

Antioxidant activity and quality of asparagus affected by microwave-circulated water combination and conventional sterilization

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Received 7 March 2005; received in revised form 28 October 2005; accepted 28 October 2005

Abstract

Green asparagus (*Asparagus officinalis* L.), as a healthy and perishable vegetable, is processed after harvest to minimize the deterioration of its physical and chemical quality. In our research, fresh asparagus was sterilized using several methods, including a pilot-scale 915 MHz microwave-circulated water combination (MCWC) heating system, pressured hot-water heating and steam-heating in a retort. Rutin content of asparagus processed by these methods did not show significant difference. But antioxidant activity of asparagus after MCWC treatment was significantly greater than that processed by other methods. Although the shear stress of asparagus spears was significantly decreased by eight times after sterilization, no significant difference existed among the textures of asparagus processed by these methods. Asparagus processed by MCWC showed greener colour with negative a^* value and greater hue value (H^0) than did hot water- and retort-treated asparagus. Therefore, asparagus sterilized by MCWC showed greater antioxidant activity and greener colour than did asparagus processed by the conventional methods.

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Keywords: Asparagus; Microwave-circulated water combination heating; Hot-water heating; Retort heating; Rutin; Antioxidant activity

1. Introduction

Green asparagus (*Asparagus officinalis* L.) is a healthy and nutritious vegetable, containing antioxidants, such as rutin, ascorbic acid, tocopherol, ferulic acid and glutathione. Rutin accounts for an important percentage of the antioxidant activity in asparagus (Tsushida, Suzuki, & Kurogi, 1994). Among 23 commonly consumed vegetables, antioxidant activity of asparagus, based on dry weight, has been ranked as the greatest (Vinson, Hao, Su, & Zubik, 1998). Reactive oxygen species (ROS), such as singlet oxygen (1O_2), hydroxyl radical ($\cdot OH$), superoxide anion ($O_2^{\cdot -}$) and peroxy radical ($R-OO\cdot$) can be generated from normal metabolism in the human body, and can cause DNA damage, cancer, cardiovascular disease and aging. Antioxidants

can reduce the damage of ROS to the human body. Therefore, intake of vegetables could significantly decrease the death rate of cardio- and cerebro-vascular diseases, immune dysfunction and cancer (Verlangieri, Kapeghian, el-Dean, & Bush, 1985).

Green asparagus has a short harvest season, between early April and late June in Washington State. Texture is an important quality index of asparagus, which deteriorates rapidly and becomes more fibrous after harvest with the development of a bitter flavour. It is, therefore, important to handle and process fresh asparagus quickly, to reduce the post-harvest losses and increase the economic return for farmers. Asparagus is preserved by canning, pickling, freezing and drying. In a traditional way, canned asparagus is sterilized by steam in the retort. Because asparagus is a low acid food with pH greater than 5.0 and water activity greater than 0.95, sterilization of asparagus is performed at 121 °C to inactivate the spoilage and pathogenic bacteria and spore-formers by destroying *Clostridium botulinum* and its spores as the main target

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(Harlfinger, 1992). The major goal of the sterilization process is to inactivate the spoilage components but minimize the quality deterioration of food; therefore, a high-temperature-short-time (HTST) process has been developed (Esteve, Frigola, Martorell, & Rodrigo, 1998). Thermal degradation rates of the desirable quality attributes of asparagus, such as nutrients, colour and texture, were lower than that of microbial spores at 121 °C. The limitation of the HTST process is that the slow heat-conduction rate can cause overheating at the solid surface when heat is transferred to the “cold-spot” of food. Microwave heating may overcome the limitation of conventional heating.

Microwave heating uses electromagnetic waves (from 300 MHz to 300 GHz) to generate heat in a material (Guan et al., 2003). Interaction of an electromagnetic field with food constituents, such as water and NaCl, causes molecular friction and excitation to generate heat rapidly. Microwave heating is of important interest to the food industry. Microwave heating is 3–5 times faster than conventional heating, therefore, it has the potential to improve product quality with the reduced heating time. Many studies have been undertaken to investigate nutrient properties of food treated by microwave, such as in blanching of asparagus (Begum & Brewer, 1997; Brewer, Begum, & Bozeman, 1995; Canet & Hill, 1987; Kidmose & Kaack, 1999), pasteurizing of pickled asparagus (Lau & Tang, 2002), inactivation of polyphenoloxidase in mushroom and lipoxygenase in soybean (Kermasha, Bisakowski, Ramaswamy, & Vandervoort, 1993; Rodriguez-Lopez et al., 1999). But, effect of microwave sterilization on food quality has been scarcely studied. In particular, no information is available on change of chemical and physical quality of asparagus during microwave sterilization.

The objective of our research was to compare the effects of microwave-circulated water combination, hot water and steam retort sterilization processes on antioxidant activity, rutin content, colour and texture of green asparagus spears.

2. Materials and methods

2.1. Preparation of asparagus spears

Green asparagus spears (*Asparagus officinalis* L. vs. Jersey Giant) were harvested from a local farm in Washington State and shipped to Washington State University overnight in a cooler packed with ice. Asparagus spears were stored at 4 °C for 4 days before performing the experiment. The length of asparagus spears was 5 in. after cutting the basal part. Asparagus was blanched in hot water at 91 °C for 2 min and immediately cooled in ice water.

2.2. Sterilization of asparagus spears by microwave-circulated water combination (MCWC) and hot-water heating

Blanched asparagus spears (200 g) were packed into the tray (14.0 cm × 10.0 cm × 2.5 cm) composed of polypropylene and EVOH (Rexam™ Union, MO, USA) and treated

with 39 g of water. A fibre-optic sensor was inserted into the asparagus spear in the tray with its tip located at the centre of the tray and was used to monitor the temperature of asparagus during heating. Then the tray was flushed with N₂ and vacuum-sealed before thermal treatment.

The pilot scale microwave-circulated water combination (MCWC) heating system included a 915-MHz microwave generating system, a multimode cavity, a pressurized microwave heating vessel and a water circulation heating and cooling system. A detailed description of the system can be found in a recent paper (Guan et al., 2003). Two asparagus trays can be treated at the same time using the microwave system. An overpressure of approximate 34 psig was provided by compressed air to maintain the integrity of the package during microwave heating. Circulation water was used to heat and cool the tray package. The process of heating and cooling in the microwave system was controlled by Think & Do™ computer software (Entivity, Ann Arbor, MI, USA). In the microwave system, asparagus spears were preheated to 75 °C with circulating water before microwave heating was combined with the circulation water of 120 °C. The sterilization process was used to inactivate *Clostridium botulinum* spores in asparagus. Asparagus was processed in the microwave oven with $F_0 = 3$ min (F_0 is the heating time at 121 °C that is required to inactivate microorganism with $Z = 10$ °C), which was equivalent to a 12D process assuming $D_{121,1} = 0.25$ min for *Clostridium botulinum* spores. After $F_0 = 3$ min and 121 °C was reached during microwave heating, microwave power was turned off and the asparagus trays in the microwave oven were cooled to 60 °C by the circulated water.

In the microwave system, only pressurized hot circulating water was used to sterilize packed asparagus without turning on microwave power for hot-water heating. After 121 °C and $F_0 = 3$ were reached for asparagus, the trays were cooled immediately by the circulating water.

2.3. Steam-heating of canned asparagus in a retort

Blanched asparagus spears (about 200 g) were packed in the electrolytic tinplate can (211 × 400) with their tips end-down. About 290 ml of brine (2.5% NaCl in water) were heated to 90 °C and poured into the cans. The cans were sealed immediately and placed in the pilot scale retort for thermal treatment. Processing time and temperature in a still retort were selected according to a published commercial procedure for canned asparagus (Lopez, 1987). During steam heating, the temperature in the retort was increased to 121 °C quickly, maintained at 121 °C for 17 min and immediately cooled to room temperature by cold circulating water in 10 min.

2.4. Analysis of chemical and physical quality of asparagus

2.4.1. General

After thermal treatment, asparagus spears and brine solution in the tray or can were separated to analyze their

antioxidant activities and rutin contents. Colour and texture of processed asparagus spears was also analyzed. Commercially canned asparagus (Seneca Foods, Dayton, WA, USA) were also purchased from a local supermarket to analyze its chemical and physical quality. The procedures of analysis are given below.

2.4.2. Analysis of rutin content by HPLC

Asparagus spears (20 g), after a thermal treatment, were homogenized with 4 volumes of methanol and centrifuged by a Beckman J2-HS centrifuge (Beckman, Palo Alto, CA, USA) at 26,712g for 15 min. Rutin content in the supernatant of asparagus extract was determined by HPLC. Agilent 1100 HPLC (Palo Alto, CA, USA) included a quaternary pump, vacuum degasser, thermostatic column compartment and a diode array detector. A Vydac 201TP (50 mm × 4.6 mm i.d., 5 μm particle size) guard column (Columbia, MD, USA) and an Agilent Eclipse XDB-C8 column (150 mm × 4.6 mm i.d., 5 μm particle size) were maintained at 40 °C for separation. The mobile phase included 5% acetic acid, 40% methanol and 55% water and the flow rate was 0.8 ml/min. The diode array detector was set at 280 and 360 nm for determination of rutin content.

2.4.3. Determination of antioxidant activity of asparagus by DPPH method

Antioxidant activity of asparagus extract was determined according to Brand-Williams, Cuvelier, and Berset (1995). DPPH solution of 0.75×10^{-4} M was prepared in ethanol. Aliquots of asparagus extract were added to 1 ml DPPH solution and the absorbance of the DPPH solution was determined at 515 nm after 30 min by an Ultrospec 4000 UV/vis spectrophotometer (Pharmacia Biotech, Cambridge, England). The reduction of the absorbance (inhibition %) for DPPH reagent was calculated according to the following equation:

$$\text{Inhibition \%} = (\text{Abs}_{t=0} - \text{Abs}_{t=30 \text{ min}}) / \text{Abs}_{t=0} \times 100,$$

where $\text{Abs}_{t=0 \text{ min}}$ was the absorbance of DPPH reagent at 0 min and $\text{Abs}_{t=30 \text{ min}}$ was the absorbance of DPPH reagent after 30 min.

The inhibition percentage of absorbance was plotted versus the amount of asparagus sample to obtain a regression line. The Trolox equivalent antioxidant activity (TEAC) was calculated by the ratio between the slopes of the sample and Trolox.

2.4.4. Instrumental measurement of colour

L^* , a^* , and b^* values of thermally-treated asparagus spears were determined by a Minolta colorimeter (Minolta Spectrophotometer CM-2002, Minolta Camera, Osaka, Japan) (Lau, Tang, & Swanson, 2000). Hue (H^0) and chroma (C^*) were calculated according to the following equations (McGuire, 1992; Voss, 1992):

$$H^0 = \tan^{-1}(b^*/a^*) \text{ when } a^* > 0 \text{ and } b^* \geq 0 \text{ and}$$

$$H^0 = 180 + \tan^{-1}(b^*/a^*) \text{ when } a^* < 0, \quad C^* = (a^{*2} + b^{*2})^{1/2}.$$

2.4.5. Instrumental measurement of texture

Texture of asparagus was measured using a TA.XT2 Texture Analyzer (Texture Technologies, Scarsdale, NY, USA; Stable MicroSystems, Godalming, Surrey, UK). The analyzer was equipped with a single blade (10 cm × 0.3 cm) and a test cell (8.8 cm × 10 cm), and monitored by computer software (Lau et al., 2000). After thermal treatment, asparagus spears with similar size were selected for texture analysis and the diameter at the middle of the asparagus spear was measured using a caliper. Asparagus was cut across the middle part of the spear with the blade at a speed of 1.0 mm/s. The maximum shear force required to cut across the asparagus was calculated according to the following equation (Lau et al., 2000):

$$S_m = F_m / [2 \times (\pi)D^2/4],$$

where S_m is the maximum shear stress (Pa), F_m is the measured maximum force (N) that cut cross the asparagus spears and D is the diameter (m) at the middle of the asparagus spear.

2.5. Statistical analysis

Rutin content, antioxidant activity, texture and colour analyses of asparagus were performed in triplicates. The average values and standard deviations of the results were calculated using Excel (Microsoft Inc., Redmond, WA, USA). The significant difference ($p < 0.05$) of the data were analyzed by one-way ANOVA and multiple comparisons (Fisher's least-significant-difference test) using SYSTAT (Systat Software Inc., Point Richmond, CA, USA) (Zar, 1996).

3. Results and discussion

3.1. Temperature–time profiles of MCWC and hot-water heating

The temperature–time histories of asparagus during MCWC and hot-water heating are shown in Figs. 1 and 2, respectively. The two heating processes had the same

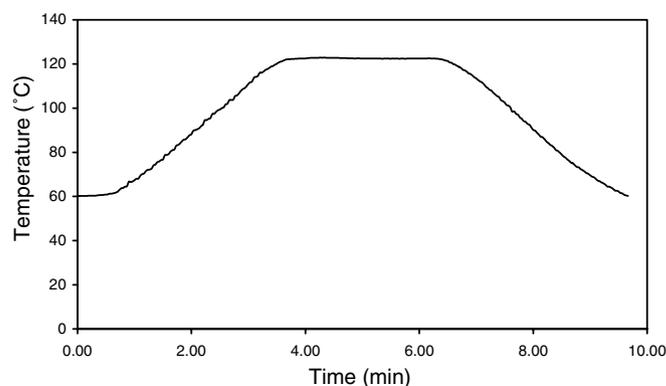


Fig. 1. Temperature–time profile of asparagus during the treatment of MCWC.

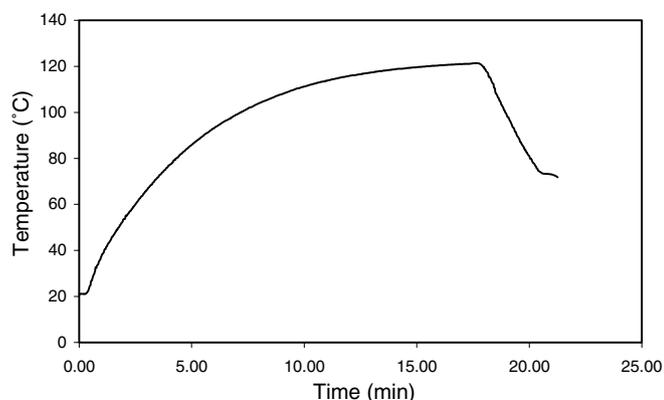


Fig. 2. Temperature–time profile of asparagus heated with pressured hot water.

F_0 value. The heating process of MCWC included four stages: preheating, combined heating, holding and cooling, totally 10 min, which was shorter than the time of hot-water heating of 22 min. The temperature of asparagus in the tray reached 121 °C in 3.6 and 18 min for MCWC and hot-water heating, respectively. Thermal degradation of food quality is affected by the time–temperature history and the kinetics of changes in a particular food component (Datta & Hu, 1992). Due to the rapid increase of temperature during microwave heating, neither nutrients nor bacteria were significantly destroyed during the come-up time (Datta & Hu, 1992). Once 121 °C was reached and asparagus was held at that temperature, the lethality of asparagus was rapidly accumulated.

3.2. Rutin content and antioxidant activity of asparagus affected by thermal treatments

Rutin content of blanched asparagus was 0.45 ± 0.05 mg/g wet weight, which was within the range of the reported value of 0.02–0.1% (w/w) in canned asparagus (Fuleki, 1999). Rutin content of asparagus did not decrease significantly after sterilization. No significant difference of rutin content was found for asparagus processed by the three sterilization methods (Table 1). The results showed that rutin was a relatively heat-stable antioxidant compared to ascorbic acid in asparagus. Commercially canned asparagus contained 0.16 ± 0.01 mg/g wet weight of rutin, which was only 42% of that in MCWC-processed asparagus.

Antioxidant activity of MCWC treated asparagus was not significantly different from that of blanched asparagus,

Table 1
Rutin content in asparagus after different thermal treatments (means \pm SD, $n = 3$)

Processed asparagus	mg/g wet weight
Blanched	$0.45 \pm 0.05b$
MCWC	$0.38 \pm 0.11b$
Hot water	$0.32 \pm 0.08b$
Retort	$0.33 \pm 0.06b$
Commercial can	$0.16 \pm 0.01a$

The data with different letters (a,b) are significantly different ($p < 0.05$).

Table 2
Antioxidant activities of asparagus after different thermal treatments (means \pm SD, $n = 3$)

Asparagus	$\mu\text{mol trolox equivalents/g wet weight}$
Blanched	$2.87 \pm 0.23c$
MCWC	$3.24 \pm 0.26c$
Hot water	$2.52 \pm 0.33b$
Retort	$2.29 \pm 0.24b$
Commercial can	$1.06 \pm 0.05a$

The data with different letters (a–c) are significantly different ($p < 0.05$).

but 28–41% greater than that of hot water- and steam retort-treated asparagus (Table 2). There was no significant difference of antioxidant activity between hot water- and steam retort-treated asparagus. Antioxidant activity of commercially canned asparagus was only 31% of that in MCWC-treated asparagus. Therefore, MCWC sterilization showed advantage in maintaining the antioxidant activity of asparagus. There was a significant correlation between rutin content and antioxidant activity of processed asparagus ($n = 15$, $R = 0.87$, $p < 0.05$), suggesting that rutin plays a key role in the antioxidant activity of asparagus (Fig. 3).

Rutin in asparagus was dissolved in the brine during heating and the antioxidant activity of the brine was increased. The obtained brines of MCWC-, hot water-, retort-treated and commercially-canned asparagus were 17, 17, 251 and 198 ml, respectively. Rutin contents and antioxidant activities of brine for MCWC- and hot water-treated asparagus were similar and greater than those of retort-treated and commercially-canned asparagus (Figs. 4 and 5). The reason was probably that a greater volume of brine for asparagus processed in a can than that in a tray diluted the rutin content and decreased the antioxidant activity of brine. In fact, the total rutin amount dissolved in brine was 6% of that in asparagus spears for MCWC- and hot water treatments, which was significantly smaller than

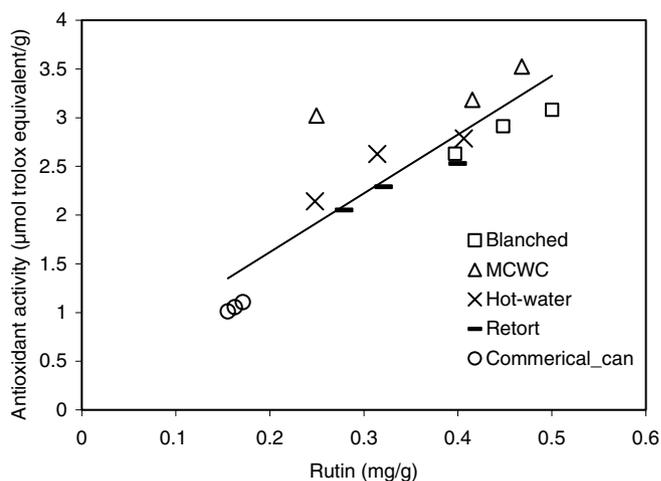


Fig. 3. The relationship between rutin content and antioxidant activity of blanched, MCWC-, hot water- and retort-treated and commercially-canned asparagus. The equation of the straight line is: $Y = 6.03X + 0.41$ ($R = 0.87$, $p < 0.01$), X is the concentration of rutin, Y is the antioxidant activity of asparagus.

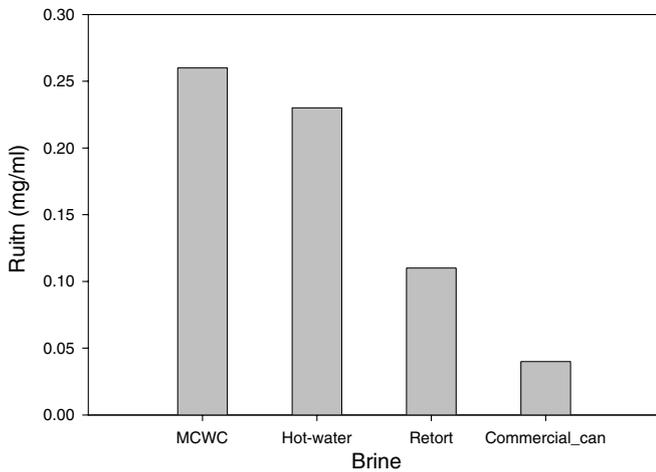


Fig. 4. Rutin content in the brine of asparagus processed by different methods.

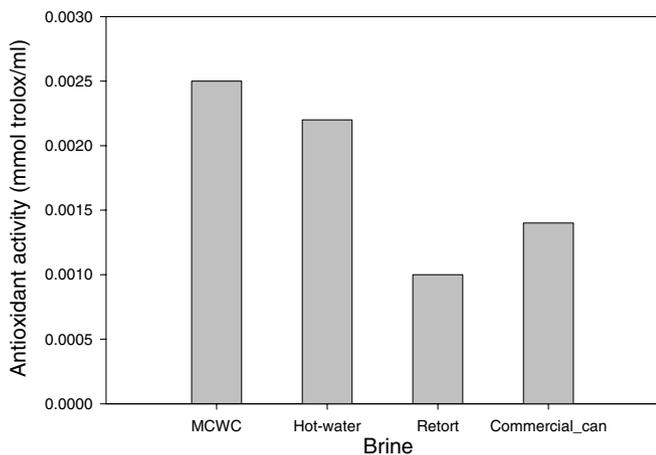


Fig. 5. Antioxidant activity of brine for asparagus processed by different methods determined by DPPH method.

the 42% for retort-treated asparagus (Fig. 6). The EVOH and polypropylene tray used in MCWC showed advantage in reducing the loss of rutin content and antioxidant activity of asparagus, because small amount of brine were added to the tray and microwave heating had a fast heating rate. Effect of thermal processing on antioxidant activity of vegetables has been investigated and the change of antioxidant activity varies with the food. For example, thermal processing increased antioxidant activity of tomatoes and sweet corn (Anese, Manzocco, Nicoli, & Lerici, 1999; Dewanto, Wu, Adom, & Liu, 2002; Dewanto, Wu, & Liu, 2002). Antioxidant activity of canned beet did not change significantly after heating. Antioxidant activity of canned green bean was decreased by 20% during commercial processing conditions (Jiratanan & Liu, 2004).

3.3. Colour of asparagus affected by thermal treatments

Green colour is an important quality characteristic of asparagus products. L^* is the degree of lightness with value

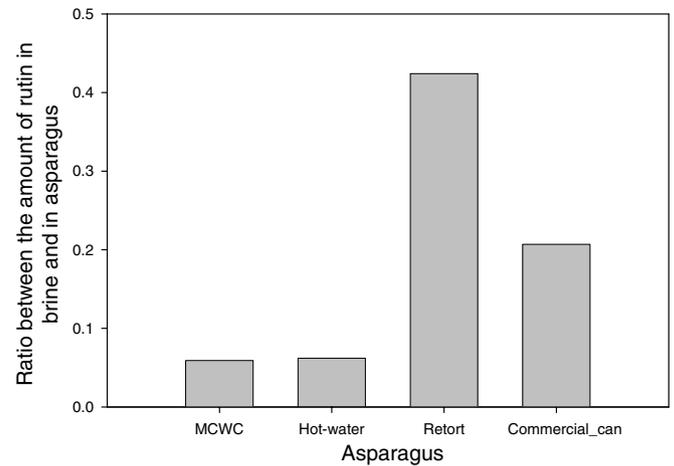


Fig. 6. Ratio between the amount of rutin dissolved in the brine and in asparagus after different thermal processings.

from zero for black to 100 for white, a^* indicates degree of greenness and redness (the more negative the more green and the more positive the more red), b^* indicates the degree of blue and yellow (the more negative the more blue and the more positive the more yellow). L^* , a^* and b^* values of blanched asparagus in our research were in the same range as previously reported data (Begum & Brewer, 1997). L^* of MCWC-treated asparagus was not significantly different from that of blanched asparagus. However, L^* of hot water- and retort-treated asparagus was significantly decreased after heating and the colour became darker (Fig. 7). Commercially canned asparagus had a greater value of L^* , showing a lighter colour than that of blanched asparagus in this research. Negative a^* for MCWC-treated asparagus indicated a green colour, although less green after processing. Positive a^* for hot water- and retort-treated asparagus indicated a less green and more red colour. Therefore, asparagus sterilized by MCWC showed a greener colour than that sterilized by hot water and retort treatment. Yellowness was significantly increased after thermal treatment with increased b^* value. MCWC-treated

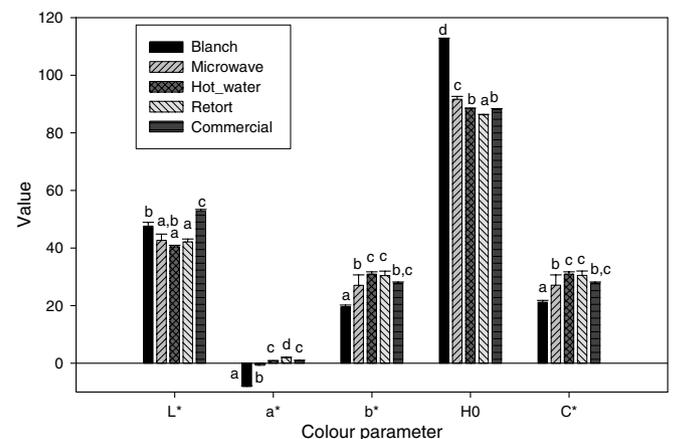


Fig. 7. The values of colour parameters (L^* , a^* , b^* , H^0 and C^*) of asparagus after different thermal treatments (means \pm SD, $n = 3$). The data with different letters (a–d) are significantly different ($p < 0.05$).

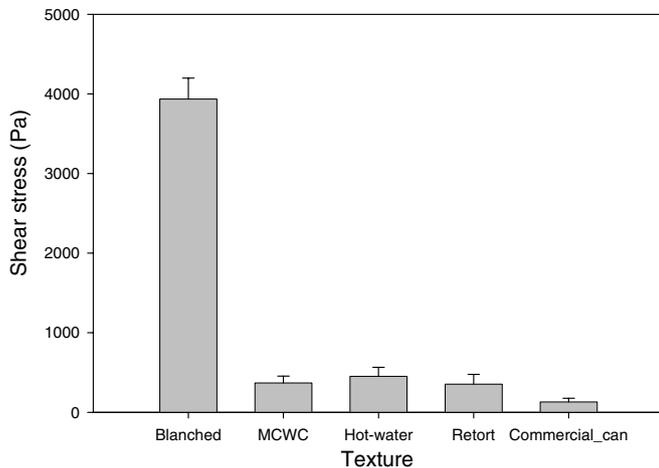


Fig. 8. Shear stress of asparagus texture after different thermal treatments (means \pm SD, $n = 3$). The data with different letters (a–c) are significantly different ($p < 0.05$).

asparagus showed less yellowness than hot water- and retort-treated asparagus. After blanching, an initial brightening of the green colour of asparagus was observed, indicating a decrease in the intensity of greenness and an increase in yellowness. After sterilization, H^0 value of asparagus was significantly decreased, which agreed with previous research that prolonged heating of asparagus caused deterioration of the chlorophyll pigments and the colour was changed from green to olive green (Lau et al., 2000). However, MCWC-treated asparagus showed significantly greater H^0 value and a better maintenance of green colour than did other treatments. C^* value of MCWC-treated asparagus was significantly greater than that of blanched asparagus, but was significantly smaller than that of hot water- and retort-treated asparagus (Fig. 7).

3.4. Texture of asparagus affected by thermal treatments

Fresh asparagus has a favourable crispy texture. However, the texture of asparagus became much softer after sterilization with the shear stress decreased by more than eight times (Fig. 8). Degradation of middle lamella and cell wall pectic substances during thermal processing caused the loss of firmness of asparagus texture. There were no significant difference among the shear stresses of MCWC-, hot water- and steam retort-treated asparagus. The shear stress of commercially canned asparagus was the smallest among the four types of asparagus products. Although pickled asparagus, pasteurized by MCWC treatment, showed a more crispy texture than did asparagus processed by pressurized hot-water heating, MCWC-sterilization did not show any advantage in maintaining the texture of asparagus (Lau & Tang, 2002).

4. Conclusion

MCWC-sterilized asparagus showed no significant differences in rutin content and texture from hot water- and

retort-treated asparagus. MCWC-treated asparagus showed greater antioxidant activity and greener colour than did asparagus heated by hot water and steam retort. Therefore, MCWC sterilization showed advantage over conventional heating in processing asparagus.

Acknowledgements

We sincerely thank Dr. Frank Liu in the Department of Biological Systems Engineering at Washington State University for assistance in MCWC processing of asparagus. We also thank the USDA Cooperative State Research, Education and Extension Service (CSREES) for providing the grant for this research.

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