THE EFFECT OF STEAMABLE BAG MICROWAVING AND STEAMABLE BAG DESIGN ON NUTRITIONAL PRESERVATION AND PHYSICAL QUALITY OF FROZEN BROCCOLI

By

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ABSTRACT

THE EFFECT OF STEAMABLE BAG MICROWAVING AND STEAMABLE BAG DESIGN ON NUTRITIONAL PRESERVATION AND PHYSICAL QUALITY OF FROZEN BROCCOLI

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This study aims to evaluate the effect of steamable bag microwaving and steamable bag designs on the nutritional value and physical properties of frozen broccoli. The results show that steamable bag microwaving performs better than traditional microwaving and is equal to steamer steaming in retaining ascorbic acid content and increasing antioxidant activity compared to thawed frozen broccoli. It tenderizes frozen broccoli faster and better maintains the broccoli's lightness and yellowness as compared to steamer steaming and traditional microwaving. These findings support that steamable bag microwaving is a cooking method that increases nutritional content, tenderizes at a quicker rate and produces minimal color changes in frozen broccoli, which satisfies current consumers' needs. Most of the studied parameters are more affected by the shape of steamable bags than by the surface area. Frozen broccoli cooked in more square-shaped steamable bags retains significantly higher ascorbic acid content, is less green and firmer than that cooked in more rectangular-shaped steamable bags. The smaller the surface area, the softer the broccoli became after cooking. Neither the shape nor the surface area of steamable bags had an effect on the lightness or the antioxidant capacity of the broccoli. These results demonstrated the importance of controlling the surface area and shape of steamable bags to optimize the qualities of frozen broccoli.

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1. Introduction

Numerous scientific evidence supports the relationship between the increase in the consumption of dietary antioxidants to the decrease in chronic illnesses (Bengtsson et al., 2006; Eberhardt, Kobira, Keck, Juvik & Jeffery, 2005; Wu et al., 2004). Vegetables are the major sources of dietary antioxidants (Kurilich, Jeffery, Juvik, Wallig & Klein, 2002). Carotenoids, fat-soluble vitamins (such as tocopherol), water-soluble vitamins (such as Vitamin C), and many phenolic compounds together contribute to the total antioxidant capacity (Kalt, 2005; Wu et al., 2004). Consumption of frozen vegetables can not only fulfill the needs of health benefits as fresh vegetables but also provides consumers with bonus convenience such as time saving, easy storage and longer shelf life. According to USDA report (2005), approximately 44 to 46.2 kg of frozen vegetables, not including frozen potatoes, has been consumed annually per person in the United State for the past 20 years. Broccoli is one of the most commonly consumed frozen vegetables because of its desired flavor and odor as well as high content in health-promoting compounds. Americans consumed approximately 1.23 kg of frozen broccoli per person during 2005. Frozen vegetables are typically cooked prior to consumption which the concentration of nutrients (i.e. ascorbic acid), their activity (antioxidant capacity) and sensory quality (i.e. color, firmness) may change through cooking practices (Wu et al, 2004). Although the effect of typical cooking methods (i.e. boiling, steaming and microwaving) on the quality of frozen vegetables has been widely investigated, the most popular cooking method for frozen vegetables, steamable bag microwaving is lack of study. Therefore, the objectives of this study are: (1) to investigate the effect of steamable bag microwaving on the nutritional value and physical properties of frozen broccoli compared to those thawed at room temperature (uncooked frozen broccoli); (2) to compare the effect of steamable bag microwaving versus steamer steaming and traditional

microwaving on the nutritional value and physical properties of frozen broccoli; and (3) to investigate how the package design (shape and surface area) of a steamable bag containing a steam release valve affects the nutritional value and physical properties of frozen broccoli during microwaving.

2. Literature Review

2.1 Broccoli

Broccoli is a member of the Brassicaceae family and belongs to the species *Brassica oleracea* (Wildman, 2001), which are recommended to provide better health benefits against cancer and cardiovascular disease than many other vegetables according to recent epidemiological studies (WCRF, 2007). Broccoli grows best in cool climates; therefore, in the United States of America the majority of broccoli is grown in cool coastal areas of California generally during the winter and early spring. The Calabrese variety and the Italian variety are the two predominate types of broccoli. Calabrese is more common in the United States of America, and the Italian variety is more common in Great Britain and other regions in Europe (Wildman, 2001). Broccoli did not become a largely consumed vegetable in the United States until the 1970's (Wildman, 2001).

2.1.1 Frozen Broccoli

Longer preparation time is required for fresh vegetables which do not fit for current consumers' need for convenience and time saving, therefore, the consumption of frozen vegetables rapidly increased in recent years (Danesi & Bordoni, 2008). Frozen vegetable is a product that has been undergone to different processes (e.g., blanching and quick freezing). Balancing is a process of exposing vegetables or fruits to high temperatures for a short period. This process not only prolongs the shelf life of vegetables by inactivating the enzymes responsible for browning (Nielsen, Larsen, & Poll, 2004), such as polyphenoloxidase, lipoxygenase and peroxidase, but also improves both color and flavor. Broccoli is one of the most consumed frozen vegetables over the past 10 years in the United States (USDA, 2005),

which is widely considered to contain high level of health benefit phytochemicals including glucosinolates, flavonoids, vitamins, and minerals (Cao, Sofic, & Prior, 1996; Plumb et al., 1996).

The quality of frozen vegetables is defined in the United States Standards for Grades with color and texture as two of the common quality attributes assigned to broccoli. The color of Grade A frozen broccoli should be "reasonably good". The color of the vegetables is important since many of the natural colorants are either precursors to antioxidants or contain antioxidant capacity themselves. Texture of Grade A frozen broccoli should be a "tender texture". The texture in vegetables consumed by today's consumers should be maintained rather than cooked to a very soft final product. Microwaving for approximately 6-8 min per pound or boiling for approximately 3-10 min per pound are recommended on most commercial packages of these vegetables sold today.

2.2 Nutritional Values

2.2.1 Ascorbic Acid

Vitamin C, a water-soluble vitamin, which includes ascorbic acid and its oxidation product-dehydroascorbic acid (DHA), has many biological activities in human body. More than 85% of vitamin C in human diets is supplied by fruits and vegetables, such as citrus fruits, broccoli and tomatoes (Davey et al., 2000; Lee & Kader, 2000). Ascorbic acid is well known for its antioxidant activity, acting as a reducing agent to reverse oxidation in liquids. Biological function of ascorbic acid can be defined as an enzyme cofactor, a radical scavenger, and as a donor/acceptor in electron transport at the plasma membrane. Ascorbic acid is able to scavenge the superoxide and hydroxyl radicals, as well as regenerate α-tocopherol (Davey et al., 2000).

The oxidation product of ascorbic acid, dehydroascorbic acid (DHA), is unstable at physiological pH and it is spontaneously and enzymatically converted to 2,3-diketogulonic acid (Davey et al., 2000).

2.2.2 Antioxidant Capacity

Antioxidants have gained lots of studies due to their potential effects in the prevention of chronic and degenerative diseases such as cancer and cardiovascular disease as well as aging (Diaz, Frei & Keaney, 1997; Ames, Shigenaga & Hagen, 1993; Ames, Gold & Willet, 1995; Young & Woodside, 2001). These health promotion effects may be related to components in the foods with antioxidant activity (Kaur & Kapoor, 2001). The ability of antioxidants to scavenge free radicals in the human body and thereby decrease the amount of free radical damage to biological molecules like lipids and DNA may be one of their protective mechanisms. Since there are hundreds of antioxidant compounds in most foods, the total antioxidant capacity of a given food may be the integrated action from different compounds instead of that from any single compound. The concept of total antioxidant capacity reflects the integrated and, if any, synergic effects of all the antioxidants. In order to evaluate the total antioxidant capacity of a given food and their health promotion effects accurately, we need to consider the possible interaction of components in their contribution to antioxidant activity. In general, components in plants can be divided into two fractions, hydrophilic and lipophilic.

2.2.2.1 Hydrophilic Antioxidants

Hydrophilic antioxidants are water-soluble antioxidants including vitamin C, phenolic compounds and others. Hydrophilic antioxidants are the main contributors to its total antioxidant

capacity in previous studies for broccoli. For example, hydrophilic extracts are responsible for 80% to 95% of the total antioxidant capacity of fresh broccoli using the ORAC assay reported by Kurilich, Jeffery, Juvik, Wallig and Klein (2002). Similarly, Wu, Beecher, Holden, Haytowitz, Gebhardt and Prior (2004) stated that hydrophilic antioxidants in *Brassica* vegetables provide more than 89% of the total antioxidant capacity while Roy, Juneja, Isobe and Tsushida (2009) found that 92% of the total antioxidant capacity of broccoli is provided by its hydrophilic extract. Most popular in vitro antioxidant measurement methods are designed primarily for hydrophilic components, and may not be suitable or adaptable for lipophilic measurements.

2.2.2.2 Lipophilic Antioxidants

Lipophilic antioxidants are lipid-soluble antioxidants including carotenoids, vitamin E and others. In order to obtain a good measurement of total antioxidant capacity for a given food, Cano, Acosta and Arnao (2000) and Arnao, Cano and Acosta (2001) suggested that separating lipophilic components from that of the hydrophilic components using similar chemical principles. The original oxygen radical absorbance capacity (ORAC) assay and the modified $ORAC_{FL}$ methods were developed using a hydrophilic environment (Cao et al., 1993, 1995; Ou, Huang, Hampsch-Woodill, Flanagan & Deemer, 2002). However, it has proven to be adaptable for lipophilic antioxidants as well. Recently, Huang, Ou, Hampsch-Woodill, Flanagan & Deemer (2002) developed a lipophilic $ORAC_{FL}$ measurement method that employed randomly methylated b-cyclodextrin (RMCD) as a solubility enhancer. This allowed for the measurement of the antioxidant capacity of lipophilic and hydrophilic components for a given sample separately, but based on the same peroxyl free radical. The $ORAC_{FL}$ method has the advantage that it combines the inhibition degree and time of inhibition into one value.

2.2.2.3 Oxygen Radical Absorbance Capacity assay

The use of different assays by research groups can result in reporting varying amounts of antioxidants found in various food products. Each assay typically employs a different free radical to use as the standard. The ability of the antioxidants to react with the free radical affects the antioxidant capacity. Oxygen radical absorbance capacity (ORAC) assay depends on the free radical (AAPH generated) damage to fluorescein. The degree of the change of fluorescent intensity indicates the amount of radical damage. The presence of antioxidants results in the inhibition in the free radical damage to the fluorescent compound. This inhibition is observed as a preservation of the fluorescent signal calculated the area-under-curve (AUC) while the results are expressed as Trolox equivalents (Prior & Cao, 1999). ORAC assay can be used for testing hydrophilic and lipophilic antioxidants. The area-under-curve (AUC) calculation combines both inhibition percentage at fixed time and the length of inhibition time of free radical action by an antioxidant into a single quantity that provides high specificity (Cao & Prior, 1998). Wu et al. (2004) found that uncooked broccoli contains 15.9 μM Trolox equivalent/g when assayed used ORAC and cooked broccoli decreased in total antioxidant capacity to a value of 12.59 μM TE/g.

2.3 Physical Quality

2.3.1 Firmness

Plant-based foods are subjected to cooking or processing to increase their edibility and palatability, especially for frozen vegetables. Once people get used to the characteristics of a food, they expect to consume similar texture when they eat it again. Changes in nutrients are excluded because they are usually not apparent to the person eating the food but change in firmness will. Firmness of food may be considered as the combined effect of mechanical

properties and behavior perceived in the mouth, which is affected by both the cell wall and then cell contents (Brown, 1977). The turgor or the internal pressure of the living cell is considered as a very important aspect of the texture of raw fruits and vegetables. Maintenance of this pressure is a function of the integrity of the semi-permeable membrane between the cell wall and the remainder of the cell contents (Brown, 1977). When the cell membrane has been damaged by cooking, water and soluble substances leak out of the cell and the rigidity of the tissues is lost. In fruits, salad greens, leafy vegetables, and other thin-walled tissues, this loss of turgor pressure causes a major change in textural characteristics. Stems, roots, and seed pods contain specialized cells that support and protect the growing plant. These can provide firmness or even crispness in the absence of turgor. Fruits that are heated in canning or in the preparation of a cooked dish, loss their crispness because the cell membranes are damaged to the extent that they are no longer able to retain the cellular fluid. In contrast to fruits, many vegetables have a crisp or firm texture after cooking because their cells have relatively thick walls. Although heating eliminates the contribution of turgor to the texture of vegetables, it causes only gradual softening of the thick cell walls. When the desired softening has been attained, the cooking is considered 'done', and the degree of crispness may still be present. Therefore, since most of vegetables are cooked before consumption, cell turgor is not a factor in their texture. So the 'proper' firmness of cooked vegetable can be achieved by a suitable cooking method including proper temperature, time and pressure, etc.

2.3.2 Color

Cooking or heat treatments have variable effect on pigments, such as chlorophyll, carotenoids and anthocyanins, which are responsible for the color of fruits and vegetables.

Chlorophyll is a green pigment found in the leaves and green stems of plants, which has different stabilities towards pressure and temperature. Butz et al. (2002) reported that a significant reduction in the chlorophyll content of broccoli juice at temperature higher than 50℃. At a constant pressure level, the values of the degradation rate constants of chlorophylls increase with increasing temperature (Van Loey et al., 1998) whereas at constant elevated temperatures, pressure increase accelerates the degradation of chlorophyll. Turkmen, Poyrazoglu, Sari and Velioglu (2006) found that the loss of greenness in vegetables after cooking could be attributed to the degradation of chlorophyll along with the formation of pheophytins. However, the increase in green color intensity of broccoli at the early stage of blanching was related to cell disruption during blanching treatment which resulted in the leakage of chlorophyll into the intercellular space yielding a more intense bright green color on the vegetable surface (Oey, Lille, Loey & Hendrickx, 2008).

Besides, structure and pigmentation of food interact with each other to affect both color and translucency/opacity. Firmness modification may result in changes in the nature and extent of internally scattered light and the distribution of surface reflectance, which in turn may produce changes of color appearance more than the effect of pigment concentration changes.

2.4 The Effect of Cooking Treatments on Broccoli

Most vegetables, especially frozen samples, are commonly cooked before being consumed. It is known that cooking induces significant changes in chemical composition, affecting the bio-accessibility and the concentration of nutrients and health-promoting compounds such as vitamin C, carotenoids, and polyphenols (Pellegrini et al., 2010).

Domestic cooking methods, including high pressure, microwaving and boiling, reduced vitamin C (AA and DHAA) content of broccoli between 20% and 46% except for steaming which no loss was found (Vallejo, Tomas-Barberan & Garcia-Viguera, 2002). Galgano, Favati, Caruso, Pietrafesa and Natella (2007) reported that boiling and steaming caused vitamin C losses of 34.2% and 22.4% in broccoli, respectively, while pressure cooking and microwaving conversely did not induce significantly loss of vitamin.

Bernhardt and Schlich (2006) evaluated the influence of different domestic cooking method (i.e. boiling, stewing, steaming, pressure steaming and microwave) on ascorbic acid content in fresh and frozen broccoli and found that all cooking methods caused small losses of ascorbic acid except boiling leaded to high losses. Zhang and Hamauzu (2004) reported that only 35% of antioxidant capacity in broccoli was retained while up to 70% of ascorbic acid lost after boiling and microwaving for 5 minutes. It is noteworthy that the process used to microwave the vegetables in the study by Zhang and Hamauzu (2004) was essentially the same as boiling since the broccoli was placed in 200 ml of boiled water and then cooked in the microwave oven. Both, microwaving and conventional cooking of broccoli with water, have been shown to decrease in antioxidant components and activity in broccoli (Gliszczynska-Swigo et al., 2006; Lopez-Berenguer, Carvajal, Moreno, & Garcia-Viguera, 2007). These findings indicated that loss of nutritional values due to nutrients being largely leached into the cooking water.

On the contrary, Turkmen, Sari, and Velioglu (2005) found that the total antioxidant capacity of fresh broccoli increased up to 17% after cooking. This increase was the same during cooking by all boiling, steaming and microwaving. Wachtel-Galor, Wong and Benzie (2008) reported the increased tendency of antioxidant capacity in broccoli after cooking while the antioxidant capacity of broccoli cooked by microwaving was about 30% lower than those cooked

by boiling but only approximately 30% -50% compared to those under steaming. The enhanced antioxidant capacity of broccoli after cooking may relate to the softening of matrix structure which improves the antioxidants extractability and formation of new antioxidant compounds such as Maillard-reaction products (Turkmen, Sari, & Velioglu, 2005). Miglio, Chiavaro, Visconti, Fogliano and Pellegrini (2008) stated that cooking treatments including boiling, steaming and frying induced softening for broccoli. In their study, broccoli cooked by steaming and frying became less green (-a* increased) while a significant increase in greenness was found in the boiled broccoli. The prolonged heating time in steaming may induce more chlorophyll degradation and then cause a decrease in greenness compared to the shorter cooking time used in boiling. Besides, oil and extreme high temperature in frying may change the light scattering and reflectance of green surfaces which leaded to loss of greenness. Pellegrini et al. (2010) claimed that fresh broccoli retained phytochemicals and total antioxidant capacity better than frozen broccoli, whereas frozen broccoli had color features more similar to raw samples because the blanching process inactivated the function of enzymes which limited the degradation of chlorophylls making the pigments more stable after cooking.

3. Effect of steamable bag microwaving versus steamer steaming and traditional microwaving on nutritional preservation and physical quality of frozen broccoli

3.1 Materials

Frozen Broccoli. Individual quick freezing (IQF) broccoli (stems and florets) was purchased from a local market in 2.5 lbs bag and stored in a freezer at −18 ºC. The frozen broccoli from the purchased bags was mixed together to obtain homogeneous samples.

Chemicals. Meta-phosphoric acid (ACS reagent, 33.5~36.5%), 2, 6-dichlorophenol sodium salt hydrate (BioReagent), sodium bicarbonate (Analytical grade, 99.0%), 6-methoxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), fluorescein, 2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH), sodium phosphate (mono and dibasic) were purchased from Sigma – Aldrich (St. Louis, MO, USA). L-ascorbic acid (U.S.P. grade, ⩾99.0% was purchased from Spectrum Chemical Manufacturing Corp. (New Brunswick, NJ, USA). Acetic acid (glacial, ACS reagent, 99.0%) was purchased from EMD Chemicals Inc. (Billerica, MA, USA). Sulfuric acid (ACS reagent, 96.4%) was purchased from J.T. Baker (Center Valley, PA, USA). Methanol (ACS reagent, 99.8%) was purchased from Macron Chemicals Inc. (Center Valley, PA, USA). Acetone (ACS reagent) was purchased from Jade Scientific Inc. (Westland, MI, USA). Distilled water was purchased from Meijer Distribution Inc. (Grand Rapids, MI, USA).

Steamable bags. Steamable bags were composed of a high-barrier two-ply lamination film (Printpack Inc., Atlanta, GA, USA) and a steam-activated steam release valve (Avery Dennison's Flexis valve, Avery Dennison, Pasadena, CA, USA). The laminated material consisted of polyethylene terephthalate (PET, outer layer) and polypropylene (PP, inner layer) and had a total thickness was 5 mil. The dimensions of the steamable bags were 23 cm × 16 cm selected according to its capacity to contain 300 ± 3 g frozen broccoli.

Cookware. A T-fal steamer (VC133851, West Orange, NJ, U.S.A.), a Pyrex glass container with glass lid (2 quart, Greencastle, PA, U.S.A.) and a Sharp microwave oven (1.4 cu. ft. 1100 watts, Sharp Model R410lk, Mahwah, NJ, USA) were purchased from a local retail store.

3.2 Methods

3.2.1 Packaging

Amounts of 300 ± 3 g frozen broccoli with an approx. equal amount of stems and florets were packed into steamable bags. The transfer of product from its original packages was made in low lighting environment at 0 ºC. The bags were sealed using a thermal heat sealer (Model 24AS/1, Sencorp Systems Inc., Hyannis, MA, USA) for 1.5 seconds at a jaw pressure of 276 kPa and temperature of 199 ± 2 ºC. The seal integrity of the steamable bags was verified using a package leak test with the ARO Non-porous package tester (F099-1080, ARO Corporation, York, PA, USA). Briefly, a filled steamable bag was immersed in water inside the testing chamber, the chamber was closed, and then vacuum was drawn. The steamable bag was considered to maintain its integrity if no bubble was escaping from the package. The sealed packages were immediately stored in a freezer at −18 ºC. All product quality tests were done within one week to avoid variations caused by extended storage time.

3.2.2 Treatments

Uncooked broccoli. Uncooked frozen broccoli was thawed to room temperature inside a corrugated box for 10 hours. The box was used to avoid light damage affecting the quality of broccoli during the thawing process.

Steamer steaming. 300 ± 3 g of frozen broccoli was steamed for 600 seconds using a T-fal steamer. Briefly, the broccoli was placed on the plastic steaming rack above the steamer base containing boiling water and covered with the steamer lid. The cooking time was determined by using a T-type handheld flexible thermocouple probe (Model 91100-40, Cole-Parmer Instrument Co., Vernon Hills, IL, USA). This was inserted into the broccoli floret (stem –towards- floret direction) via the vent of the steamer lid at time intervals between 480 and 720 seconds and then the temperatures were recorded after 2 seconds. The time needed for the broccoli to reach 74 ºC (safe minimum cooking temperature) was designated as cooking time.

Traditional microwaving. 300 ± 3 g of frozen broccoli was microwaved for 330 seconds using a rectangular-shaped Pyrex glass container with a glass lid. Briefly, the broccoli together with 100 ml of distilled water at room temperature was placed inside the glass container and then microwaved with the glass lid on. The cooking time was determined by inserting the thermocouple probe into the broccoli floret as abovementioned immediately after microwaving at time intervals between 240 and 420 seconds, and then recording the temperatures after 2 seconds. The time needed for the broccoli to reach 74 ºC was designated as the cooking time. The use of water was to prevent excessive water loss leading to serious color and firmness changes during microwaving, and an amount of 100 ml was used to avoid the "microwave boiling" phenomenon.

Steamable bag microwaving. A steamable bag containing 300 ± 3 g of frozen broccoli was heated during 300 seconds in a Sharp microwave oven. The cooking time was determined by opening the microwave oven after time intervals between 240 and 360 seconds, puncturing a small hole in the center of the steamable bag, inserting the thermocouple probe into the broccoli

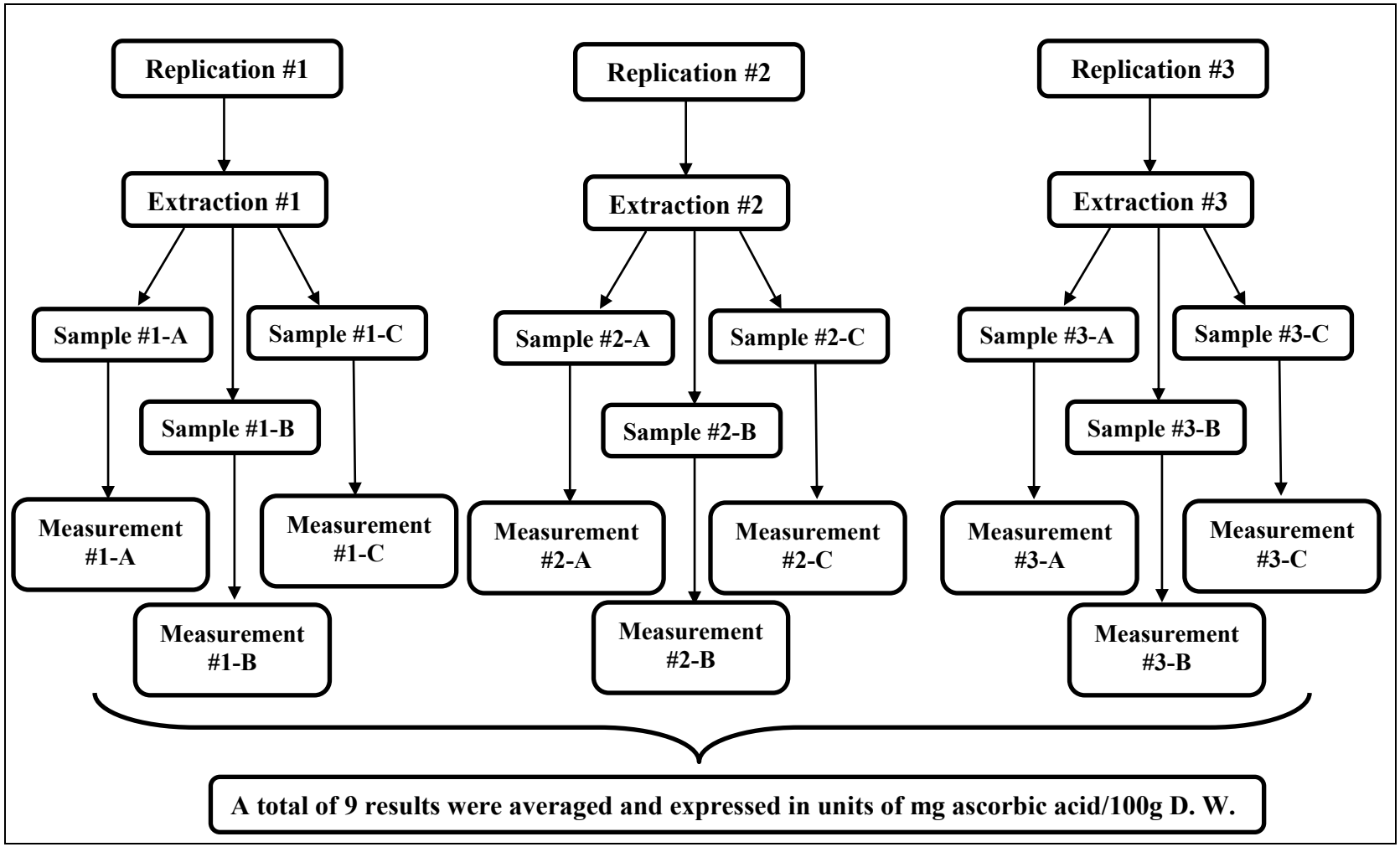
floret as abovementioned and recording the temperatures after 2 seconds. The time needed for the broccoli to reach 74 ºC was designated as the cooking time.

After each cooking process finished, some of the broccoli was cooled rapidly on ice water slurry and then drained using a paper towel for ascorbic acid and antioxidant capacity analyses. Other broccoli samples were cooled to 50 ºC (mimicking consumption temperature) for firmness analyses, and to room temperature for color analyses. Temperatures were controlled by inserting the above-mentioned thermocouple probe into the broccoli of the same batch used as the temperature control sample.

3.2.3 Ascorbic Acid

An amount of 100 g of uncooked or cooked frozen broccoli containing an approx. equal amount of stems and florets was blended with 100 ml of extraction solution (15 g meta-phosphoric acid: 40 ml acetic acid: 3.7 ml conc. sulfuric acid: 450 ml water) for 30 seconds using a food chopper (Model 72705, Hamilton Beach Brands Inc., Southern Pines, NC, USA). The homogenates were filtered using a nylon cloth and the resulting residues mixed with another 50 ml of extraction solution, blended for 30 seconds and filtered. The two filtrates were combined together and then centrifuged at 3500 rpm, 4 °C (Centrifuge 5804R, Eppendorf, Germany) for 600 seconds. The supernatants were collected and titrated against a dye solution (50 mg 2, 6-dichlorophenol Na salt, 42 mg NaHCO3 and 200 ml water) until a pink color persisted for 15 seconds (AOAC method No. 967.21; AOAC, 2000). Each treatment was replicated 3 times. Three broccoli samples of 100 g each were analyzed per replication. The results from the nine samples of each treatment were averaged and expressed as mg ascorbic acid /100 g D.W. (dry weight basis) as shown in Diagram 1.

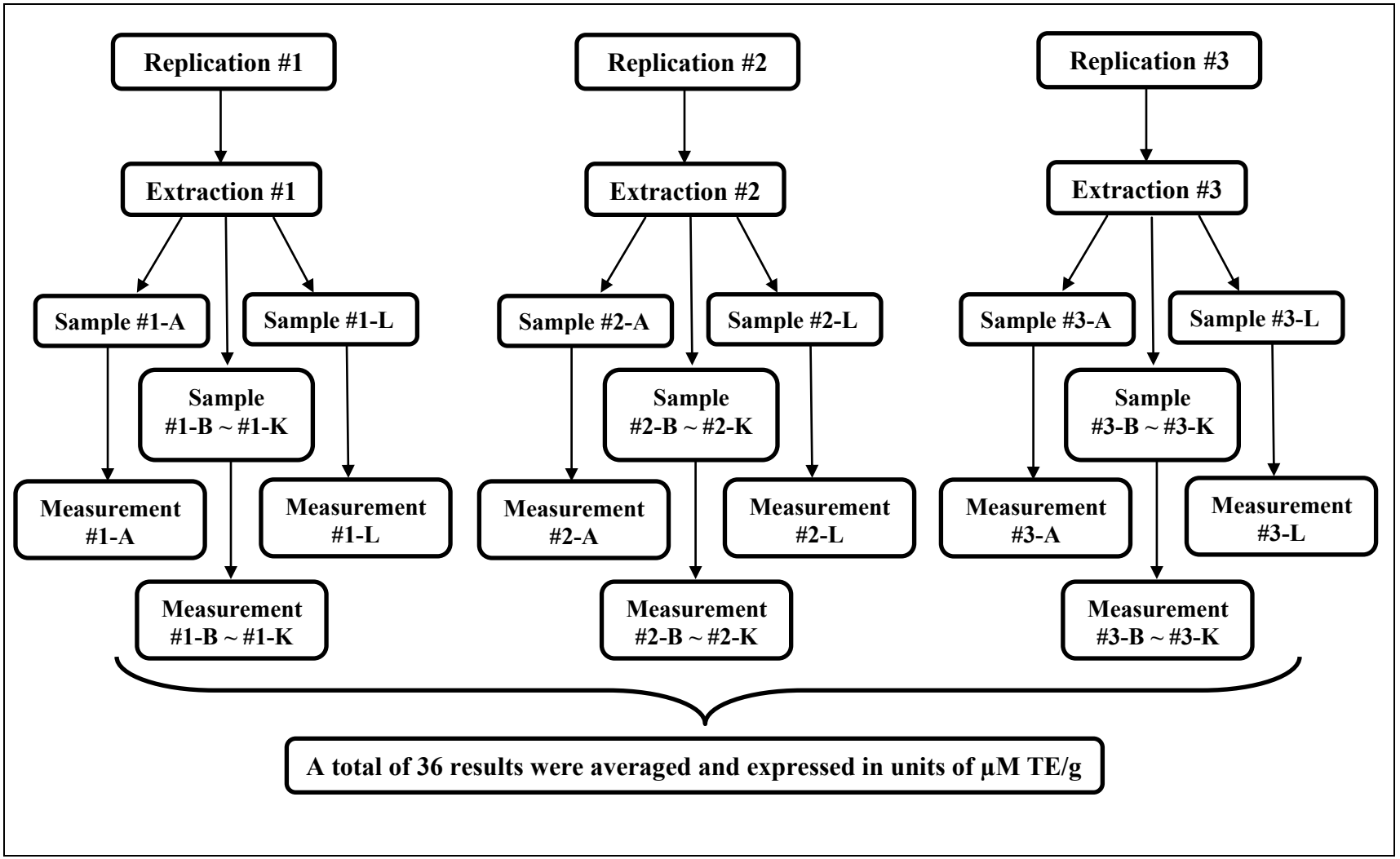
Figure 1. Sample size used to determine ascorbic acid in uncooked broccoli and in broccoli cooked using steamer steaming, traditional microwaving and steamable bag microwaving.



3.2.4 Oxygen Radical Absorbance Capacity assay

Uncooked or cooked broccoli was homogenized in a high-speed blender, and a 5 g sample was mixed with 40 ml acidic methanol/water (50:50, v/v, pH 2). The mixture was placed in a water-bath shaker for 1hour and then centrifuged at 10,000xg (Sorvall RC-5B Refrigerated Superspeed Centrifuge, Du Pont Instruments, Wilmington, DE, USA) for 600 seconds. The supernatant was collected and the residues were extracted by adding to 40 ml acetone/water (70:30, v/v) followed by 1 hour shaking and then centrifuged at 10,000xg for 600 seconds. The two supernatants were combined and then acetone/water (70:30, v/v) was added to adjust the extracted solution volume to 80 ml. The ORAC assay was done following the analytical procedures of Huang, Ou, Hampsch-Woodill, Flanagan and Prior (2002). Briefly, 150 µl flourescein (20 nM) was added to a 96-wells black plate, followed by 25 µl of each of the following solutions: blank (sodium phosphate buffer), Trolox (6-methoxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) standard and diluted sample extract (50 µl sample extracts were added to 10 ml sodium phosphate buffer). The mixture was incubated at 37 °C for 1800 seconds in Microplate Reader (Biotek Instruments, Winooski, VT, USA). After incubation, 25 µl AAPH (2,2'-azobis (2-amidino-propane) dihydrochloride) was added to all the wells. Fluorescence was monitored using 485 nm excitation and 528 nm emissions at 120 seconds intervals for 5 hours in Microplate Reader. Trolox standard (6.25, 12.5, 25, 50, 100 µM) was used to generate a standard calibration curve and ORAC values were expressed as µM TE/g D.W. (TE = Trolox Equivalent). Each treatment was replicated 3 times. Twelve broccoli samples of 5 g each were analyzed per replication. The results from the thirty-six samples of each treatment were averaged and expressed as µM TE/g D. W. as shown in Diagram 2.

Figure 2. Sample size used to determine antioxidant capacity of uncooked broccoli and of broccoli cooked using steamer steaming, traditional microwaving and steamable bag microwaving.



3.2.5 Firmness

The firmness of uncooked and cooked broccoli stems was measured using TA.XTPlus texture analyzer (Stable Micro Systems, Godalming, UK) equipped with a 10-blades Kramer shear cell-since it produces simulated results similar to those from humans chewing food. Four stems of approx. similar size and amount (about 10 ± 0.2 g) were placed in the Kramer shear cell for evaluation. Maximum peak force was recorded at a shear press speed setting of 1.50 mm/s. In order to avoid temperature effect on the firmness of samples, all samples were placed on trays and stored in an oven (Fisher ISOTEMP, 200 series, Model 230F, Wood Dale, IL, USA) under controlled condition of 50 ºC. Each treatment was replicated 3 times and for each replication six samples consisting of four stems each were tested. The results from the eighteen samples of each treatment were averaged and expressed in units of kg-force/g broccoli as shown in Diagram 3.

3.2.6 Color

The color of uncooked and cooked broccoli florets was measured using a Hunter LabScan XE colorimeter (LX17582, Reston, VA, USA) calibrated using standard black and white tiles. The color parameters values, L* (lightness, black = 0, white = 100), a*(redness > 0, greenness < 0), and b*(yellowness > 0, blue < 0), of the pieces were recorded. Results were also expressed by hue angle (h° = arctan (b*/a*), red = 0°, yellow = 90°, green = 180°, blue = 270°) and total color difference △E. Florets were individually placed in the standard sample cup and duplicate readings were taken at room temperature. Each treatment was replicated 3 times and for each replication 6 florets were tested as shown in Diagram 4.

Figure 3. Sample size used to determine the firmness of uncooked broccoli and of broccoli cooked using steamer steaming, traditional microwaving and steamable bag microwaving.

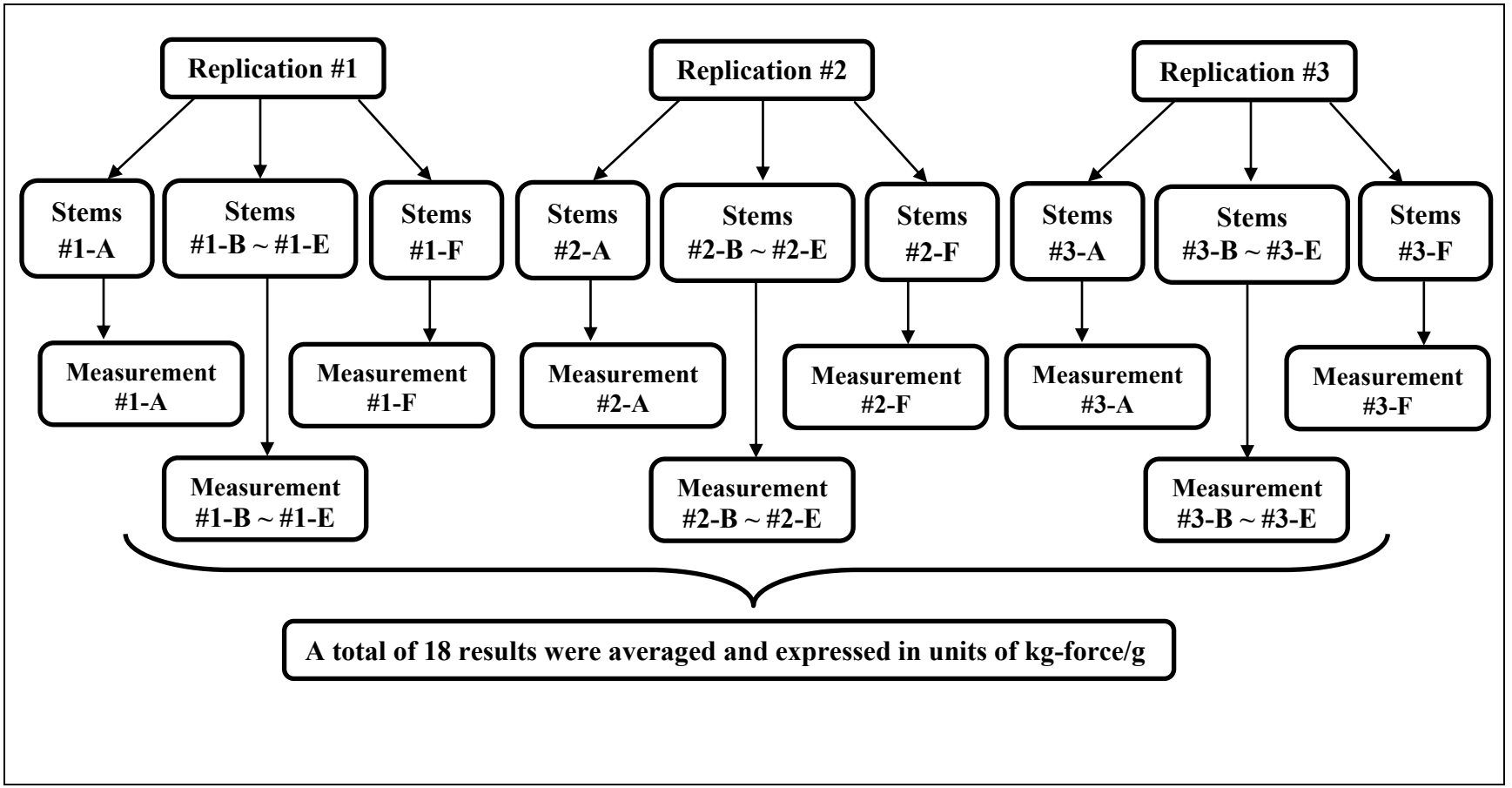
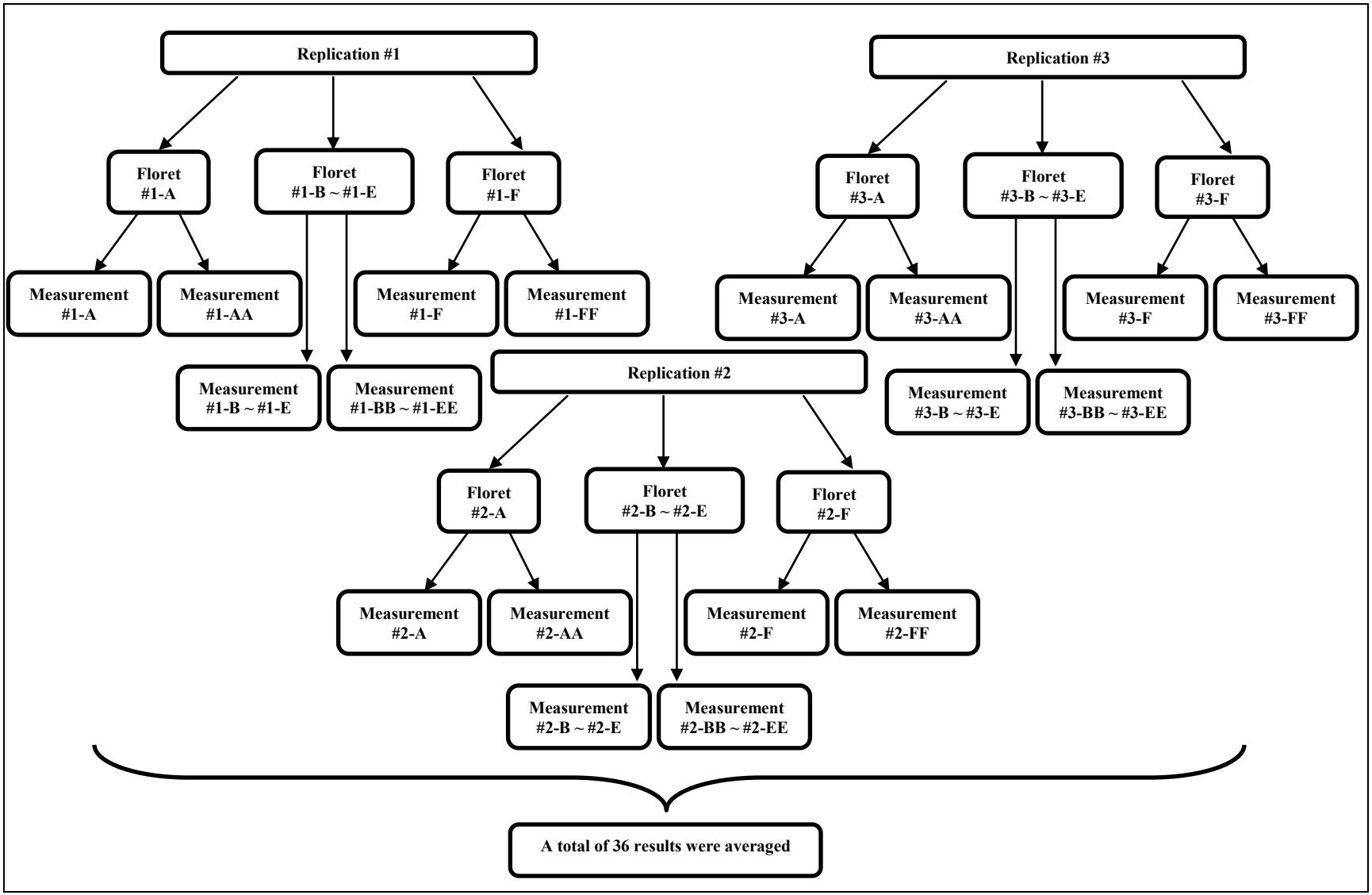


Figure 4. Sample size used to determine the color of uncooked broccoli and of broccoli cooked using steamer steaming, traditional microwaving and steamable bag microwaving.



3.2.7 Moisture Content

The moisture contents of all analyzed samples (uncooked and cooked frozen broccoli) were determined by a moisture analyzer (MX-50, AND Instruments Ltd., Abingdo, UK) and used to convert data from fresh weight basis to dry weight basis. Briefly, an amount of 5-6 g of homogenized broccoli was dried in the moisture analyzer at 105 °C using its standard drying program until reaching constant weight. Results were obtained in triplicate and averaged. A moisture content of 90.96%, 91.17%, 90.69% and 89.53% for uncooked, steamer steaming, traditional microwaving and steamable bag microwaving, respectively, was obtained.

3.2.8 Statistical Analysis

One-way analysis of the variance (ANOVA) in combination with Tukey's test was used to compare the ascorbic acid, antioxidant capacity, firmness and color of uncooked and steamable bag microwaving cooked broccoli as well as the effect of steamable bag microwaving versus steamer steaming versus traditional microwaving on the abovementioned parameters of frozen broccoli. The significance level used was $p \leq 0.05$. MINITAB® 16.1.1 Statistical Software (Minitab Inc., PA, US) was used for all statistical assessments.

3.3 Results and Discussions

3.3.1 Ascorbic Acid

Figure 1 shows the ascorbic acid content of frozen broccoli after steamable bag microwaving, steamer steaming, and traditional microwaving as well as of thawed frozen broccoli. The latter was used to determine the ascorbic acid retained in broccoli after cooking because of the known negative effect of heat on ascorbic acid preservation in vegetables

(Erdman & Klein, 1982). The retained ascorbic acid was 90%, 90% and 84% for the broccoli cooked by steamable bag microwaving, steamer steaming, and traditional microwaving retained, respectively. The ascorbic acid content of frozen broccoli cooked by steamer steaming and steamable bag microwaving was the same (573 ± 16 mg/100g D.W. and 571 ± 17 mg/100g D.W., respectively), and was significantly ($p \leq 0.05$) higher than that of frozen broccoli cooked by traditional microwaving (535 ± 18 mg/100g D.W.). The difference can be attributed to the leaching of ascorbic acid into water during traditional microwaving. Erdman and Klein (1982) reported that both, presence and amount of water, can significantly affect ascorbic acid retention, with a larger quantity of cooking water resulting in more loss of water soluble vitamins due to leaching. Miglio, Chiavaro, Visconti, Fogliano and Pellegrini (2008) also observed a loss of ascorbic acid in steamed broccoli while Vallejo, Tomas-Barberan and Garcia-Viguera (2002) and Gliszczynska-Swiglo et al. (2006) reported no effect of steamer steaming on ascorbic acid content. The difference between their results and our results can be attributed to the different methodologies used to extract and determine ascorbic acid. Several studies have also observed a higher loss of ascorbic acid in microwaved broccoli than in steamed broccoli (Hudson, Dalal & Lachance, 1985; Vallejo, Tomas-Barberan & Garcia-Viguera, 2002; Pellegrini et al. 2010). In summary, steamable bag microwaving performs better than traditional microwaving and is equal to steamer steaming in retaining ascorbic acid content. However, steamable bag microwaving notably reduces cooking time compared to steamer steaming (300 and 600 seconds for steamable bag microwaving and steamer steaming, respectively).

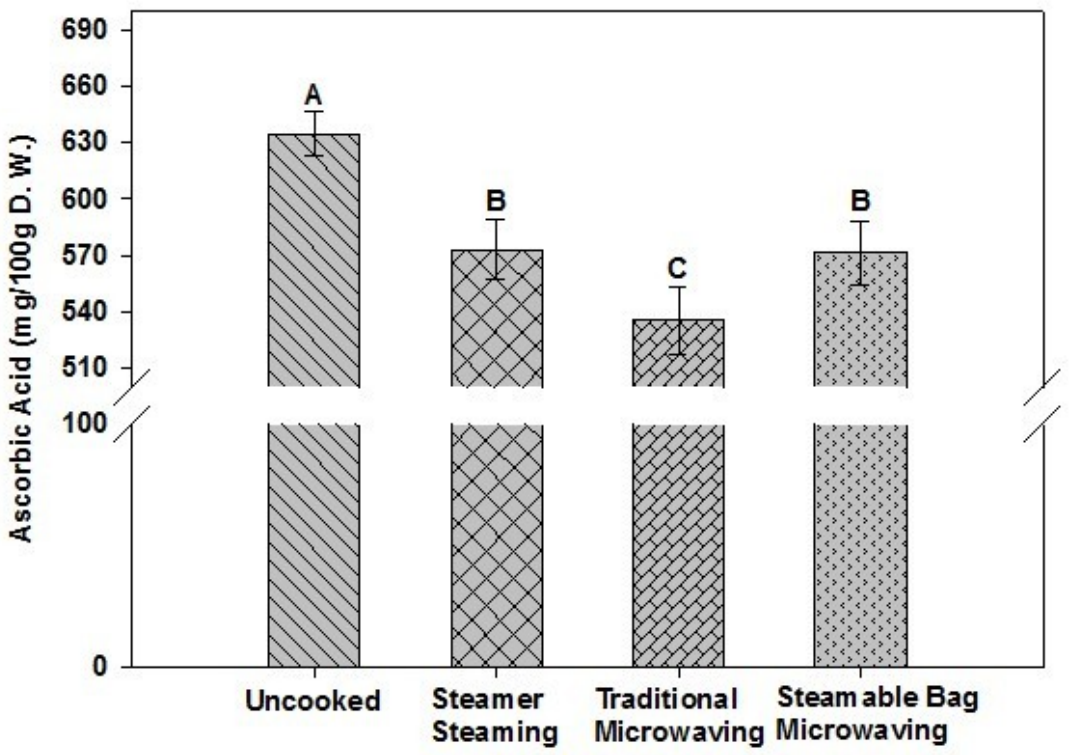


Figure 5. Ascorbic Acid Content of Frozen Broccoli after Steamable Bag Microwaving, Steamer Steaming, and Traditional Microwaving as well as of Thawed Frozen Broccoli (different letters indicate significant differences ($p \leq 0.05$)).

3.3.2 Antioxidant Capacity

Hydrophilic antioxidants have been reported to be the main contributors to the antioxidant capacity of broccoli (Kurilich, Jeffery, Juvik, Wallig & Klein, 2002; Wu, Beecher, Holden, Haytowitz, Gebhardt & Prior, 2004; Roy, Juneja, Isobe & Tsushida, 2009) and therefore, only these were extracted from thawed and cooked frozen broccoli and evaluated using oxygen radical absorbance capacity (ORAC) assay. Figure 2 shows the obtained ORAC values in a dry weight basis. The ORAC values of frozen broccoli upon steamer steaming and steamable bag microwaving were significantly ($p \leq 0.05$) higher than those of thawed frozen broccoli. An increased antioxidant capacity in vegetables upon cooking has been attributed to cooking promoting the release of antioxidant compounds from the vegetable matrix and determining the

formation of new antioxidant compounds, such as Maillard-reaction products (Rechkemmer, 2007; Miglio, Chiavaro, Visconti, Fogliano & Pellegrini, 2008). The antioxidant capacity of frozen broccoli cooked by traditional microwaving was the same as that of thawed frozen broccoli, and 53% and 48.0% lower than that of frozen broccoli cooked by steamer steaming and steamable bag microwaving, respectively. The different antioxidant capacity could in part be due to the major retention of ascorbic acid in broccoli cooked by steamer steaming and steamable bag microwaving compared to traditional microwaving (Figure 1). In addition, the leaching of other water-soluble nutritional compounds such as glucosinolates into cooking water in traditional microwaving might have contributed to the lower antioxidant capacity of the microwaved frozen broccoli (Wachtel-Galor, Wong & Benzie, 2008; Miglio, Chiavaro, Visconti, Fogliano & Pellegrini, 2008).

This increased or maintained antioxidant capacity of the cooked frozen broccoli contrasts with the losses of antioxidant capacity of frozen broccoli during boiling, microwaving and steaming reported by Pellegrini et al. (2010). But it is of note that different extraction solvents, procedures and analytical measurements might lead to results that are not easily compared (Perez-Jimenez et. al., 2008). However, Turkmen, Sari and Velioglu (2005) and Wachtel-Galor, Wong and Benzie (2008) observed an increased antioxidant capacity in boiled, microwaved and steamed fresh broccoli, which aligns with the results of this study.

Our results partially disagree with the lower nutritional value attributed to processed vegetables compared to uncooked ones (Zhang & Hamauzu, 2004; Danesi & Bordoni, 2008; Mazzeo et al., 2011). According to this study, the final nutritional value of processed broccoli depends on the cooking treatment. In the case of steamer steaming and steamable bag

microwaving, some of the ascorbic acid of the frozen broccoli was lost during cooking but its antioxidant capacity was increased.

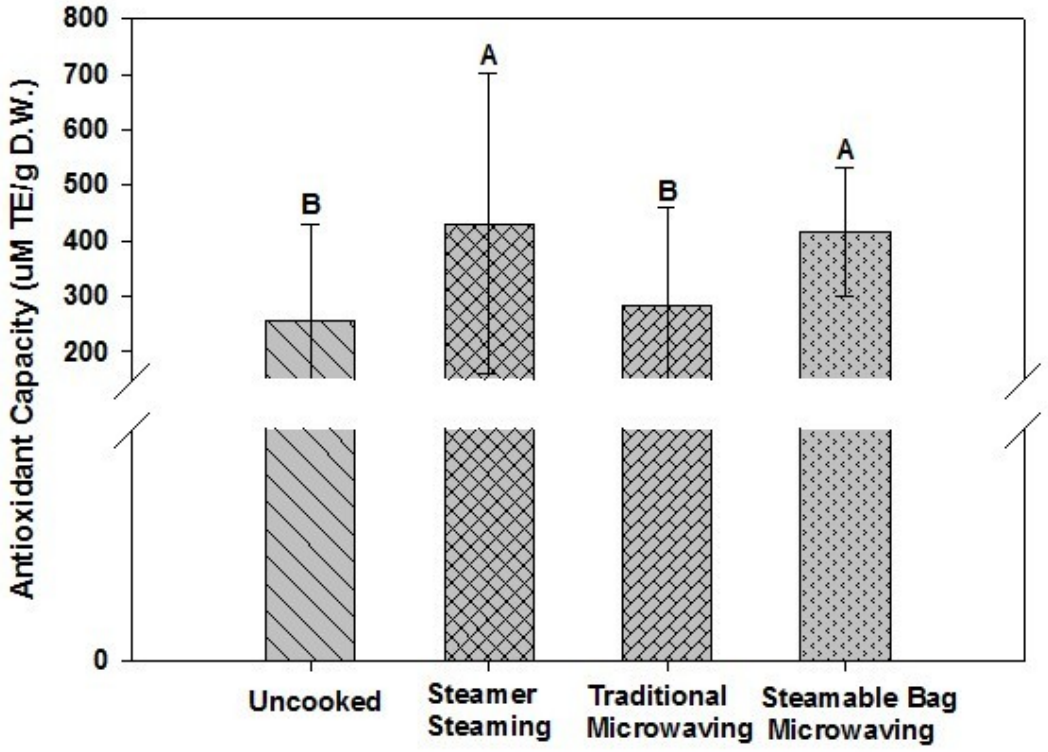


Figure 6. Antioxidant Capacity of Frozen Broccoli after Steamable Bag Microwaving, Steamer Steaming, and Traditional Microwaving as well as of Thawed Frozen Broccoli (different letters indicate significant differences ($p \leq 0.05$)).

3.3.3 Firmness

The firmness of frozen broccoli after steamable bag microwaving, steamer steaming, and traditional microwaving was determined and compared to that of thawed frozen broccoli to evaluate the effect of these cooking methods on broccoli tenderization. Table 1 shows that the firmness of frozen broccoli after steamer steaming, traditional microwaving and steamable bag microwaving was significantly ($p \leq 0.05$) lower than that of thawed frozen broccoli, indicating the softening of the broccoli after all three cooking methods. By comparing the cooking methods, traditional microwaving and steamer steaming softened the broccoli equally, and the softening

was less than that of the steamable bag microwaving. These results indicate the time-saving in tenderizing broccoli of steamable bag microwaving compared to traditional microwaving and steamer steaming (300, 330, 600 seconds for steamable bag microwaving, traditional microwaving, and steamer steaming, respectively). In addition, steamable bag microwaving does not require cooking water to tenderize broccoli as traditional microwaving and steamer steaming do. Thus, steamable bag microwaving can soften broccoli faster and without the use of additional water, which upgrades microwave cooking to a higher level of efficacy and convenience.

Table 1. Color and Firmness of Frozen Broccoli after Steamable Bag Microwaving, Steamer Steaming and Traditional Microwaving as well as of Uncooked Thawed Frozen Broccoli

	Color[1]					Firmness[2] (kg-force/g)
	L*	a*	b*	Hue°	ΔE	
Uncooked	20.85 ± 2.18B	–13.19 ± 1.39B	20.07 ± 2.79B	123.43 ± 1.68A	31.83 ± 3.60B	3.29 ± 0.36A
Steamer Steaming	23.14 ± 2.50A	–12.77 ± 1.01AB	23.43 ± 2.41A	118.66 ± 2.35C	35.35 ± 3.34A	2.31 ± 0.35B
Traditional Microwaving	23.77 ± 2.04A	–14.17 ± 0.92C	23.29± 1.98A	121.34 ± 1.82B	36.19 ± 2.71A	2.61 ± 0.46B
Steam Bag Microwaving	21.65 ± 1.16B	–12.50 ± 0.88A	20.42 ± 1.66B	121.48 ± 1.27B	32.31 ± 1.69B	1.84 ± 0.36C

Means in rows followed by different letters differed significantly ($p \leq 0.05$)

[1]Values presented as mean ± SD (n = 18)

[2]Values presented as mean ± SD (n = 18)

3.3.4 Color

Cooked vegetables exhibit poor color quality in comparison with fresh ones (Turkmen, Poyrazoglu, Sari & Velioglu, 2006). Thus, the color of broccoli florets after steamable bag microwaving, steamer steaming and traditional microwaving was determined and compared to that of thawed frozen broccoli (Table 1). Steamer steaming and traditional microwaving significantly ($p \leq 0.05$) increased the L* value of the broccoli florets while steamable bag microwaving maintained the intense darkness of the broccoli florets compared to thawed frozen broccoli. The use of shortest cooking time in steamable bag microwaving might have caused this difference since less cell juice released by cell membrane deterioration replaced intercellular air (Tijskens, Schijvens & Biekman, 2001). In contrast to our results, Pellegrini et al. (2010) observed a decreased and maintained L* value in steamed and microwaved frozen broccoli, respectively. The difference between their and our results can be attributed to the longer cooking times used for steaming and microwaving by Pellegrini et al. (2010) that might have increased cell membrane deterioration and consequently, more cell juice replacing intercellular air. Broccoli florets under steamer steaming and traditional microwaving became yellower (increased + b* value) while those under steamable bag microwaving maintained yellowness (maintained + b* value) compared to thawed broccoli florets. The use of shortest cooking time in steamable bag microwaving might have also caused this difference. A loss of greenness (increased -a* values) was found in broccoli florets cooked by steamer steaming and steamable bag microwaving. Pellegrini et al. (2010) also found that steaming causes loss of greenness in frozen broccoli. Loss of greenness in cooked broccoli has been attributed to chlorophyll pigment degradation along with the formation of pheophytins caused by the exchange of Mg^{+2} by H^{+} in the porphyrin ring of the chlorophyll (Turkmen, Poyrazoglu, Sari & Velioglu, 2006). In agreement, Pellegrini et al.

(2010) found that 29% chlorophylls broke down while 274% pheophytins were generated for frozen broccoli under steaming. In contrast to the aforementioned cooking methods, traditional microwaving yielded greener (decreased -a*) broccoli florets. This greenness increase has been related to an alteration of surface reflecting properties and light penetration into the vegetable tissue caused by replacement in cells of air and other dissolved gases with cooking water (Miglio, Chiavaro, Visconti, Fogliano & Pellegrini, 2008; Turkmen, Poyrazoglu, Sari & Velioglu, 2006). Other factors could have contributed to this difference in greenness. The shorter cooking time of traditional microwaving (330 seconds) compared to steamer steaming (600 seconds) could have reduced chlorophyll degradation and pheophytins formation. The different matrix structure (softness) between broccoli cooked under similar cooking times (traditional microwaving (330 seconds) and steamable bag microwaving (300 seconds)) could have been the reason for their different greenness since changes in matrix structure of vegetables have been reported to affect their color (Oey, Lille, Loey & Hendrickx, 2008). In contrast to our results, Pellegrini et al. (2010) reported a 45% loss of greenness in frozen broccoli after microwaving. The difference between their and our results could be attributed to the longer cooking time used for microwaving by Pellegrini et al. (2010). The hue angle of the broccoli florets decreased (shift toward yellow values) for all cooking methods, with steamed broccoli having the lowest hue angle values. A decreased hue angle was observed for all cooking methods due to the combined effects of a* and b* values since one or both changed but in a different way depending on the cooking method. The total color difference ($\triangle$E) of the broccoli florets cooked by steamable bag microwaving was the same as that of the uncooked florets while an increased $\triangle$E value was observed in the florets cooked by steamer steaming and traditional microwaving. Therefore, steamable bag microwaving is a better cooking method compared to steamer steaming and

traditional microwaving in terms of minimizing color changes. This will most likely have an effect on consumers' sensory acceptance since consumers prefer eating fresh-like vegetables.

4. Effect of steamable bag design on the nutritional and physical quality of cooked frozen broccoli

4.1 Materials

Frozen broccoli. Individual quick freezing (IQF) broccoli (stems and florets) was purchased from a local market in 2.5 lbs bag and stored in a freezer at −18 ºC. The frozen broccoli from the purchased bags was mixed together to obtain homogeneous samples.

Chemicals. Meta-phosphoric acid (ACS reagent, 33.5% ~ 36.5%), 2,6-dichlorophenol sodium salt hydrate (BioReagent), sodium bicarbonate (Analytical grade, 99.0%), 6-methoxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), fluorescein, 2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH), sodium phosphate (mono and dibasic) were purchased from Sigma–Aldrich (St. Louis, MO, USA). L-ascorbic acid (U.S.P. grade, ≥99.0%) was purchased from Spectrum Chemical Manufacturing Corp. (New Brunswick, NJ, USA). Acetic acid (glacial, ACS reagent, 99.0%) was purchased from EMD Chemicals Inc. (Billerica, MA, USA). Sulfuric acid (ACS reagent, 96.4%) was purchased from J.T. Baker (Center Valley, PA, USA). Methanol (ACS reagent, 99.8%) was purchased from Macron Chemicals Inc. (Center Valley, PA, USA). Acetone (ACS reagent) was purchased from Jade Scientific Inc. (Westland, MI, USA).
Steamable bags. Steamable bags were composed of a high –barrier two-ply lamination film (Printpack Inc., Atlanta, GA, USA) and a steam activated release valve (Avery Dennison's Flexis valve, Avery Dennison, Pasadena, CA, USA) with an approximately central location. The laminated material consisted of polyethylene terephthalate (PET, outer layer) and polypropylene (PP, inner layer), and had a total thickness was 5 mil. The material was shaped into bags differing in shape and surface area (length × width) as shown in Table 2. The dimensions of the steamable bags were selected according to their capacity to contain the same amount of frozen

broccoli (330 ± 3 g). Different bag headspaces were obtaining by placing a same amount of broccoli in bags differing in dimensions.

Table 2. Package Specification and Cooking Time.

Steamable Bag Type	Length (cm)	Width (cm)	Surface Area (cm^2)	Cooking Time (seconds)
SB-I	28.58	18.42	526.44	300
SB-II	22.23	18.42	409.48	315
SB-III	24.13	15.88	383.18	300

*Time taken for broccoli to reach 74 °C at full microwave power.

4.2 Methods

4.2.1 Packaging

Amounts of 330 ± 3 g frozen broccoli with an approx. equal amount of stems and florets were packed into the three steamable bags. The transfer of product from its original packages was made in low lighting environment at 0 ºC. The bags were then sealed using a thermal heat sealer (Model 24AS/1, Sencorp Systems Inc., Hyannis, MA, USA) for 1.5 seconds at a jaw pressure of 276 kPa and temperature of 199 ± 2 ºC. The seal integrity of the steamable bags was verified using a package leak test with the ARO Non-porous package tester (F099-1080, ARO Corporation, York, PA, USA). Briely, a filled steamable bag was immersed in water inside the chamber, the chamber was closed and then vacuum was drawn. The steamable bag was considered to maintain its integrity if no bubble was escaping from the package. The sealed packages were immediately stored in a freezer at −18 ºC. All product quality tests were done within one week to avoid variations caused by extended storage time.

4.2.2 Cooking Time

Each of the steamable bags containing frozen broccoli was heated up in a microwave oven (1.4 cu. ft. 1100 watts; Sharp Model R410lk, Mahwah, NJ, USA) at different interval times

between 240 and 360 seconds. Then, the microwave oven was opened and a small hole in the center of the steamable bag was punctured. A T-type handheld flexible thermocouple probe (Model 91100-40, Cole-Parmer Instrument Co., Vernon Hills, IL, USA) was inserted via the puncture hole into the broccoli floret (stem-towards-floret direction) and the temperature was recorded after 2 seconds. The time needed for the broccoli to reach 74 ºC (safe minimum cooking temperature) was designated as the cooking time. Four packages of each type of steamable bag were used to confirm the cooking time. Results are presented in Table 2.

4.2.3 Ascorbic Acid

An amount of 100 g of steamable bag microwaved frozen broccoli containing an approx. equal amount of stems and florets was blended with 100 ml of extraction solution (15 g meta-phosphoric acid: 40 ml acetic acid: 3.7 ml conc. sulfuric acid: 450 ml water) for 30 seconds using a food chopper (Model 72705, Hamilton Beach BrandsInc., Southern Pines, NC, USA). The homogenates were filtered using a nylon cloth and the resulting residues mixed with another 50 ml of extraction solution, blended for 30 seconds and filtered. The two filtrates were combined together and then centrifuged at 3500 rpm, 4 °C (Centrifuge 5804R, Eppendorf, Germany) for 10 minutes. The supernatants were collected and titrated against a dye solution (50 mg 2, 6-dichlorophenol Na salt, 42 mg $NaHCO_3$ and 200 ml water) until a pink color persisted for 15 seconds (AOAC, No. 967.21, 2000). Three replicates from each package were evaluated, and the results were averaged and expressed as mg ascorbic acid/100g F.W. (fresh weight) and mg ascorbic acid /100 D.W. (dry weight). Broccoli from a total of nine packages was tested (three packages per type of steamable bag).

4.2.4 Oxygen Radical Absorbance Capacity assay

The cooked broccoli was removed from the steamable bag and homogenized in a high-speed blender. 5 g of the homogenized broccoli was mixed with 40 ml acidic methanol/water (50:50, v/v, pH 2). The mixture was placed in a water-bath shaker for 1 hour and then centrifuged at 10,000x*g* (Sorvall RC-5B Refrigerated Superspeed Centrifuge, Du Pont Instruments, Wilmington, DE, USA) for 10 minutes. The supernatant was collected and the residues were extracted by adding to 40 ml acetone/water (70:30, v/v) followed by 1 hour shaking and then centrifuged at 10,000x*g* for 10 minutes. The two supernatants were combined and then acetone/water (70:30, v/v) was added to adjust the extracted solution volume to 80 ml. The ORAC assay was done following the analytical procedures of Huang, Ou, Hampsch-Woodill, Flanagan and Prior (2002). Briefly, 150 µl of flourescein (20 nM) was added to a 96-wells black plate, followed by 25 µl of each of the following solutions: blank (sodium phosphate buffer), Trolox (6-methoxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) standard and diluted sample extract (50 µl sample extracts were added to 10 ml sodium phosphate buffer). The mixture was incubated at 37 °C for 30 minutes in Microplate Reader (Biotek Instruments, Winooski, VT, USA). After incubation, 25 µl of AAPH (2,2'-azobis(2-amidino-propane) dihydrochloride) was added to all the wells. Fluorescence was monitored using 485 nm excitation and 528 nm emissions at 2 minutes intervals for 300 minutes in Microplate Reader. A Trolox standard (6.25, 12.5, 25, 50, 100 µM) was used to generate a standard calibration curve and ORAC values were expressed as µM TE/g F.W. and µM TE/g D.W. (TE = Trolox Equivalent). A total of nine packages were tested (three packages per type of steamable bag).

4.2.5 Color

The color of the frozen broccoli after steamable bag microwaving was measured using a Hunter LabScan XE colorimeter (LX17582, Reston, VA, USA) calibrated using standard black and white tiles. Five florets of similar size from each package were analyzed immediately after microwaving. The floret pieces were individually placed in the standard sample cup and duplicate readings were taken per piece. The color parameter values, L*(lightness, black = 0, white = 100), a*(redness > 0, greenness < 0), and b*(yellowness > 0, blue < 0), of the pieces were recorded. Results were also expressed by hue angle (h° = arctan (b*/a*), red = 0°, yellow = 90°, green = 180°, blue = 270°). Broccoli from a total of nine packages was tested (three packages per type of steamable bag).

4.2.6 Firmness

The firmness of the frozen broccoli after microwaving the steamable bags was measured using a TMS-TP Texture Press Analyzer (Model FTA-300 Force Tr.; Food Technology Corp., Sterling, VA, USA), equipped with a 10-blade Kramer shear cell (C-332; 67 x 67 mm), because it produces simulated results similar to those from humans chewing food. Four stems of approx. similar size and amount (about 10 ± 0.2 g) were placed in the Kramer shear cell for evaluation and a total of twenty pieces from each package were evaluated. In order to avoid a temperature effect on the firmness of the samples, all samples were placed on trays and stored in an oven (Fisher ISOTEMP, 200 series, Model 230F, Wood Dale, IL, USA) under controlled conditions of 50 ºC. Maximum force was recorded at a shear press speed setting of 0.424 cm/s. The results were averaged and expressed in units of kg-force/g broccoli. Broccoli from a total of twelve packages was tested (four packages per type of steamable bag).

4.2.7 Moisture Content

The moisture content of frozen broccoli cooked in the microwave steamable bags was determined using a moisture analyzer (MX-50, AND Instruments Ltd., Abingdo, UK) with a standard drying program. An amount of 5-6 g of cooked homogenized broccoli (in triplicate) from each steamable bag was dried in the moisture analyzer at 105 °C until reaching constant weight. The average moisture content of broccoli from each type of steamable bag was then determined and used to convert the data from wet basis to dry basis.

4.2.8 Statistical Analysis

One-way analysis of the variance (ANOVA) in combination with Tukey's test was used to evaluate the effect of the steamable bag design on the physical quality and nutritional content of frozen broccoli. The significance level used was $p \leq 0.05$. MINITAB® 16.1.1 Statistical Software (Minitab Inc., PA, US) was used for all statistical assessments.

4.3 Results and Discussions

4.3.1 Cooking Time

The time necessary for the frozen broccoli located in the center of each steamable bag to reach a temperature of 74 ºC (minimum cooking temperature recommended as safe) was determined and the results are presented in Table 2. 300 seconds was sufficient for the SB-I and SB-III to reach 74 ºC while 315 seconds were required for SB-II. These different cooking times were due to the different shape of the packages. Even though the SB-I and SB-III differed in microwavable surface area (526.44 cm^2 vs. 383.18 cm^2) both had a rectangular shape and cooked the broccoli located in the center of the steamable bag 15 seconds faster than SB-II. The latter

had a microwavable surface area (409.48 cm^2) similar to that of SB-III (383.18 cm^2) but a more square shape. Therefore, the shape of a steamable bag significantly affected the time necessary for a specific temperature to be reached and, therefore, the speed at which a steamable bag can cook a frozen product. Since the temperature reached in all the steamable bags at the cooking times determined was the same, these cooking times were used for cooking the broccoli for the quality and nutritional evaluations.

4.3.2 Ascorbic Acid

Broccoli is known to be a significant source of vitamin C (Vallejo, Tomas-Barberan & Garcia-Viguera, 2002). L-ascorbic acid is the predominant form of vitamin C found in broccoli and other foods of plant origin (Erdman et al., 1982). It is a common practice to use ascorbic acid as an indicator of the effect of food processing (heating) since vitamin C is considered one of the most labile vitamins. Thus, the effect of the different steamable bags on the loss of ascorbic acid in frozen broccoli during microwaving was determined and the results are presented in Figure 3. Significant differences ($p \leq 0.05$) in ascorbic acid content were found between the broccoli cooked by different steamable bags. Broccoli from SB-II (square-shaped bag) had significantly ($p \leq 0.05$) higher ascorbic acid content in both fresh weight (F.W.) basis and dry weight (D.W.) basis than broccoli from other steamable bags (rectangular-shaped bag). This was due to the lower heating environment reached inside the SB-II since this steamable bag was the one that cooked the broccoli the least as supported by the firmness results (Table 3). Since the broccoli in SB-II was cooked longer and yet had the highest ascorbic acid content, cooking the broccoli for additional 15 seconds did not affect its ascorbic acid content. Steamable bags are designed to control the amount of heat generated by releasing steam once a specific internal temperature in

the product is achieved. Comparing the remaining two steamable bags, the broccoli from SB-I had significantly ($p \leq 0.05$) higher ascorbic acid content than the broccoli from SB-III (73.2 $\pm$ 0.5 mg/100g F.W. and 669.3 $\pm$ 4.6 mg/100g D.W. vs. 70.7 $\pm$ 0.9 mg/100g F.W. and 658.6 $\pm$ 8.3 mg/100g D.W. for SB-I and SB-III, respectively). The higher loss of ascorbic acid in SB-III may be correlated to its smaller surface area, which cooked the broccoli the most as supported by the firmness results (Table 3). The specific internal temperature necessary to activate the valve was achieved faster in SB-III than in SB-I due to its smaller headspace which promoted faster hot air cycling which resulted in a higher amount of steam released from SB-III.

Vitamin C is a water-soluble vitamin and therefore, it was lost with the loss of steam through the valve (leaching). This loss of vitamin C due to leaching is supported by the differences in vitamin C content between fresh weight basis values and dry weight basis values. While the ascorbic acid content in fresh weight basis differed by 3.51% between the broccoli from SB-III and SB-I and by 2.34% between the broccoli from SB-I and SB-II, the ascorbic acid content in dry weight basis differed by 1.62% between the broccoli from SB-III and SB-I and by 2.99% between the broccoli from SB-I and SB-II. This difference between the ascorbic acid content in fresh weight basis and in dry weight basis results from the loss of water through the valve since the water loss is included in the dry weight basis calculations but not in the fresh weight basis calculations. Therefore, the low ascorbic acid content in the frozen broccoli from SB-III resulted from a higher amount of water loss from this steamable bag due to its capability to reach a specific internal temperature and thus able to activate the valve to release the steam earlier than the other steamable bags. Loss of vitamin C associated to its water loss has previously been reported for frozen broccoli. Pellegrini et al. (2010) reported that retention of ascorbic acid in frozen broccoli after cooking via microwaving without additional water, basket

steaming and boiling were 80%, 59% and 41%, respectively. However, these same authors found that fresh broccoli cooked by microwaving without additional water has almost complete loss of ascorbic acid while fresh broccoli cooked via basket steaming and boiling maintains 77% and 82% of its ascorbic acid. This different retention of ascorbic acid between frozen broccoli and fresh broccoli cooked using a microwave was attributed to the presence of ice on the surface of the frozen broccoli which prevented a large amount of water loss during microwaving (Erdman et al., 1982).

Besides the loss of water-soluble nutritional compounds, a significant evaporation of water from broccoli can result in flavor changes (Vallejo et al., 2003). Therefore, the retention of water in broccoli during cooking plays an important role in the preservation of its nutritional compounds and physical quality. Since microwave steamable bags offer a cooking technology which protects the frozen food from large losses of water during cooking, these bags most likely minimize changes in flavor in addition to maintaining nutritional content. However, this minimal flavor change will depend on the design of the steamable bag system containing a valve.

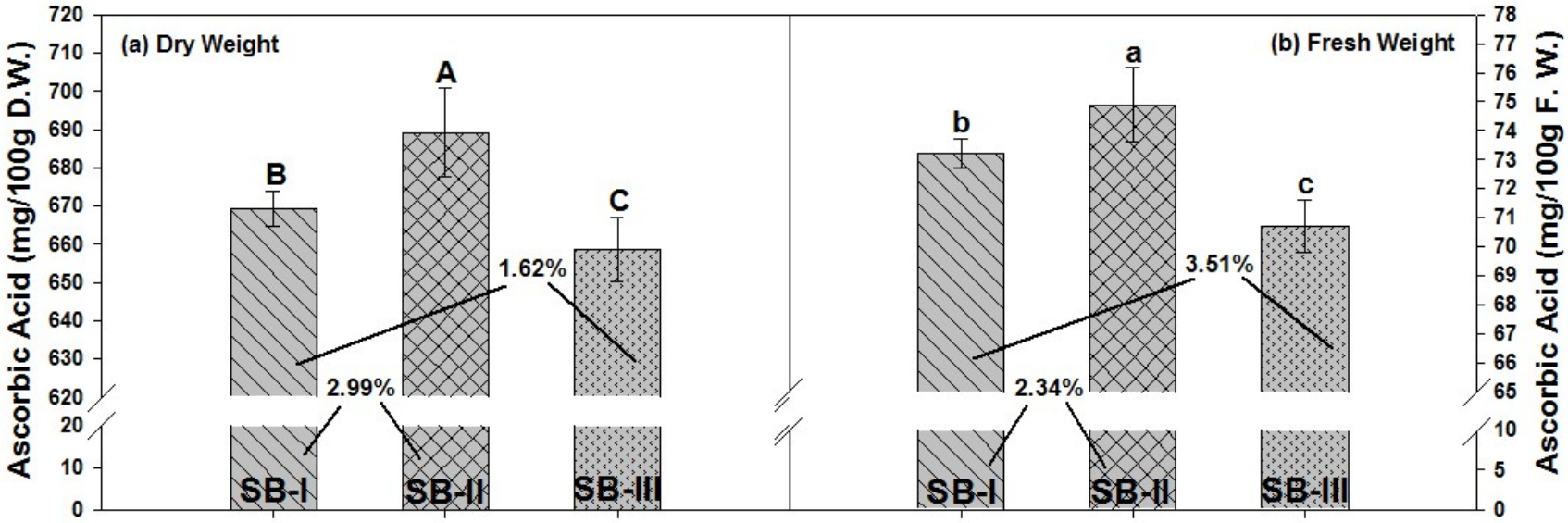


Figure 7. Ascorbic Acid Content of Frozen Broccoli cooked in Different Steamable Bag Designs in (a) Dry Weight Basis vs. (b) Fresh Weight Basis (numbers between columns indicate ascorbic acid percentage differences and different letters indicate significant differences ($p \leq 0.05$)).

4.3.3 Antioxidant Capacity

Antioxidant capacity is used to evaluate the integrated and synergic effects of the several different antioxidants found in a food product (Danesi & Bordoni, 2008). In the case of broccoli, hydrophilic antioxidants are the main contributors to its total antioxidant capacity. Kurilich, Jeffery, Juvik, Wallig and Klein (2002) determined that hydrophilic extracts are responsible for 80% to 95% of the total antioxidant capacity of fresh broccoli using the ORAC assay. Similarly, Wu, Beecher, Holden, Haytowitz, Gebhardt and Prior (2004) stated that hydrophilic antioxidants in *Brassica* vegetables provide more than 89% of the total antioxidant capacity and Roy, Juneja, Isobe and Tsushida (2009) found that 92% of the total antioxidant capacity of broccoli is provided by its hydrophilic extract. Taking this into consideration, only the hydrophilic compounds of the broccoli were extracted and evaluated in this study.

Figure 4 summarizes the ORAC values of the frozen broccoli cooked in the three microwave steamable bags. These ORAC values are similar to the ORAC values reported for uncooked broccoli. Kurilich et al. (2002) determined that the antioxidant capacity of 8 genotypes of uncooked fresh broccoli ranged between 38.1 and 121.6 μM TE/g D.W. using the ORAC assay. Similarly, Ou, Huang, Hampsch-Woodill, Flanagan and Deemer (2002) reported ORAC values for uncooked fresh broccoli between 23 and 208 μM TE/g D.W. As observed in Figure 2, the different designs of the microwave steamable bag did not have an effect on the antioxidant capacity of the frozen broccoli during microwaving. This means that the differences in cooking time between bags, temperature achieved inside the bags and/or steam released from the bags were not enough to produce a change in the antioxidant capacity of the cooked frozen broccoli.

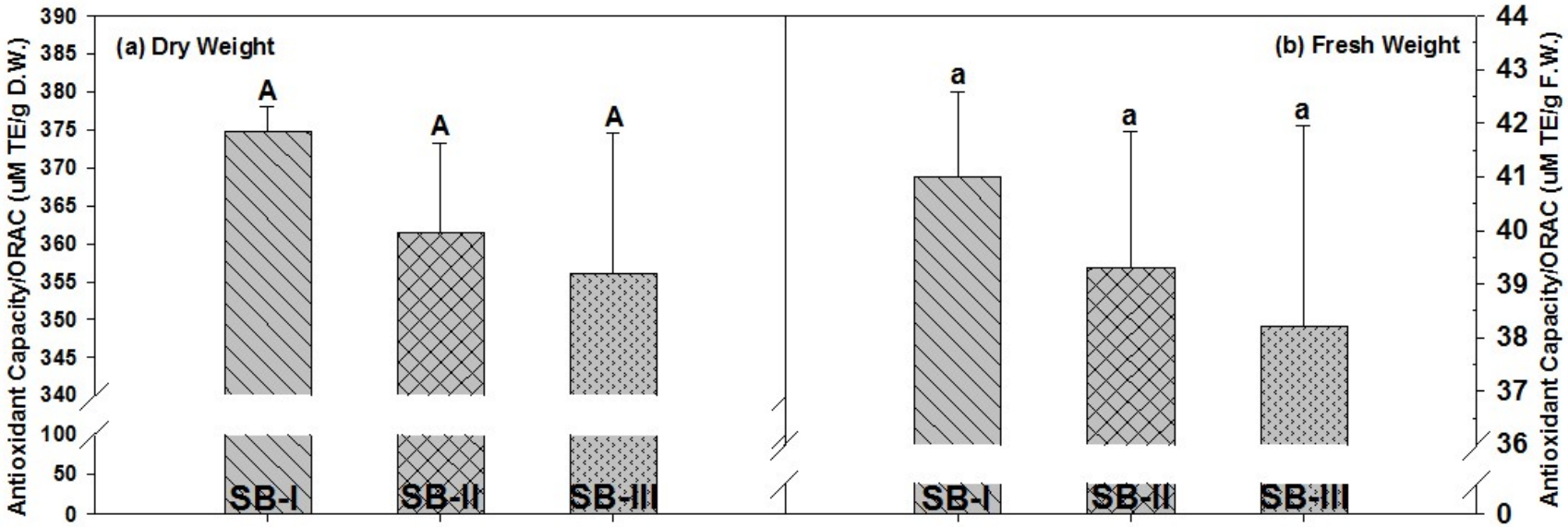


Figure 8. Antioxidant Capacity (ORAC) of Frozen Broccoli cooked in Different Steamable Bag Designs in (a) Dry Weight Basis vs. (b) Fresh Weight Basis (different letters indicate significant differences ($p \leq 0.05$)).

4.3.4 Firmness

The heating of vegetables is intended to tenderize them for consumption (Tijskens, Schijvens & Biekman, 2001). Thus, the firmness of the cooked frozen broccoli was evaluated to determine the effect of the steamable bag design on its tenderization. Significant differences ($p \leq 0.05$) between the shear force values of the frozen broccoli cooked in SB-I, SB-II and SB-III were found as shown in Table 3. Frozen broccoli cooked in SB-I and SB-II was 27% and 13% firmer, respectively, than that cooked in SB-III. The difference in firmness between the broccoli in SB-I and SB-III was due to the different surface area of the packages, 526.44 cm^2 and 383.18 cm^2, respectively. The smaller the surface area of the steamable bag, the softer the broccoli is after cooking. This result can be explained by the role of the surface area of the steamable bag in the loss of water from the broccoli. Under the same microwaving conditions (microwaving power and cooking time), the amount of steam necessary to activate the valve was generated faster in SB-III than in SB-I due to its smaller headspace. Consequently, more steam was released from SB-III than from SB-I. This resulted in a higher loss of water for the frozen broccoli cooked in SB-III. Changes in the firmness of plants have been correlated to loss of water. The water content of plants has a direct effect on the turgor of their cells and the degree of cellular hydration is known to result in noticeable changes in plant firmness (Sams, 1999; Jacobsson, Nielsen & Sjoholm, 2004). Therefore, a steamable bag like SB-III that allows a faster release of steam would tenderize the frozen products faster under the same microwaving conditions.

The difference in firmness between broccoli in SB-II and SB-III resulted from a combined effect of surface area and shape of the steamable bag. SB-II and SB-III had very similar surface areas, 409.48 cm^2 and 383.18 cm^2, respectively. This difference in surface area of 26 cm^2 seems

not enough to cause a 13% difference in broccoli firmness if compared with the 27% difference in broccoli firmness caused by a difference in area of about 143 cm^2 (SB-I vs. SB-III). Therefore, the different shapes of the steamable bags probably had an effect on tenderizing the frozen broccoli. The square-shaped steamable bag seems to provide a lower heating environment for the frozen broccoli during microwaving that leads to less tender broccoli. This agrees with the cooking time results which showed that more time is necessary for cooking the frozen broccoli in a square-shaped steamable bag than in a rectangular-shaped steamable bag. Therefore, there appears to be a relation between broccoli tenderization and steamable bag's surface area and shape. Both of these parameters significantly affected the firmness of the cooked broccoli and would most likely affect consumer acceptance. This shows the importance of controlling the surface area and shape of the steamable bag.

4.3.5 Color

The color of frozen broccoli florets cooked in the three studied microwave steamable bags is presented in Table 3. No differences was found between the L* values of the florets cooked in the three different steamable bags. Zhong, Dolan and Almenar (2014) reported that the frozen broccoli cooked in steamable bag maintains its intense darkness (L* value) compared to thawed frozen broccoli. The design of the steamable bags did affect other color parameters of the frozen broccoli after steamable bag microwaving. The florets cooked in SB-II were significantly ($p \leq 0.05$) less green (less -a* value) than those cooked in SB-I and SB-III. No differences between the green color of the florets cooked in SB-I and SB-III were observed. The reason for the frozen broccoli cooked in SB-II to be less green than those cooked in SB-I and SB-III is most likely due to the relative lower heating environment in the SB-II that resulted in 15 seconds additional time

of cooking. Boekel (1999) and Turkmen, Poyrazoglu, Sari and Velioglu (2006) reported that the green color of vegetables is mainly related to chlorophyll pigment content and the loss of greenness is generally associated with the degradation of chlorophyll pigments and formation of pheophytins during heat processing. Therefore, the cooking of the frozen broccoli for 15 seconds more in SB-II induced more chlorophyll degradation and pheophytin formation which led to an increased a* value. Broccoli florets cooked in SB-I became yellower (increased +b* value) than those cooked in SB-II and SB-III. This difference could be attributed to differences in matrix structure (softness) between the broccoli florets, as supported by the firmness results, which changed the light penetration and surface-reflecting properties of the broccoli florets (Miglio, Chiavaro, Visconti, Fogliano & Pellegrini, 2008; Oey, Lille, Loey & Hendrickx, 2008). The hue angle of the florets cooked in SB-II shifted more towards yellow ($p \leq 0.05$) than that of the broccoli cooked in SB-I and SB-III. This lower hue angle could be attributed to the combined effects of a* and b* values since both of them decreased and this did not happen in broccoli florets cooked in the other steamable bag designs. Therefore, the color results show that the design of the steamable bag has an important effect on color maintenance and consumers' acceptance of the cooked vegetables.

Table 3. Color and Firmness of Frozen Broccoli Cooked in Different Steamable Bags Designs

	Color[1]				Firmness[2] (Kg-force/g)
	L*	a*	b*	Hue°	
SB-Ⅰ	19.87 ± 1.20a	−11.44 ± 0.94b	19.30 ± 1.91a	120.70 ± 1.70b	3.40 ± 0.39a
SB-Ⅱ	19.78 ± 1.76a	−10.22 ± 0.92a	18.42 ± 2.08ab	119.09 ± 1.95c	3.02 ± 0.34b
SB-Ⅲ	20.06 ± 1.25a	−11.31 ± 1.14b	17.97 ± 2.28b	122.28 ± 2.22a	2.68 ± 0.49c

Means in rows followed by different letters differed significantly ($p \leq 0.05$)

[1]Values presented as mean ± SD (n = 15)

[2]Values presented as mean ± SD (n = 20)

5. Conclusions

This is the first time that the effect of steamable bag microwaving and the shape and surface area of a steamable bag on changes in nutritional content and physical properties of a frozen food product during cooking has been investigated. The nutritional content of frozen broccoli can be increased using steamable bag microwaving since this cooking method decreases ascorbic acid only slightly while it notably increases antioxidant capacity of cooked frozen broccoli compared to thawed frozen broccoli. Steamable bag microwaving also affects the physical properties of frozen broccoli since the broccoli is tenderized and changes in color but only in terms of greenness and not lightness or yellowness compared to thawed frozen broccoli. The nutritional content of frozen broccoli cooked by steamable bag microwaving is the same as that of frozen broccoli cooked by steamer steaming and higher than that of frozen broccoli cooked by traditional microwaving. Less color change and faster tenderization of frozen broccoli are obtained using steamable bag microwaving compared to steamer steaming or traditional microwaving. These findings show that steamable bag microwaving is a cooking method that increases nutritional content, tenderizes fast and produces minimal color changes in frozen broccoli, which fulfills current consumers' needs.

Furthermore, both the shape and the surface area of a steamable bag (with a steam release valve) can significantly affect the nutritional content and physical properties of frozen broccoli. Frozen broccoli cooked in a more square-shaped steamable bag was significantly less green and more yellow in color, less soft, and retained higher vitamin C content than frozen broccoli cooked in a more rectangular-shaped steamable bag. The lower heating environment created by the square shape of the steamable bag results in less loss of vitamin C and hardness due to less water loss from the steamable bag but a greater change in color due to the longer cooking time.

The smaller the surface area of the steamable bag the softer the broccoli became after cooking, independent of the shape of the steamable bag. However, this trend was not observed for either color or vitamin C content. For steamable bags with a more rectangular shape, a smaller surface area results in less time needed to achieve an internal temperature at which the steam release valve opens and releases water. This results in softer broccoli and lower ascorbic acid retention, but does not affect the color of the broccoli. Neither the shape nor the surface area of the steamable bag had an effect on the lightness and antioxidant content of the broccoli. It is concluded that the design of a steamable bag (with a steam release valve) can optimize the preservation of nutrients and the physical properties of cooked frozen food, such as frozen broccoli.

6. Future Work

Only one type of steam release devices (steam release valve) and a constant location of the valve (in the center of the steamable bag), were studied. However, the steam release valve location may affect its activated time which leads to new changes of nutrition value and physical quality of frozen broccoli after cooking. Moreover, different steam release devices, such as contaminated seal, mechanical score and perforations, have different influences on the packaging hermeticity and generate different steaming environment inside the steamable bag. Therefore, future study can be conducted to better evaluate the performance of steamable bag microwaving and steamable bag designs.

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