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Effects of Sea Lettuce (*Ulva Prolifera*) Extracted via Subcritical Water on the Physicochemical Properties of Pork Patties

Abstract

This study investigated the effects of sea lettuce (SL, *Ulva prolifera*) extracted using various subcritical water (SW) temperatures (100°C–300°C) on the physicochemical properties of pork patties. The thiobarbituric acid reactive substances (TBARS) and total volatile basic nitrogen (TVBN) of patties prepared with SL extracted at ≥200°C were significantly lower than those of the control after 2 weeks of chilled preservation ($p < 0.05$). In addition, the extracts subjected to ≥250°C temperatures exhibited lower total aerobic microbial count (TAC) than the control ($p < 0.05$). Although the brownish appearance originating from the SL extracts influenced the color of the pork patties, the patties containing SL extracted at temperatures of ≥200°C maintained a stable color even after preservation. The water-binding properties of the patties tended to increase as the extraction temperature of SL increased, and the extracts obtained at temperatures of 200°C–250°C exhibited texture characteristics similar to the fresh group after preservation. Although the optimal SW extraction temperature for SL in relation to the physicochemical properties of the patties was not distinguished, SL extracted at temperatures of 200°C–250°C generally demonstrated potential as an additive for extending the freshness of pork patties. Consequently, the results of this study revealed that SL extracted at 200°C–250°C effectively inhibited the oxidative deterioration of the patties.

Keywords: subcritical water, Maillard conjugates, *Ulva prolifera*, pork patties, antioxidant

Introduction

The prevention of oxidative deterioration has always been challenging in the meat processing industry. One of the primary concerns is lipid oxidation, which is easily catalyzed by heme iron oxidation during the chilled preservation of meat and meat products (Ganhão et al., 2010). Lipid oxidation affects the flavor and shelf-life of meat products. It induces protein oxidation, leading to high drip loss, low water-holding capacity, and undesirable textural changes in meat products (Ribeiro et al., 2019). For these reasons, the addition of antioxidants in meat formulation is essential for maintaining the quality of meat products. Ascorbic acid and its stereoisomer erythorbic acid are commonly used antioxidants in meat processing. From an economic perspective, numerous investigations have been conducted to replace ascorbic acid with synthetic or natural additives (Yehye et al., 2015; Zahid et al. 2019). Owing to negative biochemical reactions and health concerns associated with synthetic additives such as butylated hydroxytoluene and butylated hydroxyanisole, natural additives extracted from spices, fruits, and vegetable residues have attracted significant attention as antioxidants for meat products (Ribeiro et al., 2019). However, these natural antioxidants have exhibited limited antioxidative activity, and thus cannot completely replace synthetic antioxidants.

Recently, there has been growing interest in converting food and agro-industrial byproducts and waste into bioactive compounds. In particular, subcritical water (SW) is regarded as a novel green technology for upcycling organic biomass (Carr et al., 2011). SW is defined as compressed hot water with a temperature between the normal boiling point (100°C at 0.1 MPa) and critical point (374°C at 22.1 MPa) of water (Carr et al., 2011). Owing to its high thermal energy, SW can effectively liquefy poorly soluble biomass through the hydrolysis of organic compounds (Yüksel Özşen, 2020). These hydrolysates provide strong bioactivities, including antioxidant, anti-inflammatory, antihypertensive, and anticancer properties (Lee et al., 2024a; Ramachandraiah et al., 2017). In addition, the low dielectric constant of SW enables it to act like an organic solvent, such as methanol and acetonitrile, enabling the extraction of nonpolar or less polar phytochemicals, including phenolic acids and flavonoids (Carr et al., 2011). Therefore, SW can be an effective technique for converting biomass into bioactive ingredients.

Ulva prolifera, commonly referred to sea lettuce (SL), is a green macroalga present in coastal areas worldwide (Li et al., 2024). Traditionally, SL has been used in various oriental cuisines due to its health benefits. SL is rich in chlorophyll and phenolic acids, which are known as natural antioxidants (E et al., 2023). Additionally, polysaccharides and proteins account for ca. 40% and 20% of the dry weight of SL, respectively, making the SW extraction favorable for promoting the Maillard reaction (Fan and Gao, 2022; Lee et al., 2024b). Melanoidins, which are brown pigment compounds formed by the Maillard reaction, are strong antioxidants with various bioactivities, including antimicrobial, antihypertensive, and anticarcinogenic effects (Mesías and Delgado-Andrade, 2017). This suggests that SL extracted using SW is a promising candidate for functional food ingredients. However, unharvested SL can negatively impact aquaculture and contribute to serious coastal pollution (Shretha et al., 2021). Therefore, strategies to expand the utilization of SL in the food industry are necessary.

Trials have utilized marine algae as additives in meat products. However, these investigations have primarily focused on the extraction of algal polysaccharides and the textural improvements of low-fat meat formulations (Cofrades et al., 2017). Recently, research has expanded to explore the application of raw or dried marine algae as taste enhancers and nutritional supplements in meat products (Baek and Kim, 2024; Cofrades et al., 2017; Gupta and Abu-Ghanam, 2011). Despite the potential for the bioactive conversion of SL through SW, the impact of SL extracted via SW on the quality characteristics of actual meat products has rarely been investigated. Burger patties are known to be highly sensitive to oxidative deterioration, hence, these products are desirable for exploring the antioxidative impact of biomaterials (Ganhão et al., 2010). Therefore, this study investigated the effects of SL extracted via various SW temperatures as additives on the physicochemical properties of pork patties.

Materials and Methods

Materials

SL was purchased from a local market (Seoul, Korea). After washing gently in running water, which was done twice, SL was dried at 60°C for 24 h using a hot-air dryer (LD-918H5, L’equip, Seoul, Korea). The dried

SL was vacuum packaged with a polyethylene pouch and kept at −30°C before use (within 2 weeks). Six pork loins (*longissimus lumborum*) from each carcass and pork lard were purchased randomly at 48 h post-mortem from a local meat mart (Seoul, Korea). After removing the connective tissue and fat from pork loins, the loins and lard were separately ground twice through a 6 mm plate using a food processor (KMX51, Kenwood Co. Ltd., Havant, UK) and used to prepare pork patties without further storage. All chemicals used in this study were analytical grade and obtained from a local supplier (Seoul, Korea).

Sample preparation

The SL extracts were prepared using a lab-assembled SW system described in our previous study (Lee et al., 2024b) at the Biopolymer Research Center for Advanced Materials (Seoul, Korea). The dried SL was suspended in distilled water, and each 120 mL of 5% (w/v) suspension was applied to the reactor (130 mL working volume). After closing the reactor tightly, the sample suspension was heated from the ambient to target temperature (100°C, 150°C, 200°C, 250°C, and 300°C) at a heating rate of 4°C/min. The pressure inside the reactor was built via the self-vaporization of the suspension. When the temperature inside the reactor reached the target, the reactor was immersed directly into a 4°C water bath for 20 min. The suspension was centrifuged at 3,000 ×g for 15 min at ambient temperature (~20°C), and the supernatant was used as SL extracts. For water extraction (WE) treatment, the SL suspension was gently stirred for 30 min at ambient temperature and centrifuged in the same condition described above. All SL extracts were cooled in a 4°C refrigerator overnight.

Pork patties were formulated with 60% pork loins, 20% pork lard, 1.5% NaCl, and 18.5% SL extracts based on the total weight. After mixing pork loins and NaCl for 2 min using the food processor (KMX51, Kenwood Co. Ltd.), SL extracts and lard were added to the mixture and further mixed for 2 min. Aliquots 80 g of mixture were shaped cylindrically using a petri dish and wrapped. For control, the SL extract was replaced by distilled water. A total of 6 patties per treatment was prepared, while control was prepared 12 patties to compare characteristics of fresh group (FG) and those after preservation. Two patties of each treatment were randomly selected to measure freshness parameters, color, moisture content, and expressible moisture. The remaining four patties were heated to determine cooking loss and texture profiles. For experimental replications, the entire procedure described above was repeated three times on different days using a new batch of SL extracts, pork loins, and lard.

Freshness indicators

Lipid oxidation of each pork patty was evaluated in duplicate using thiobarbituric acid reactive substances (TBARS), as described in Song et al. (2024), with slight modifications. A sample (5 g) was suspended in 45 mL distilled water using a homogenizer (PH91, SMT, Tokyo, Japan) for 60 s and filtered through a Whatman No. 1 filter paper (GE Healthcare Life Science, Buckinghamshire, UK). The filtrate (0.5 mL) was mixed with 4.5 mL of reagent consisting of 0.25 M HCl, 15% (w/v) trichloroacetic acid, and 0.375% (w/v) 2-thiobarbituric acid. The mixture was heated in boiling water for 15 min and centrifuged at 3,000 ×g for 10 min under 4°C. The absorbance of the supernatant was taken at 535 nm. The TBARS of pork patties were calculated using a standard curve prepared with malonaldehyde (MA) as a reference and expressed as mg MA/kg sample.

Each sample's total volatile basic nitrogen (TVBN) was measured in duplicate using Conway's microdiffusion method described by Park et al. (2021). The total aerobic microbial count (TAC) of pork patties was determined using the method of Lu et al. (2024). From each patty, a 2-g sample was aseptically taken in duplicate and suspended in 18 mL sterilized saline. The suspensions were subjected to a stomacher (WS-400, Shanghai Zhisun Equipment Co. Ltd., Shanghai, China) for 1 min and diluted serially to 10^9 level using saline. Each dilute was spread on a petri film (aerobic count plate, 3M Co., St. Paul, MN, USA) and incubated at 37°C for 48 h. Films with 30–300 colonies were selected and counted. The TAC of the samples was expressed as log colony-forming units (CFU)/g sample.

Instrumental color

The color of pork patties was determined from two random surfaces of each sample immediately after preparation and preservation using a chroma meter (CR-400, Konica-Minolta, Tokyo, Japan) calibrated using a standard whiteboard (L* = 96.8, a* = 0.30 and b* = 1.67). CIE L*, a*, and b* were recorded as indicators of lightness, redness, and yellowness, respectively, and the color data from each treatment were averaged.

Water-binding properties

The moisture content of each sample was measured in duplicate based on the 105°C hot-air drying method. The expressible moisture of pork patties was evaluated using the method described by Park et al. (2021), with minor modifications. A 2 g sample was collected from each patty in duplicate and placed in a centrifuge tube with gauze as the moisture absorber. The sample was centrifuged at 3,000 ×g for 15 min under 4°C, and the sample pieces were carefully removed from the tube. The tube with gauze was weighed and dried at 105°C for 24 h. Expressible moisture was calculated using the percentage of moisture loss relative to the initial sample weight.

To measure cooking loss, four patties from each treatment were weighed and separately placed in a plastic bag. The patties were cooked in a 75°C water bath for 15 min and cooled at ambient temperature for 30 min. Surface exudates were gently wiped off, and the cooked patties were weighed again. The cooking loss of the samples was calculated using the percentage weight loss of the patties before and after cooking.

Texture profile analysis

After measuring cooking loss, the cylindrical samples were obtained from the center of the patties using a cork borer (26.25 mm in diameter). The samples were subjected to a texture analyzer (CT-3, Brookfield Engineering Labs Inc., Middleboro, MA, USA) equipped with a standard probe (50.8 mm in diameter, TA-25/1000, Brookfield Engineering Labs Inc). The samples were compressed twice to 70% of their height under 5 g of trigger load and 1 mm/s head speed.

Statistical analysis

A completely randomized design was employed to estimate the main effect (extraction temperature). Data collected from each experiment were averaged, and the means and standard deviations were calculated using the averages obtained from three independent experiments (n = 3). The statistical significance of the main effect was determined by one-way analysis of variance using SPSS software (ver. 25, IBM Inc., Armonk, NY, USA). Duncan's multiple range test was conducted for the post hoc test when the main effect was significant ($p < 0.05$).

Results and Discussion

Freshness indicators

Compared with 0.28 mg MA/kg of TBARS of FG (Fig. 1A), all patties showed significantly higher TBARS after 2 weeks of preservation regardless of treatments ($p < 0.05$). Among preserved treatments, SL extracted at lower than 150°C did not exhibit antioxidative activity. The TBARS of these treatments ranged from 1.43 to 1.63 mg MA/kg. Meanwhile, patties prepared with SL extracted at 200°C or higher exhibited 0.46–0.55 mg MA/kg of TBARS, lower than those of former groups ($p < 0.05$). In general, biomass subjected to SW at greater than 200°C was reported to possess high antioxidative activities (Lee et al., 2024b). The current study indicates that SL could be converted into strong antioxidants by SW extraction. As described earlier, the hydrothermal conversion of SL would be manifested not only by the generation of melanoidins but also by the extraction of phenolic compounds (Carr et al., 2011; Lee et al., 2024b; Mesías and Delgado-Andrade, 2017). In particular, SL extracted at 250°C could extend the shelf-life of pork patties for 2 weeks since the TBARS of this treatment was still lower than 0.5 mg MA/kg, which was recognized as an upper limit for rancidity in meat products (Hansen et al., 2004).

The TVBN as an indicator of protein spoilage showed a similar pattern to those of TBARS (Fig. 1B). The TVBN of FG ranged from 6.20 to 8.40 mg/100 g, and those of all patties increased after 2 weeks of preservation ($p < 0.05$). Compared with patties after preservation, the addition of SL extracted at higher temperatures tended to decrease the TVBN of pork patties, and the addition of SL extracted at 200°C–250°C lowered the TVBN of pork patties to 11.47–12.60 mg/100 g ($p < 0.05$). Conversely, SL subjected to 300°C increased the TVBN of pork patties to 16.34 mg/100 g ($p < 0.05$). TVBN is related to the action of microbial and intrinsic enzymes (Bekhit et al., 2021). As polysaccharides account for most organic compounds in SL, the high SW temperature could manifest the generation of simple sugars. Under an elaborated SW environment, the simple sugars are converted into thermal derivatives such as 5-hydroxymethylfurfural (HMF) and furfural (Lachos-Perez et al., 2017; Yüksel Özşen, 2020), and these metabolites were easily absorbed on the surface of enzymes and bacteria inhibiting their actions (Chai et al., 2013). In most biomass such as ginseng roots and rice husks, the thermal derivatives were intensively generated at a SW temperature of 200°C–250°C (Lee et al., 2024a, 2024b), which could contribute to the low TVBN of pork patties through inhibiting endogenous and exogenous proteases. Conversely, these hydrothermal metabolites were further degraded into formic and acetic acids at a greater SW temperature (Yüksel Özşen, 2020), possibly resulting in higher TVBN at a 300°C treatment.

For antimicrobial activities, the TAC of the FG ranged from 3.00–3.31 log CFU/g (Fig. 1C), whereas all treatments showed significantly higher TAC after 2 weeks of preservation ($p < 0.05$). There were no differences in the TAC of patties prepared using SL extracted at temperatures of ≤200°C, and the TAC of these treatments ranged from 7.73 to 8.10 log CFU/g. Meanwhile, SL subjected to temperatures of ≥250°C exhibited antimicrobial activities. A 300°C treatment showed 5.97 log CFU/g of the lowest TAC among treatments ($p < 0.05$). These changes in TAC with SW temperatures explained that the generation of acetic acid, a thermal product from degradation of intermediate derivatives such as HMF and furfural, suppressed the growth of bacteria in pork patties during preservation. According to the previous literature, biomass subjected to 250°C–300°C completely prevented microbial growth (Lee et al., 2024a, 2024b). However, the high TAC level of the 300°C treatment compared with that of the FG in the present study would be due to low concentrations of SL extracts in the formulation of pork patties. Nevertheless, it was demonstrated that SL extracted at temperatures of ≥200°C effectively improved the stability against the lipid oxidation in pork patties. In addition, SW at temperatures of ≥250°C could convert SL into an effective antimicrobial agent for meat products. The bioactivities of SL extracts observed in pork patties were likely related to the compositional changes in SL under the applied SW temperature. The findings of this study indicated that SL extracts had potential applications in extending the shelf-life of pork patties.

Visual appearance and instrumental color

For the appearance of SL extracts (Fig. 2A), WE treatment exhibited a bright yellow appearance, resulting from an extraction of chlorophylls (E et al., 2023). In addition, SL had no visual change when subjected to 100°C–150°C. However, SL extracted at 200°C showed a dark brown appearance, and the browning discoloration was more intense at 250°C. As reported, proteins and polysaccharides in biomass underwent hydrothermal hydrolysis under SW at 190°C–220°C (Lee et al., 2024b; Ramachandraiah et al., 2017). The intense dark brown appearance of the latter SL extracts would be evidenced by the generation of Maillard conjugates between these hydrolysates (Mesías and Delgado-Andrade, 2017). Still, the SL extracted at 300°C showed a dark brown appearance, but the color intensity was slightly lower than that obtained at 250°C. According to the report of Fan and Gao (2022), primary Maillard products were polymerized into secondary products, and the polymers were partially eliminated by centrifuging, leading to a lower intensity of the Maillard pigmentation in SL extracts.

Despite its known physiological impacts, the unique color of the Maillard conjugates in the SL extract influenced the color of fresh pork patties (Fig. 2B). Compared with the control, the addition of WE or SW treatments at 100°C–200°C did not affect the visual appearance of fresh pork patties. However, the pork patties prepared with SL subjected to 250°C or higher could be distinguishable visually. Based on instrumental color measurement as depicted in Fig. 3, the visual color of the latter group was characterized by higher a* and b* values compared to control ($p < 0.05$).

After chilled preservation, the appearance of all patties changed to brownish, possibly due to the oxidation of myoglobin (Fig. 2C). In contrast to control of which L* tended to decrease, the L* value of all SL treatment tended to increase compared to the corresponding counterparts with significant decreases in a* and b* values ($p<0.05$). In comparison of treatments, the intensity of the brown appearance depended on the SW temperature applied to extract SL. According to the instrumental color, treatments with SL subjected to ≤200°C showed higher L* value than control ($p<0.05$), while there was no difference in L* value among treatment group. The a* and b* values showed a different pattern based on the extraction temperature. The a* and b* values of all SL treatments were higher than those of control ($p<0.05$), reflecting that SL had an ability to stabilize color of pork patties during chilled preservation. Among treatment group, the SL subjected to 250°C manifested the highest a* value of pork patties among treatments ($p < 0.05$), whereas the b* of pork patties tended to be high in patties prepared with SL subjected to 150°C–250°C. Conversely, the lowest b* value among treatments was obtained by 300°C treatment.

The results indicated that the antioxidative activities of SL extracted at 200°C–300°C could stabilize the color of pork patties during chilled preservation. As reported previously, SW could yield Maillard products and extract phenolic compounds due to its low dielectric constant (Carr et al., 2011; Lee et al., 2024b; Mesías and Delgado-Andrade, 2017), and these factors were closely related to inhibiting myoglobin oxidation. Although the color of pork patties was affected by the Maillard pigments originating from SL extracts, the results indicated that myoglobin oxidation could be delayed by the addition of SL extracted at 200°C–300°C, which could also inhibit the oxidative reactions of pork patties during preservation.

Water-binding properties

The moisture content of the pork patties in the FG was 67.9% (Fig. 4A). All patties showed a slight decrease in moisture content after 2 weeks of preservation, and a significant reduction in moisture content was observed only in the control and WE treatment ($p < 0.05$). By contrast, the moisture content of the SW treatment groups did not differ significantly from that of FG. Based on these findings, it could be presumable that the SW-treated SL had the advantage of minimizing changes in the water-binding capacity of pork patties during 2 weeks of chilled preservation. The impact of SL extracted via SW on the water-binding properties of pork patties was likely due to their strong antioxidant activity, which would suppress protein oxidation. Reportedly, lipid oxidation manifested protein oxidation. The oxidized proteins were susceptible to aggregation owing to their enhanced hydrophobicity, resulting in reduced water-binding properties (Nawaz et al., 2022). Additionally, the result showed that SW effectively extracted polysaccharides in the cell walls of SL, thereby improving the water-binding properties of pork patties. Expressible moisture was significantly lower in all the patties after 2 weeks of preservation than that in the FG (Fig. 4B). Although the 250°C treatment tended to show lower expressible moisture than other treatments, the overall difference among the preserved treatments was not significant.

Herein, no significant differences in moisture content and expressible moisture among preserved treatments might indicate that the relationship between degree of oxidation and the water-binding properties of pork patties was not clearly observed unless the patties were thermally processed. A clear difference in water-binding properties among preserved treatments was found in terms of cooking loss (Fig. 4C). Overall, cooking loss tended to increase slightly after 2 weeks of preservation compared with that in the FG. In particular, the WE treatment exhibited significantly higher cooking loss than FG ($p < 0.05$). This was likely due to the oxidation of polyunsaturated fatty acids in SL, which influenced protein oxidation and reduced the water-binding properties of pork patties. The comparison among the treatments showed that the 150°C–200°C treatment exhibited a significantly lower cooking loss than the WE treatment ($p < 0.05$). In addition to yielding melanoidins, the dielectric constant of SW at 150°C–200°C was similar to that of methanol, providing an effective environment for extracting phenolic compounds from SL (Carr et al., 2011). These compositional changes would inhibit and delay protein oxidation during preservation, possibly resulting in less cooking loss of pork patties. Furthermore, these SW extraction conditions effectively extracted carbohydrates from SL. Although the carbohydrates in the cell walls of SL were poorly soluble in water, they could be hydrolyzed and extracted more readily in the SW environment (Álvarez-Viñas et al., 2021). Notably, the final products of the hydrothermal degradation of polysaccharides were organic acids (Yan et al., 2021), which could explain why cooking loss at SW temperatures of ≥250°C was similar to that in the WE treatment. Eventually, the present study indicated that SL extracted via SW had the potential to suppress protein oxidation, thereby minimizing a change in the water-binding properties of meat proteins during preservation.

Texture profile analysis

After preservation, the hardness of all treatments preserved for 2 weeks tended to be higher than the FG (Fig. 5A), and particularly patties with SL subjected to ≤150°C had a significantly higher hardness than FG ($p < 0.05$). Among treatments, addition of SL extracted at ≥150°C showed lower hardness than those extracted at lower SW temperature, and the lowest hardness was obtained at 250°C treatment ($p<0.05$). The cohesiveness of patties preserved for 2 weeks did not differ from that of FG (Fig. 5B), still 150°C–200°C treatments showed low cohesiveness compared to patties with SL subjected to ≤100°C ($p<0.05$). The springiness of pork patties also showed a similar pattern to cohesiveness (Fig. 5C). Excluding 300°C treatment, all patties exhibited no significant change in springiness after 2 weeks of preservation compared to the FG. In comparison among the treatments, the lowest springiness was observed in 200°C treatment whereas the highest in 300°C treatment. For gumminess, significant differences were observed between the preserved treatments and the FG (Fig. 5D). Among the treatment group, gumminess tended to decrease gradually as the extraction temperature of SL increased which was similar to those of hardness. According to the above results, SL extracted at relatively low temperatures (< 200°C) had no effect on the texture of pork patties during chilled preservation, while SL extracted at temperatures in the range of 200°C–250°C had an inhibitory effect on the textural changes of the patties during preservation. Conversely, the texture-stabilizing ability shown by the latter group was diminished due to the SW at 300°C.

It was believed that the textural changes in pork patties after preservation were likely due to protein oxidation. Since protein oxidation promotes cross-linking and aggregation among proteins, negatively affecting the water-binding properties and texture of meat products (Zhang et al., 2013). Therefore, the increase in toughness of the former group (< 200°C) appeared to be a result of the protein oxidation during preservation. Meanwhile, the textural stabilization of SL subjected to 200°C–250°C could be influenced by the content and composition of the solid components derived from SL. SL primarily comprised polysaccharides, which significantly affect the water-binding properties and texture of meat products (Han et al., 2023). Since the SW temperature in these ranges was favorable for breaking down the cell wall structure of SL, it would promote the extraction of algal polysaccharides (Li et al., 2024). By contrast, polysaccharides underwent hydrothermal hydrolysis into oligomers or monomers under the SW environment, which could enhance water-binding properties but manifest the loss of the textural contributions of meat products. As a result, significant changes in textural properties of preserved patties would be rarely observed by addition of SL extracted at 200°C–250°C. Furthermore, the high antioxidant capacity of SL, resulting from the generation of Maillard conjugates and the extraction of phenolic compounds at these temperature ranges, could inhibit protein oxidation in pork patties during preservation, minimizing textural changes (Mesías and Delgado-Andrade, 2017; Wu et al., 2023).

From this perspective, the textural changes observed in the 300°C treatment could be explained as a result of compositional changes of Maillard conjugates and phenolic compounds. In other words, the hydrothermal polymerization of these components at extreme SW temperature caused an elimination of the polymer during centrifugation of SL extraction, thereby resulting in a loss of antioxidative activity. Consequently, the results demonstrated that SL extracts could be utilized as novel biomaterials for inhibiting oxidative deterioration in meat products during chilled preservation, and the SW extraction at temperatures of 200°C–250°C appeared to be a promising technology for maximizing the antioxidative activities of SL extracts, which could effectively suppress protein oxidation and maintain the textural properties of meat products during preservation.

Conclusion

Based on the results of this study, the SL extracted via SW could extend the freshness of pork patties during 2 weeks of chilled preservation. The SL extracts not only inhibited lipid oxidation in the patties but also affected color stability of pork patties during preservation. The ability of SL to inhibit lipid oxidation effectively prevented the loss of water-binding properties and textural modifications caused by meat protein oxidation. Although the brown appearance of the SL extract resulting from high SW extraction temperatures could negatively affect the color of the raw patties, these unattractive features were likely to be offset during chilled preservation. Therefore, this study demonstrated that SW extraction effectively converted SL into valuable additives for meat processing. Although, the impact of SL extracts on sensorial properties of pork patties warranted further explorations, SW extraction at temperatures of 200°C–250°C imparted inhibitory ability of oxidative deteriorations to SL, suggesting its potential application in various meat products.

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References

Álvarez-Viñas M, Rodríguez-Seoane P, Flórez-Fernández N, Torres MD, Díaz-Reinoso B, Moure A, Domínguez H. 2021. Subcritical water for the extraction and hydrolysis of protein and other fractions in biorefineries from agro-food wastes and algae: a review. Food Bioprocess Technol 14:373-387.

Baek UB, Kim HY. 2024. Physicochemical properties of restructured black goat Jerky with various types of ultra-ground seaweed powders. Food Sci Anim Resour 44:483-497.

Bekhit AEDA, Holman BWB, Giteru SG, Hopkins DL. 2021. Total volatile basic nitrogen (TVB-N) and its role in meat spoilage: A review. Trend Food Sci Technol 109:280-302.

Carr AG, mammucari R, Foster NR. 2011. A review of subcritical water as a solvent and its utilisation for the processing of hydrophobic organic compounds. Chem Eng J 172:1-17.

Chai WM, Liu X, Hu YH, Feng HL, Jia YL, Guo YJ, Zhou HT, Chen QX. 2013. Antityrosinase and antimicrobial activities of furfuryl alcohol, furfural and furoic acid. Int H Biol Macromol 57:151-155.

Cofrades S, Benedí J, Carcimartin A, Sánchez-Muniz FJ, Jimenez-Colmenero F. 2017. A comprehensive approach to formulation of seaweed-enriched meat products: From technological development to assessment of healthy properties. Food Res Int 99:1084-1094.

E HM, S M, Baskaran R. 2023. The sea lettuce Ulva sensu lato: Future food with health-promoting bioactives. Algal Res 71:103069.

Fan R, Gao Y. 2022. Maillard and hydrolytic reactions in subcritical water extraction of bioactive compounds from licorice. Molecules 27:6851.

Ganhão R, Morcuende D, Estévez M. 2010. Protein oxidation in emulsified cooked burger patties with added fruit extracts: Influence on colour and texture deterioration during chilled storage. Meat Sci 85:402-409.

Gupta S, Abu-Ghannam N. 2011. Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods. Innov Food Sci Em 12:600-609.

Hansen E, Lauridsen L, Skibsted LH, Moawad RK, Andersen ML. 2004. Oxidative stability of frozen pork patties: Effect of fluctuating temperature on lipid oxidation. Meat Sci 68:185-191.

Kendel M, Wielgosz-Collin G, Bertrand S, Roussakis C, Bourgougnon N, Bedoux G. 2015. Lipid composition, fatty acids and sterols in the seaweeds *Ulva armoricana*, and *Sllieria chordalis* from Brittany (France): An analysis from nutritional, chemotaxonomic, and antiproliferative activity perspectives. Mar Drugs 13:5606-5628.

Lachos-Perez D, Tompsett GA, Guerra P, Timko MT, Rostagno MA, Martínez J, Forster-Carneiro T. 2017. Sugars and char formation on subcritical water hydrolysis of sugarcane straw. Bioresour Technol 243:1069-1077.

Lee JW, Lee MY, Lee SY, Hong GP. 2024a. Compositional changes and physiological activities of fresh ginseng extracts prepared at various temperatures in subcritical water. J Ginseng Res (in press).

Lee JW, Nam J, Lim W, Lim TG, Hong GP. 2024b. Subcritical water mediated hydrolysis of rice husk lignocellulose and upcycling applications of the hydrolysates. Food Bioproc Technol (in press).

Li C, Wang H, Zhu B, Yao Z, Ning L. 2024. Polysaccharides and oligosaccharides originated from green algae: structure, extraction, purification, activity and applications. Bioresour Bioprocess 11:85

Lu J, Li R, Chen H, Sun D, Yu Z, Liu Y, Zhang B, Jiang W. 2024. Effect of brine concentration on the quality of salted large yellow croaker during processing and refrigeration. Food Sci Biotechnol 33:3257-3267.

Mesías M, Delgado-Andrade C. 2017. Melanoidins as a potential functional food ingredients. Curr Opin Food Sci 14:37-42.

Nawaz A, Sana I, Ali Khan I, Khalifa, I, Walayat N, Aadil RM, Kumar M, Wang M, Chen F, Cheng KW, Lorenze JM. 2022. Protein oxidation in muscle-based products: Effects on physicochemical properties, quality concerns, and challenges to food industry. Food Res Int 157:111322.

Park DH, Lee S, Kim EJ, Ji YR, Wi G, Choi MJ. 2021. Freshness of deep frozen mackerel and croaker during long-term storage. Int J Food Prop 24:89-104.

Ramachandraiah K, Koh BB, Davaatseren M, Hong GP. 2017. Characterization of soy protein hydrolysates produced by varying subcritical water processing temperature. Innov Food Sci Em 43:201-206.

Ribeiro JS, Santos MJMC, Silva LKR, Pereira LCL, Santos IA, Lannes SCDS, da Silva MV. 2019. Natural antioxidants used in meat products: A brief review. Meat Sci 148:181-188.

Shretha A, Acharya B, Farooque AA. 2021. Study on hydrochar and process water from hydrothermal carbonization of sea lettuce. Renew Energy 163:589-598.

Song HG, Lee S, Han J. 2024. Enhanced oxygen barrier properties of sodium alginate coatings in humid environments: ionic crosslinking of sodium alginate by calcium ions released from calcium hydrogen phosphate and calcium carbonate. Food Sci Biotechnol (in press).

Wu J, Zhao S, Chen X, Jiu Y, Liu J, Gao J, Wang S. 2023. Physicochemical properties, multi-elemental composition, and antioxidant activity of five unifloral honeys from *Apis cerana cerana*. Food Sci Biotechnol 32:1821-1829.

Yan Z, Lian J, Feng Y, Li M, Long F, Cheng R, Shi S, Guo H, Lu J. 2021. A mechanistic insight into glucose conversion in subcritical water: Complex reaction network and the effects of acid-base catalysis. Fuel 289:119969.

Yehye WA, Rahman NA, Ariffin A, Hamid SBA, Alhadi AA, Kadir FA, Yaeghoobi M. 2015. Understanding the chemistry behind the antioxidant activities of butylated hydroxytoluene (BHT): A review. Eur J Med Chem 101:295-312.

Yüksel Özşen A. 2020. Conversion of biomass to organic acids by liquefaction reactions under subcritical conditions. Front Chem 8:24.

Zahid MA, Seo JK, Parvin R, Ko J, Yang HS. 2019. Comparison of butylated hydroxytoluene, ascorbic acid, and clove extract as antioxidants in fresh beef patties at refrigerated storage. Food Sci Anim Resour 39:768-779.

Zhang W, Xiao S, Ahn DU. 2013. Protein oxidation: Basic principles and implications for meat quality. Crit Rev Food Sci Nitr 53:1191-1201.

Figure Captions

Fig. 1. Effect of subcritical water extraction temperature on the freshness parameters of pork patties prepared with sea lettuce extracts. (A) Thiobarbituric acid reactive substances (TBARS), (B) total volatile basic nitrogen (TVBN), and (C) total aerobic microbial count (TAC) of patties after 2 weeks of chilled preservation. FG, fresh group; C, control; WE, water extraction. Vertical bars indicate standard deviations (n = 3). $^{a-c}$ Means with different letters are significantly different ($p < 0.05$).

Fig. 2. Effect of subcritical water extraction temperature on the visual appearance of sea lettuce extracts and pork patties. (A) Sea lettuce extracts, (B) fresh pork patties prepared with sea lettuce extracts, and (C) the patties after 2 weeks of chilled preservation.

Fig. 3. Effect of subcritical water (SW) extraction temperature on the CIE color of pork patties prepared with sea lettuce extracts. (A) L*, (B) a*, and (C) b* values of fresh patties and those after 2 weeks of chilled preservation. C, control; WE, water extraction. Vertical bars indicate standard deviations (n = 3). $^{a-c,\ A-D}$ Means with different letters are significantly different ($p < 0.05$).

Fig. 4. Effect of subcritical water (SW) extraction temperature on the water-binding properties of pork patties prepared with sea lettuce extracts. (A) Moisture content, (B) expressible moisture, and (C) cooking loss of patties after 2 weeks of chilled preservation. FG, fresh group; C, control; WE, water extraction. Vertical bars indicate standard deviations (n = 3). $^{a-b}$ Means with different letters are significantly different ($p < 0.05$).

Fig. 5. Effect of subcritical water (SW) extraction temperature on the texture profiles of pork patties prepared with sea lettuce extracts. (A) Hardness, (B) cohesiveness, (C) springiness, and (D) gumminess of patties after 2 weeks of chilled preservation. FG, fresh group; C, control; SW, water extraction. Vertical bars indicate standard deviations (n = 3). $^{a-d}$ Means with different letters are significantly different ($p < 0.05$).

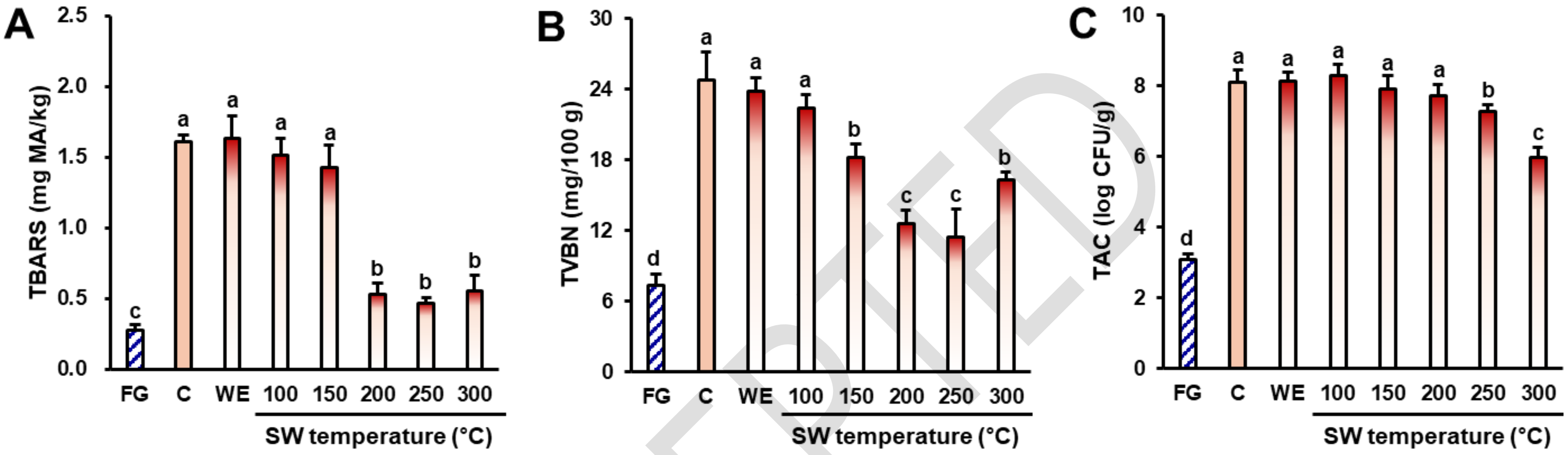

A
TBARS (mg MA/kg)
2.5
2.0
1.5
1.0
0.5
0.0
c
a
a
a
a
b
b
b
FG
C
WE
100
150
200
250
300
SW temperature (°C)
B
TVBN (mg/100 g)
30
24
18
12
6
0
d
a
a
a
b
c
c
b
FG
C
WE
100
150
200
250
300
SW temperature (°C)
C
TAC (log CFU/g)
10
8
6
4
2
0
d
a
a
a
a
a
b
c
FG
C
WE
100
150
200
250
300
SW temperature (°C)

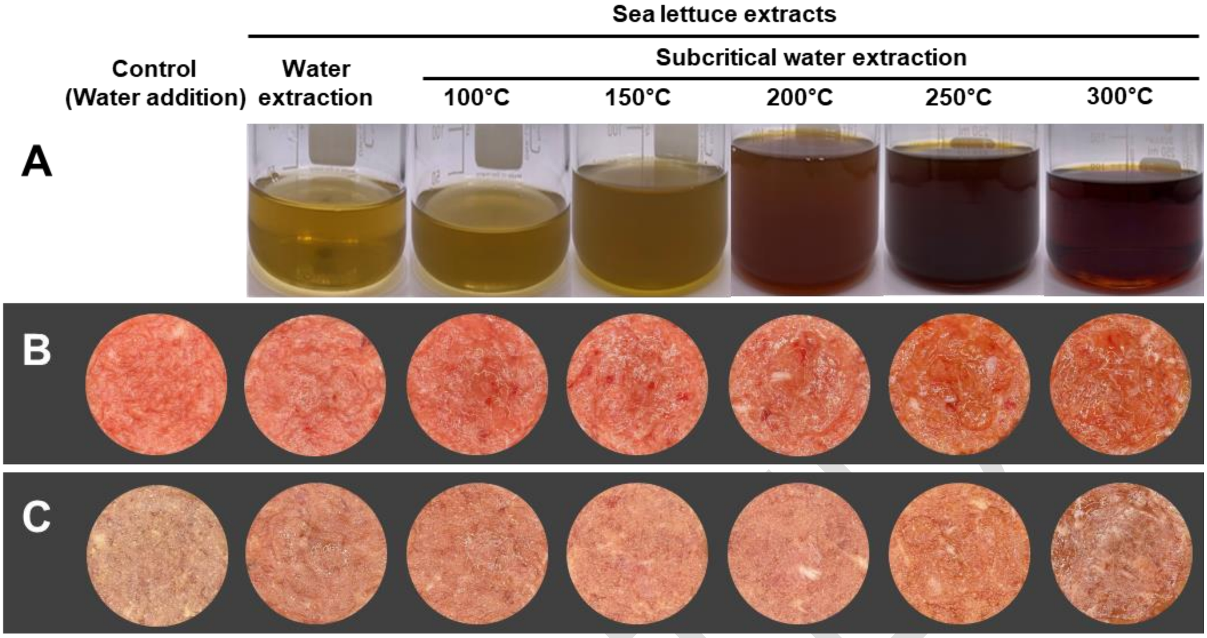
Fig. 1.

P

A

G

448



449

450 **Fig. 2.**

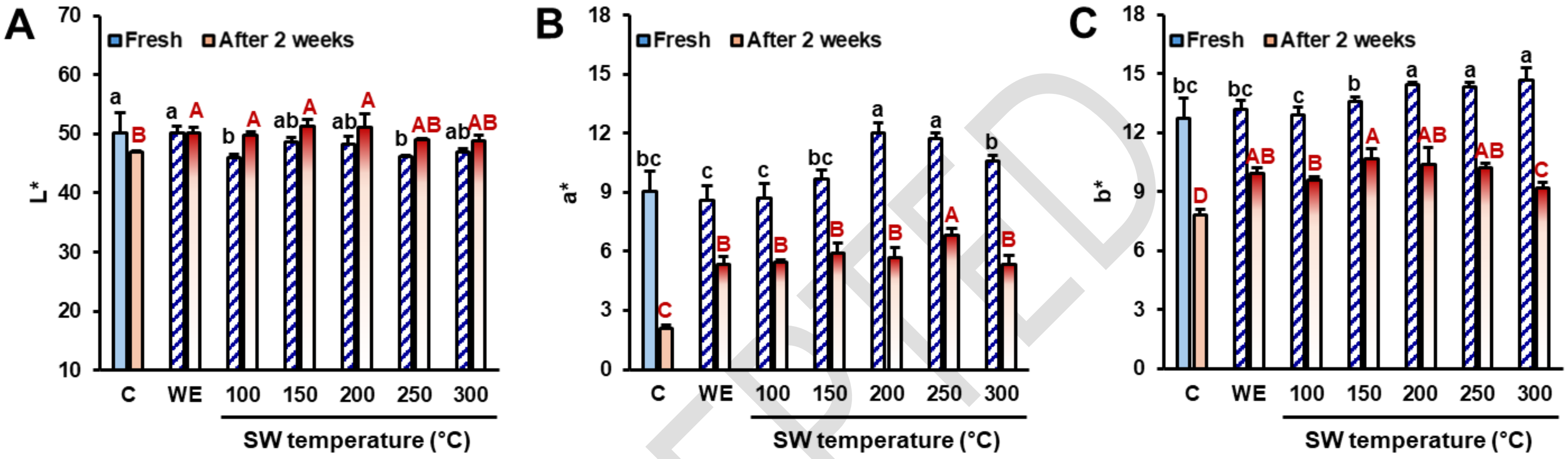

A
70
60
50
40
30
20
10
L*
■Fresh ■After 2 weeks
a
B
a
A
b
A
ab
A
ab
A
b
AB
ab
AB
C
WE
100
150
200
250
300
SW temperature (°C)
B
18
15
12
9
6
3
0
a*
■Fresh ■After 2 weeks
bc
C
c
B
c
B
bc
B
a
B
a
A
b
B
C
WE
100
150
200
250
300
SW temperature (°C)
C
18
15
12
9
6
3
0
b*
■Fresh ■After 2 weeks
bc
D
bc
AB
c
B
b
A
a
AB
a
AB
a
C
C
WE
100
150
200
250
300
SW temperature (°C)

Fig. 3.

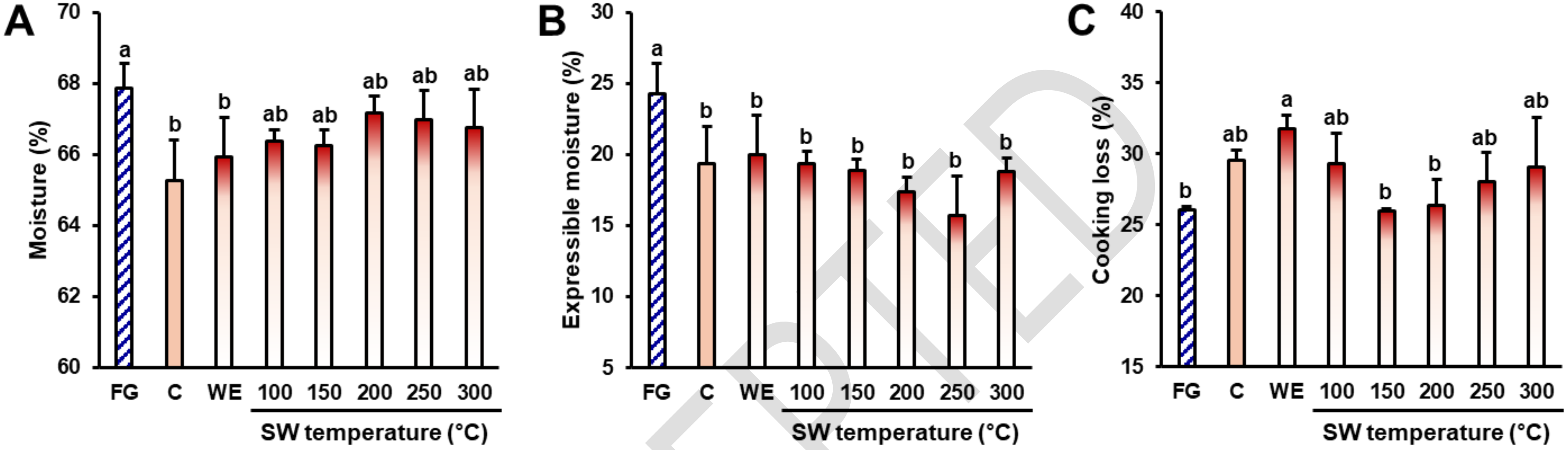

A
Moisture (%)
70
68
66
64
62
60
a
b
b
ab
ab
ab
ab
ab
FG
C
WE
100
150
200
250
300
SW temperature (°C)
B
Expressible moisture (%)
30
25
20
15
10
5
a
b
b
b
b
b
b
b
FG
C
WE
100
150
200
250
300
SW temperature (°C)
C
Cooking loss (%)
40
35
30
25
20
15
b
ab
a
ab
b
b
ab
ab
FG
C
WE
100
150
200
250
300
SW temperature (°C)

Fig. 4.

P

A

G

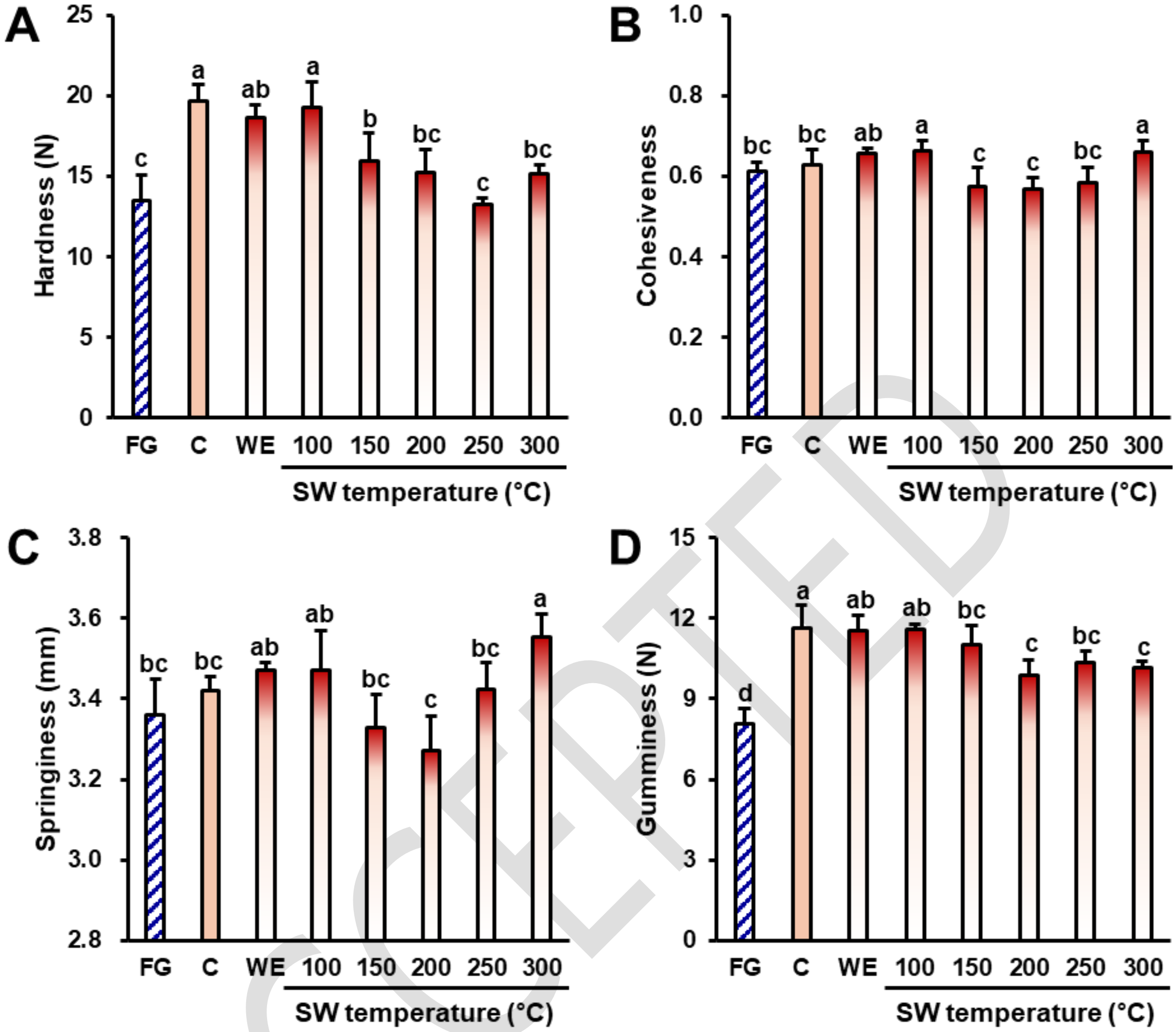

A
Hardness (N)
25
20
15
10
5
0
c
a
ab
a
b
bc
c
bc
FG
C
WE
100
150
200
250
300
SW temperature (°C)
B
Cohesiveness
1.0
0.8
0.6
0.4
0.2
0.0
bc
bc
ab
a
c
c
bc
a
FG
C
WE
100
150
200
250
300
SW temperature (°C)
C
Springiness (mm)
3.8
3.6
3.4
3.2
3.0
2.8
bc
bc
ab
ab
bc
c
bc
a
FG
C
WE
100
150
200
250
300
SW temperature (°C)
D
Gumminess (N)
15
12
9
6
3
0
d
a
ab
ab
bc
c
bc
c
FG
C
WE
100
150
200
250
300
SW temperature (°C)

Fig. 5.

P

A

G