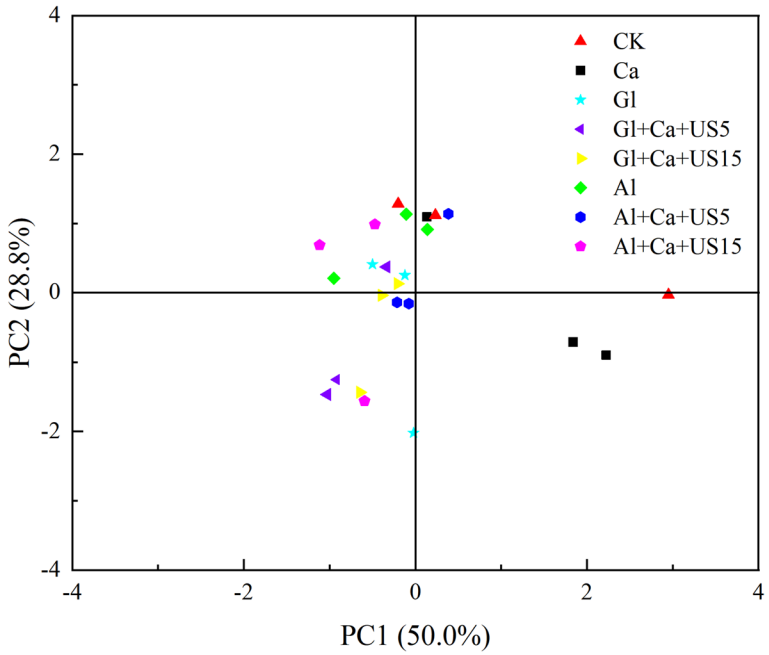


Fig. 3 PCA of the response signal of the electronic nose of blanched *Shepherd's purse*

alcohols, with 1-Pentanol being the dominant compound at approximately 21.04 ng/g content.

The addition of different impregnation treatments in this study significantly affected the composition of flavor profile components of blanched *Shepherd's purse*, leading to significant increase in the overall content of volatile substances. Additionally, 24 volatile organic compounds were identified in blanched *Shepherd's purse* treated with Al including 5 aldehydes and 4 alcohols. The identified volatile substance species had a significant impact and were higher than those in the control group. This study involved pretreatment through cutting, impregnating, and blanching, which caused the loss of flavor substances to varying degrees during processing. Besides, the high temperature led to chemical changes in various flavor substances, including the thermal degradation of unsaturated fatty acids, oxidation of fats, and degradation of amino acids and meladic reaction. However, the mechanism of production and release of each volatile substance differs, resulting in a diverse pattern that requires further exploration.

Effect of US-assisted impregnation on antioxidant activity

Antioxidant activity in plant foods depends on how many bioactive components it contains. From the above findings, it is evident that diverse impregnation treatments notably augmented the phenolic content in the blanched *Shepherd's purse*. This is in line with the outcomes that different impregnation treatments significantly amplified the blanched *Shepherd's purse*'s antioxidant activity, further proposing phenolic as vital components in assuring *Shepherd's purse*'s robust antioxidant activity. According to the data in Table 4 ,in the US-assisted impregnation group, the antioxidant capacity tended to increase with the duration of US. The free radical scavenging activity of ABTS + was increased by 10.6–67.4%. At 5 min of US, Gl and Al exhibited the strongest antioxidant activity, with an increase of 53.8% and 67.4%, respectively, compared to the CK. And the ability to reduce iron was significantly increased by 16.7–122% compared to the CK, and the antioxidant activity of Gl + Ca + US5 increased significantly by 38.9% compared to the CK. The strongest antioxidant activity was found in the Al-treated group, which may be due to the strong stabilization of sodium alginate. Both US and impregnation solution treatments augmented the antioxidant activity of blanched *Shepherd's purses* depending on the duration of ultrasound and impregnation treatment. It was noted that prolonged US led to a comparable decrease in antioxidant activity. Extended exposure to US might cause the generation of stronger reactive oxygen species, which catalyze the oxidation of phenolic, and consequently reduce antioxidant activity (Yeoh & Ali., 2017).

Conclusion

Effects of sodium alginate and calcium on quality attributes of *Shepherd's purse* under ultrasound impregnation during blanching processing were investigated in this study. The results showed that ultrasound-assisted sodium alginate and calcium impregnation treatments significantly reduced the thermal degradation of the heat-sensitive bioactive components during the blanching process. Color changes for *Shepherd's purse* during blanch processing were decreased significantly for US-assisted impregnation treatments. Meanwhile, the microstructural analysis carried out by PAS revealed that the US-assisted impregnation treatments had a bio-protective effect on *Shepherd's purse* tissues subjected heat stresses. The combination of GC-MS and electronic nose results showed that impregnation treatments can reduce the loss of volatiles during the blanching process. Calcium chloride synergized with sodium alginate impregnation and glucose treatments can reduce the undesirable odours caused by conventional calcium impregnation. Thus, the quality characteristics of blanched *Shepherd's purse* can be improved dramatically by combining sodium alginate with calcium treatment under ultrasound impregnation. This strategy can also applied to other materials to extend their application in vegetable processing fields.

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Authors' contributions
Qihui Wu: Conceptualization, Investigation, Writing - original draft, Methodology, Formal analysis. Zhongyuan Zhang, Yihong Bao: Conceptualization, Supervision, Funding acquisition, Writing – review & editing. Dajing Li: Conceptualization, Supervision, Funding acquisition. Xin Wen: Investigation, Formal analysis. Lei Feng: Investigation, Methodology. Meimei Nie: Software, Validation. Zhuqing Dai: Funding acquisition. Yayuan Xu: Funding acquisition.

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Data availability
The data that support the results reported in this paper can be found in the manuscript. Additional information about the data and materials can be obtained by contacting the corresponding author.

Declarations

Ethics approval and consent to participate
This study did not involve human or animal research. Institutional ethical approval was not required. Also, there was no need for consent to participate.

Consent for publication
All the authors agreed on publishing the manuscript in the present format in this journal.

Competing interests
The authors declared that there is no conflict of interest on the manuscript. All the author agreed on writing andsubmission of the manuscript.

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