EFFECTS OF DIFFERENT DRYING METHODS ON THE TOTAL PHENOLICS, ANTIOXIDANT PROPERTIES, AND FUNCTIONAL PROPERTIES OF APPLE POMACE

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ABSTRACT

EFFECTS OF DIFFERENT DRYING METHODS ON THE TOTAL PHENOLICS, ANTIOXIDANT PROPERTIES, AND FUNCTIONAL PROPERTIES OF APPLE POMACE

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Apple pomace was dried using three different drying methods: freeze drying at air temperature of 20ºC, drum drying at drum temperature of 140ºC, and cabinet drying at air temperatures of 60 ºC, 80 ºC, and 100 ºC. The dried samples were measured using Hunter Color CIE L*,a*, and b* values. The total color difference ($\triangle E$) of apple pomace was affected by the drying temperature and the type of drying method. The freeze dried pomace was significantly higher in L* value compared to all of the other samples, whereas cabinet drying at 100℃ showed the highest average color a* and b* values. The dried apple pomace was analyzed for total phenolics and antioxidant capacity using ORAC, and DPPH. The total phenolics of dried pomace, with the highest value (3.05 mg GAE/g) observed in freeze dried and the lowest (1.85 mg GAE/g). In the cabinet dried pomace, total phenolics decreased gradually as the process temperature was increased from 60 ℃ to 80 ℃ or 10 0 ℃. Freeze dried pomace had the highest antioxidant activity as exhibited by ORAC and DPPH results, 350.27 and 278.8 µmol TE/g, db, respectively. Drum drying of pomace at 140 ℃ reduced activity to 158.06 and 216.45 µmol TE/g, db, by ORAC and DPPH assays, respectively. Cabinet drying of pomace resulted in lower antioxidant values as drying temperature increased from 60 ℃ to 80 ℃ or 100 ℃.

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INTRODUCTION

Fruit juices are considered “healthy” drinks. In recent years, consumption and exporting of juices have increased because of the improvement in processing methods and transportation (Askar 1998). In 2006-2007 the world produced 46.1 million tons of apples. The country that produces the most apples is China, which harvests more than 50 % of the world’s apples followed by the USA. The world processes 25-30% of the fruit into juice (Figure 1) (Bhushan and others 2008). Brazil produces 800,000 tons of apple pomace each year (Protas 2003).Michigan ranks second in apple production in USA. Many of the apple-growing farmers have small orchards. According to Michigan Apple Committee, in 2013, Michigan harvested approximately 1260 million pounds of apple, and the average of harvest is 828 million pounds of apples per year. Besides fresh consumption, a large portion of production is used to produce apple juice/ cider. The remaining residue after juice extraction (skins, flesh, and stems) is considered to be a waste product, and is called pomace. Apple pomace is nutritionally rich and contains bioactive compounds, such as antioxidants. To process apple pomace into a value-added ingredient, moisture must be removed by drying, which can be done using different drying methods (e.g., freeze drying, cabinet drying, and drum drying). These methods are different with respect to cost, processing time, heat application, and production rate.

The objectives of this research were to compare the effects of three different drying methods (drum drying at one drum temperature at 140 ℃, freeze drying at 20 ℃, and cabinet drying at three different temperatures 60 ℃, 80 ℃ and 100 ℃) :1) on the

nutritional characteristics of total phenolics and antioxidant activity as assayed by ORAC and DPPH; and 2) on color values for the quality of apple pomace (Hunter Color CIE).

1 LITERATURE REVIEW

1.1 Apple Pomace

Apple pomace is a by-product that is generated by processing apples into different apple products such as juice, cider, and wines (Figure 1) (Vendruscolo and others 2008). Recently, the emphasis on apple pomace has been to utilize it for the extraction of value-added products, such as antioxidants and dietary fiber (Bhushan and others 2008). Other uses for apple pomace include extraction of pectin, animal feed, and more recently fermentation to produce citric acid or alcohol (Hang 1987). Apple pomace has started to be increasingly used as source of apple fiber and bioactive compounds (Walter and others 1985), which in turn has been incorporated into cookies, granola bars, and muffins to add to the overall fiber value (Ingredients 2012); (Carson and others 1994). Although the addition of apple pomace is good for products nutritionally, it lowers the overall appearance, texture, and flavor (Beereboom and Glicksman 1979). Of these issues, only the flavor can be addressed by the addition of spices, and use of flavors such as vanilla (Belshaw 1978).

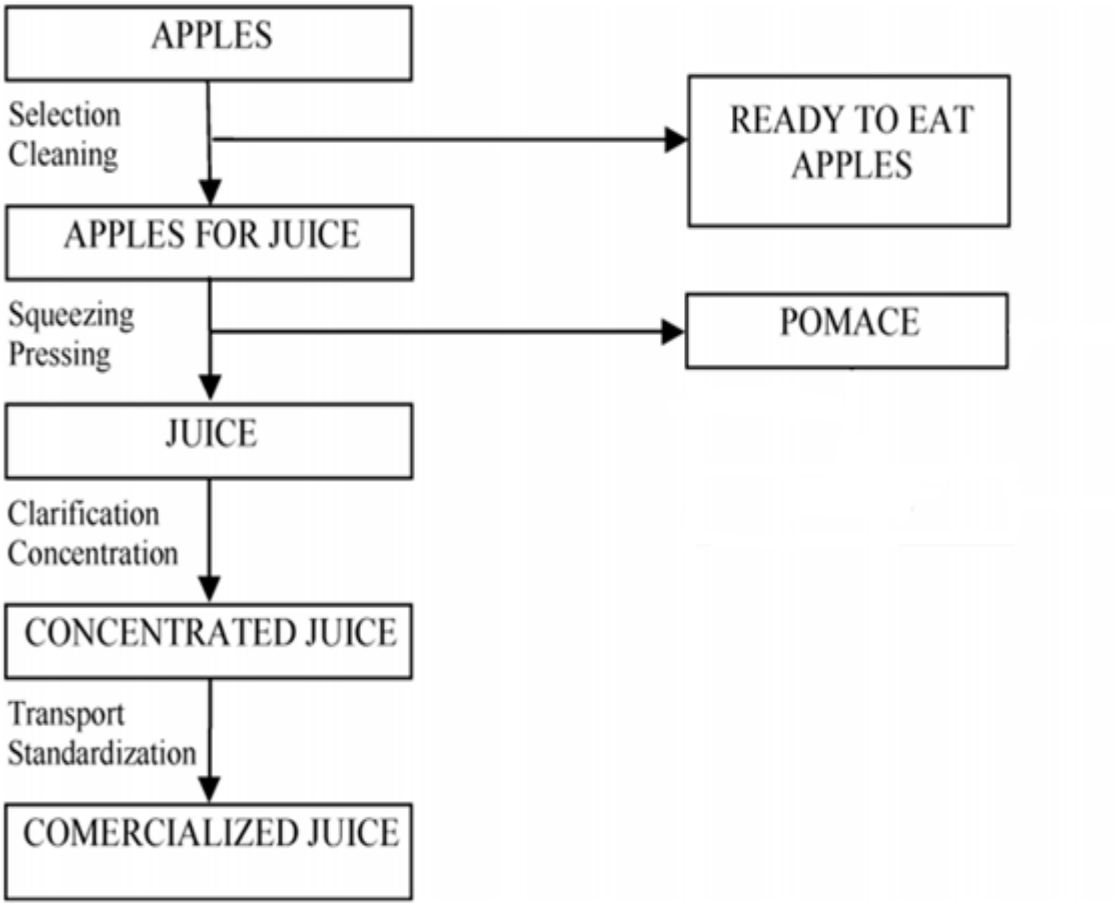

Figure 1. Apple processing for juice production

1.2 Antioxidants

Antioxidants play an important role in our health; as they may protect us from serious diseases such as cancer (Borek 1997). An antioxidant is defined as “any substance, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell 1989).” Oxygen is important for life, and is used for energy production in our bodies. However, 1-3% of oxygen we breathe has detrimental effects that make “reactive oxygen species” (ROS), which include superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2). Through providing contact with metal ions that make free radicals, which are atoms with an

unpaired electron created by interaction between oxygen and molecules. Free radicals attack and damage almost everything in our bodies If O_2^- and H_2O_2 combine with transition metal ions, the resulting free radical species can damage the human body (Halliwell 1997).

The antioxidants come from different sources, such as enzymes (catalase), large molecules (albumin), small molecules (polyphenols) and hormones (melatonin) (Prior and others 2005). Also, fruits and vegetables contain many antioxidants such as vitamin C + E, found in berries, tomato garlic, ginger, carotenoids and apple pomace (Moure and others 2001).

The antioxidants are divided into two classes: 1) primary antioxidants that are called chain-breaking antioxidants, they can interact with lipid radicals which result in more stable products , and 2) secondary or preventative antioxidants, which retard the oxidation rate (Antolovich and others 2002). The difference between them is that the secondary antioxidants postpone the oxidation by interfering with the prooxidant system, and the secondary antioxidants disable the conversion of free radical species to a more stable product (Abulude and others 2013) .

1.3 Phenolic Components

Phenolic compounds are free radical scavenging molecules present in fruits and vegetables, and include phenolic acids, flavonoids, coumarins (Larson 1988). There are some studies showing that phenolic compounds from plants are more efficient than vitamins E or C in our bodies as antioxidants (Rice-Evans and others 1997). Therefore, phenolic compounds may have an important role in protecting our health (Rice-Evans

and others 1997). Recently, many research studies have focused on the importance of phytochemical components such as phenolic acids, phenylpropanoids and flavonoids because they have an active role in antioxidant activity (Rice-Evans and others 1997).

Phenols are divided into different groups. The first group is simple phenols that contain an aromatic ring with one or more -OH groups (Schwannecke 2009). Phenolic acids in the form of substituted derivatives of hydroxybenzoic and hydroxycinnamic acids are the predominant phenolic acids in fruits. These derivatives differ in patterns of hydroxylations and methoxylations of their aromatic rings (Lule and Xia 2012). The second group, phenol carboxylic acids, include simple phenols, that bear a carboxyl group. The third group of compounds, phenyl propane, have an aromatic ring that has 3 carbon atoms. The fourth group, flavan derivatives, is a flavan skeleton that consists of 3 rings. A and B are an aromatic ring and the ring in the center contains oxygen (figure 2) (Hess 1975).

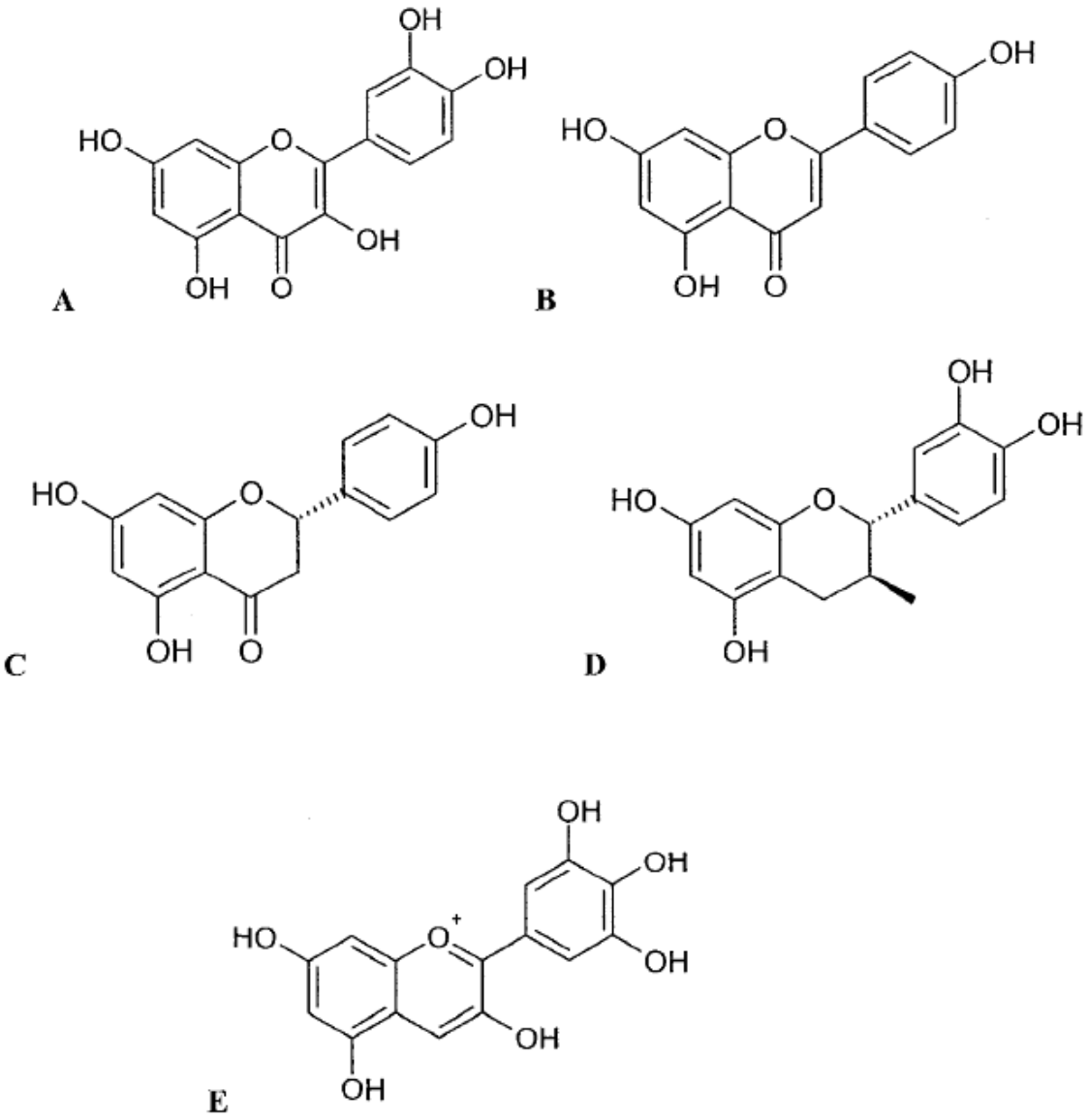

Figure 2. A quercetin (flavonol); B apigenin (flavone); C naringenin (flavanone); D (+)-catechin (flavan-3-ol); E cyaniding (anthocyanidins)

1.4 Pomace drying

The drying of pomace has been done by other groups. Yan and Kerr (2012) dried apple pomace by vacuum-belt drying at 80 ºC, 95 ºC and 110 ºC, they measured total phenolics content, anthocyanins, dietary fiber content and color properties. Also, Sogi and others (2013) dried mango peel and kernel by different drying methods (freeze drying at , hot air drying, vacuum and infrared drying), they measured total phenolics, antioxidant activity, and functional properties. However, in this study, the drum drying method has been added. This drying method has favorable characteristics for commercial production, such as low cost, rapid drying time, and large throughput.

1.5 Drying Methods

There are many drying methods that are commonly used for fruits, such as spray drying, hot air drying, drum drying, freeze drying, and microwave-vacuum drying. Fresh fruits have a short shelf life because they have a high moisture content and high sugar content, allowing microorganisms to grow.. Thus, drying will help extend the shelf life of fruits through reducing water activity. In industry, much attention is paid to the quality of products. As mentioned, the fruits have valuable components of vitamins, minerals, fiber, etc. Thus, these components can be preserved through drying processing. Polyphenolic compounds in fruits are sensitive to high temperature. For example, drum drying exposes the product to temperature from 95 to 100 C (Hsu and others 2003) or as high as 130℃. Due to sublimation, freeze drying does not damage the structure or quality properties of product (Mejia-Meza 2008). Principles of Drum Drying

Drum drying is one of the most common methods used worldwide for drying the food in pureed form. This method is appropriate for pureed foods or foods that become

pureed after treatment such as milk, mashed potato and fruit pulps (Falagas 1985). There are many products coming from the drum drying method, such as breakfast cereals, yeast and fruit puree (Moore and Dekker. 1995). Compared to other drying methods; drum drying has the best efficiency in terms of high rate of production and low labor requirements (Moore and Dekker. 1995).

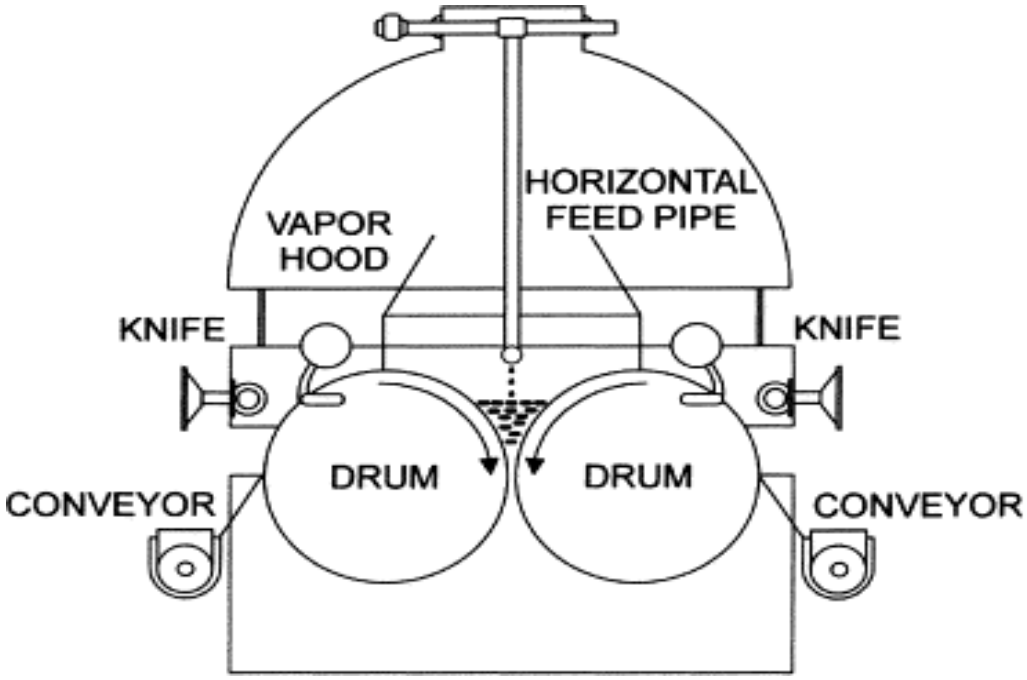

Figure 3. Schematic representation of a top loading double drum dryer (GOUDA)

A drum dryer usually consists of two drums that have the same diameter that are oriented close to each other (Figure 3) (Matsumoto and others 2000). These drums are heated by steam that goes inside the drums to make the surfaces hot. These drums rotate in opposite directions. The product is continuously poured in the crevice between the drums to create a pool of product that is squeezed to the thickness of the space

between the drums, and begins to dry on the drum surfaces. Two fixed knives touch the surfaces of drums to continuously scrape and remove the dried product to a container below the drums. The important factors that affect the product are: steam pressure inside the drums, the clearance or gap between the drums, rotational speed, pool level between the drums and chemical and physical characteristics of sample being fed (Gavrielidou and others 2002).

1.5.1 Principles of Hot-air Drying

The hot-air drying method is used to remove water from fruit. The purpose of this drying method is to decrease the moisture content in the fruit, to avoid growth of microorganisms and reduce the activity of enzymes, so that the products can be stored for a long time (Schwannecke 2009) ; (Feng and others 2002). Hot-air drying removes most of the water from the product by evaporation (Fellows 2000). In hot-air drying, removing the first 33% of moisture uses about 66% of the total time of drying. Also, the components of the product may not be stable under thermal processing, possibly damaging the structure of the crust of vegetables (Zhao 2000). In China, because of the low cost, hot-air drying is used for over 90% of dried vegetables. However, the quality of these dried products is poor (Zhang 1999).

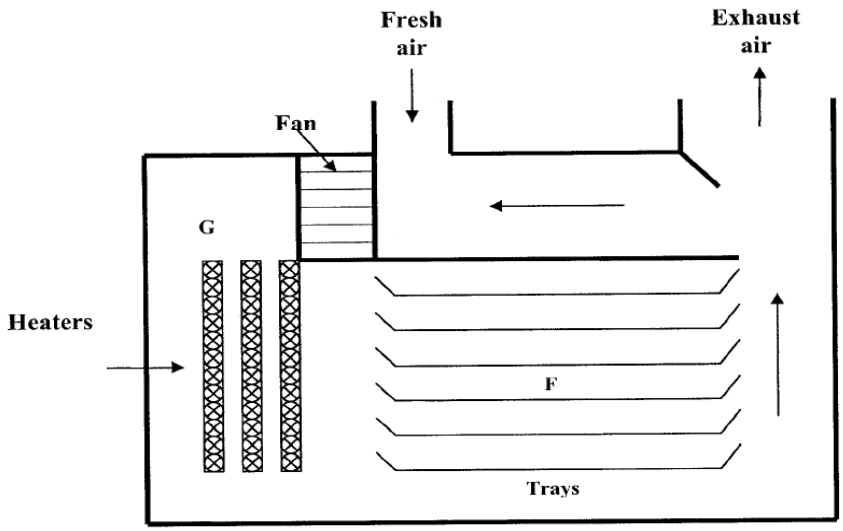

Figure 4. Schematic Diagram of a Hot-Air Dryer

Trays containing the product are exposed to hot air, which passes along the surface of the product (Figure 4) (Tang and Yang 2003). Trays have the ability to hold a depth of 2-6 cm of product per tray (Tang and Yang 2003).The passage of hot air in an enclosed cabin from above and below the product will increase the effectiveness of drying. There are influential factors on the rate of drying efficiency, such as the air speed and heaters (Mejia-Meza 2008). Low relative humidity maintains hot air drying efficiency, through the integration of fresh air and hot air in the enclosed cabin, which are connected with the product to remove moisture (Mejia-Meza 2008).

1.5.2 Principles of Freeze Drying

The main purpose of freeze drying is to preserve fruits for an extended time, especially for fruits that are sensitive to normal or high temperature. This drying method is expensive and takes a relatively long time of 12 to 24 hours (Mejia-Meza 2008). The principle of the freeze drying method is based on two phases. The first one is to freeze the fruit and the second one is to remove the moisture via sublimation of the ice from

the fruit that is frozen (Oetjen 2004). Packaging materials are excellent barriers to prevent the oxidation if the dried fruit is frozen. If dried fruit is not frozen the dried fruit can easily be rehydrated, which may cause the oxidation. (Oetjen 2004).

The drying cabinet provides vacuum and refrigeration, and contains a heating shelf and trays. Heating temperature in the drying cabinet is 20 ºC and in the condenser cabinet from −30 ºC to -60 ºC (Mejia-Meza 2008). In this method we have two phases: The first phase is the freezing of fruits quickly at atmospheric pressure and at low temperature of −20 ºC**.** The second phase is the reduction of the pressure to below the triple point of water, when moisture will leave the fruit as sublimated vapor, which will minimize damage to the fruit structure (Fellows 2000) that occurs with other drying methods.

1.6 Analysis of Total Phenlics and Antioxidant Capacity

1.6.1 Total Phenolic Assay

There are many methods to determine total phenolics such as traditional methods that depend on absorbed radiation measurement in the ultraviolet region (Somers and Ziemelis 1972). Folin–Ciocalteu's reagent has been used to determine total phenolics since 1965, and was developed by Singleton and Rossi (Singleton and Rossi 1965). Gallic acid is a popular reagent to estimate total phenolics as molar equivalents (Arnous and others 2001). Also, gallic acid and 3,4,5-trihydroxybenzoic acid ($C_7H_6O_5$) are commonly used to determine total phenolics in fruits and vegetables (Protas 2003).

1.6.2 Oxygen Radical Absorbance Capacity (ORAC)

ORAC assays are used to determine the activity of antioxidants and their relationship with total carotenoid content, total phenolics and ascorbic acid in fruit (Thaipong and others 2006). This assay is based on the inhibition of the peroxyl-radical induced oxidation, which is initiated by heat-induced or thermal decomposition of azocompounds [e.g., 2,2'-azobis(2-amidino-propane) dihydrochloride or AAPH] (Ganske and Dell 2006) (Glazer 1990). On the basis of this technique, the ORAC assay utilizes a biological relevant radical source thus uniquely combining both the inhibition time and the degree of inhibition into one quantity (Ou and others 2001). Some modifications have been made to the ORAC assay, which include the use of fluorescein as the probe (Ou and others 2001).

Advantages of the ORAC assay include: the free radicals are used from the biological perspective; and the ORAC method is a good comparison tool between different results from different laboratories and it combines reaction time and reaction degree of antioxidants, . Disadvantages: It requires expensive equipment, it is possible to get data with large variability, it is sensitive to pH, and it is time-consuming (Frankel and Meyer 2000).

1.6.3 Diphenylpicrylhydrazyl Assay (DPPH)

(DPPH) is a stable free radical (diphenylpicrylhydrazyl) that is used to determine antioxidant activity (Gil and others 2002). DPPH is a common method to estimate the antioxidant activity (Sharma and Bhat 2009). DPPH measures the efficiency of antioxidants at room temperature to avoid the deterioration of molecules from the heat, but structural conformation of the antioxidant plays an important role in mechanical

interaction between DPPH and antioxidants (Bondet and others 1997). Compounds can be divided into two groups based on reaction with DPPH: (1) there is a minority that react very rapidly with DPPH (2) there is a the majority of compounds that react slowly with DPPH and they have a complicated mechanism (Bondet and others 1997). Also, there is a combination of factors that affect absorption of DPPH such as pH, oxygen and light (Ozcelik and others 2003).

2 MATERIALS AND METHODS

2.1 Apple Powder Preparation

Raw apple pomace (Rome,Spy, Ida red, Jonathan, Joagold, empire and York) was obtained from Peterson Farms (3104 West Baseline Road, Shelby, MI 49455. The raw material was grounded by coffee grinder (Cuisinart, Model DCG-20, East Windsor, NJ) at room temperature 25 ºC. Dried samples (15 g each) were loaded into the pulverizer. Samples were prepared for antioxidant extraction, by crushing the sample, placing in dark bags and keeping in the freezer. Samples were taken from four locations of the batch. Moisture content was measured by moisture analysis (Santorius Corporation, Bohemia, NY, 11716), 1.5 – 3 grams were loaded on the analyzer. Drying measurements took approximately 30 minutes for each sample (Raw and dried sample).

2.2 Sample Preparation

The pomace was kept in a freezer at – 9 ºC in dark bags to avoid losses of antioxidants by light; each bag contained approximately 45g. The sample was thawed at room temperature of 25 ºC before drying.

2.3 Drying Methods

Three drying methods were used; drum drying, hot air drying, and freeze drying. The reason for drying was to remove moisture from apple pomace while saving valuable quality components, such as antioxidants and phenolic compounds, the levels of the components were compared using chemical assays.

2.3.1 Drum Drying

Samples of 1.6 Kg of apple pomace were mixed with water (1:2) to pour the slurry onto a drum dryer (American Drum Dryers, Overton Machine Company, Dowagiac, Michigan USA). The experiment was conducted three times. Drum drying was run continuously for 15 minutes at a drum surface temperature of 140 ℃. The temperature of the drum was estimated using an infra-red thermometer (Model 9645, CEN-Tech Company). The sample slurry was dried by pouring it on to the hot surface of the drums, then by using knives to continuously scrape off the sample from the drum so that the dried product was collected in stainless steel trays.

2.3.2 Hot-air Drying

The sample was dried at different air temperatures at 60 ℃, 80 ℃ , or 100 ℃. The drying time was 25, 20 and 15 minutes respectively. The perforated tray was loaded with approximately 150g for each temperature. The velocity of air was 15.2 m/s measured with a hot-wire anemometer (Model number 407001, Extech Instruments Corporation, the address is 9 Townsend West Nashua, NH 03063 U.S.A). The relative humidity was 30.7%, 35.3% and 39% respectively. After drying, the content of moisture in apple pomace was 3.0%, 3.0% and 3.2% respectively.

2.3.3 Freeze Drying

The freeze drying machine that used to dry apple pomace was Virtis Genesis (25L Genesis SQ Super XL-70, 2010). The freeze dryer had a sample chamber, seven shelves, a vacuum pump and a refrigerated condenser chamber. The shelf temperature was lowered to−30 ℃, and the sample was placed ont o shelves with the chamber closed. The condenser was lowered to –60 ºC and the vacuum pump was turned on

until it pulled a near complete vacuum, approximately 50 mTorr. Then, the shelf heater was turned on to warm up the samples to around 20 ºC. Samples were dried for 3 days under these conditions, until the dryer had a stable reading of around 10 mTorr pressure inside the freeze dryer. The samples were removed and kept frozen in dark colored polyethylene bags at −20 ℃.

2.4 Hunter Color CIE

Raw apple pomace's color depends on the kind of apple. This color plays an important role in food additives, in making the food attractive. Apple pomace color is affected by drying method. The color is sensitive to heat, oxygen, enzymes. For example, in the drum dryer, the high temperature will affect the color because the temperature of drums is around 135 ºC to 140 ºC . Color was measured before and after the treatment. The change of color indicates that the product loses part of its quality through degradation of some important components of apple pomace.

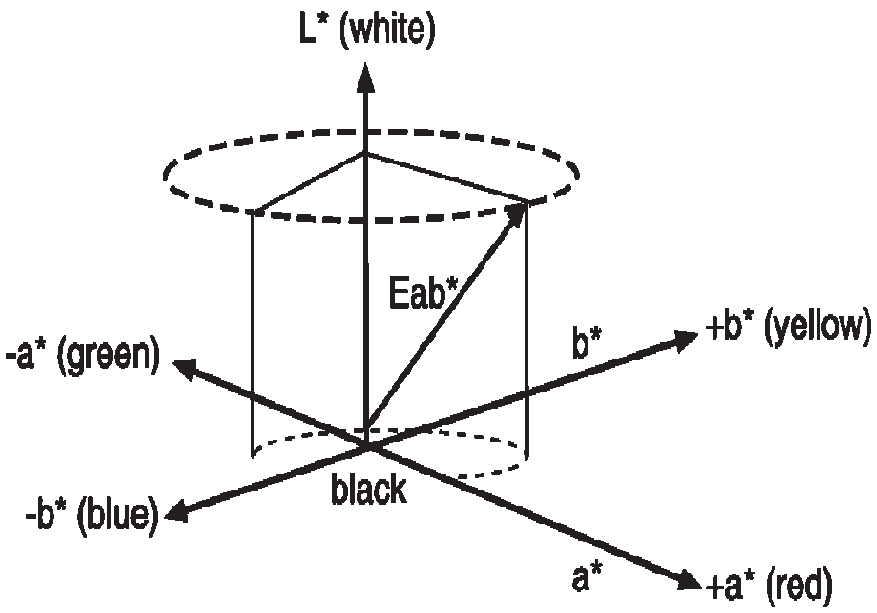

Figure 5. Scheme of L*a*b* model

There are different models to measure the color, such as the L*a*b* model, where RGB represents red, green and blue, and CMYK means cyan, magenta, yellow and black (Yam and Papadakis 2004). In this work, the Hunter color parameters and L*a*b* model (Minolta Color Reader CR-10, Ramsey, NJ) was used for color evaluation. where, *L** means lightness component that has specific range from 0 to 100, *a** means the component from green to red and b* means the component from blue to yellow (Figure 5) (Okubo and others 1998) (Yam and Papadakis 2004).

2.5 Antioxidants Extraction

For extraction of phenolics and antioxidants, the method of Sogi and others (2014) was followed. Briefly, dried pomace samples (2.5 g) were placed into separate tubes and 20mL of 80% methanol solution was added. The tubes were covered with aluminum foil. Then, they were put in a shaker for one hour at 200 g.

The samples were centrifuged for 10 min at 11,180 g . The supernatant was poured off into separate tubes. Then, 10 mL of 80% methanol was added into the residue, placed back in the centrifuge for an additional 10 min at 10,000 g and the supernatant was poured off again into a separate tube. This step was done twice and approximately 40 mL of product was obtained.

2.6 Determination of Total Phenolic Content

2.6.1 Preparation of Reagent

The total phenolic compounds test contains two reagents. Saturated sodium carbonate 7.5%; 7.5 g of anhydrous sodium carbonate was weighed and dissolved in 92.5 mL water. Gallic Acid (0−500 mg/L) was prepared in two steps (1) stock solution: 0.1 g of Gallic acid was weighed and dissolved in 10 ml ethanol to obtain 10 mg/ml, (2) working solution (100 mg/L): 200 µL of stock solution was weighed and ethanol was added to make the volume 20 ml to get 100 mg/L. The standard curve was made on these concentrations, as shown in Table 3.

Table 1. Gallic acid concentrations for the standard curve

Working solution (ml)	Ethanol (ml)	Gallic acid (mg/L)
0	5	0
1	4	20
2	3	40
3	2	60
4	1	80
5	0	100

2.6.2 The procedure of experiment

A volume of 0.5 mL of apple pomace extract was taken (1 part apple pomace extract: 10 part water) and added to 0.5 m Folin-Ciocalteu reagent (1+9 water) in a test

tube that was mixed thoroughly, and the solutions were incubated for 3 minutes. After incubation, 1 ml of saturated sodium carbonate (7.5%) was added followed by 1 ml of distilled water. The solution was then incubated for 2 hours in the dark at room temperature. A spectrophotometer was used at 750 nm and the absorbance was compared to 0.5 ml of gallic acid (0−100 mg/L) standard curve.

2.7 Determination of Antioxidant Activity: Oxygen Radical Absorbance Capacity (ORAC)

There are many ways to determine antioxidant level. ORAC assays are used to determine the activity of antioxidants and their relationship with total carotenoids contents, total phenolics and ascorbic acid in fruit (Thaipong and others 2006).

2.7.1 Preparation of Reagent

Preparation of stock solutions: Three stock solutions were prepared, which were sodium phosphate buffer (pH 7.4), using 11.741 g of dibasic sodium phosphate Na_2HPO_4 and 4.306 g of monobasic monohydrate sodium phosphate 1M NaH2PO4 that was mixed and stirred in 900-1000mL of distilled water, pH was adjusted to 7.4 by 1M NaOH and 1M HCl. Trolox (6-hydroxy -2,5,7,8-tetramethylchroman-2-carboxylic acid), from hydrophilic group (Zulueta and others 2009), Trolox stock 2.0 mM was prepared from 0.025g Trolox and 50 ml sodium phosphate buffer (pH 7.4) that were mixed and stirred in glass flask, which were then wrapped in foil and stored in the refrigerator. The final stock solution fluorescein was prepared from 0.1g Fluorescein and 100 ml sodium phosphate buffer (pH 7.4), which was wrapped in foil and placed in refrigerator. These stock solutions were stored for no more than three months due to their stability.

Dilutions of the three stock solutions were prepared on the day of the experiment. Fluorescein dilution was prepared by adding 10 ml of sodium phosphate buffer (pH 7.4) 10 μL of Fluorescein stock into test tube, then by vortexing the solution. Trolox was diluted to several different concentrations, 100μM (10mL sodium phosphate + 529 μL Trolox), 50 μM (10 mL sodium phosphate + 264 μL Trolox), 25 μM (10 mL sodium phosphate + 132 μL Trolox), 12.5 μM (10mL sodium phosphate + 66 μL Trolox) and 6.25 μM (10 mL sodium phosphate + 33 μL Trolox). Apple pomace dilution was prepared by adding 10 mL of sodium phosphate buffer (pH 7.4) and 100 μL of sample extraction into test tubes then mixing by vortex. Finally, AAPH (2,2′-azobis (2-amidinopropane) dihydrochloride) was prepared by adding 0.414g AAPH and 10 ml of sodium phosphate buffer into a test tube, then mixing thoroughly by vortex (Sogi and others 2014).

2.7.2 The procedure of experiment

Fluorometer Operation: The Biotek Plate Reader was equilibrated to 37 ℃. The wells were loaded as shown in Appendix Table 13: Blank wells were loaded with 25μL sodium phosphate buffer (pH 7.4). Trolox wells were loaded with 25μL of Trolox dilutions. Sample wells were loaded with 25μL of sample dilutions and 150μL diluted fluorescein solution was added to all experimental wells. Plates were placed in the fluorometer. After 30 minutes, 25μL of AAPH was added to all experimental wells and the plate was put again in the fluorometer for 3 hours to get the result.

2.8 Diphenylpicrylhydrazyl (DPPH) Antioxidant Assay

DPPH analysis: DPPH is stable free radical diphenylpicrylhydrazyl that was used to determine the antioxidants (Gil and others 2002). DPPH analysis is common method to measure antioxidant activity (Sharma and Bhat 2009).

2.8.1 Preparation of Reagent

The DPPH test uses 2.0 mM Trolox. Thus, the standard curve was made from Trolox concentrations listed in Table 2.

Table 2. Trolox concentrations used for the standard curve

Trolox 2.0 mM (ml)	Ethanol (ml)	Trolox concentration (µM)
0	4	0
0.1	3.9	50
0.2	3.8	100
0.3	3.7	150
0.4	3.6	200
0.5	3.5	250

$DPPH^+$ was prepared from 10 ml of DPPH stock and 50 ml of Ethanol. distilled water was used to obtain 100% Transmittance at 515 nm to get absorbance of 0.7±0.01.

2.8.2 The procedure of experiment

A volume of 3 mL $DPPH^+$ was taken with 0.6 mL of apple pomace extract (1 apple pomace extract: 10 water) and placed into a test tube. It was incubated for 20 minutes in the dark and measured at 515 nm. An absorbance reading was obtained and compared to the Trolox standard curve (0−250 µM).

2.9 Data Analysis

All data were analyzed using JMP 9.0 software (SAS Institute, Inc., Cary, North Carolina, USA). One-way analysis of variance (ANOVA) was used to analyze the data on the effects of drying techniques on the physical and antioxidant properties of dried mango pomace. The significant difference comparisons were made by Tukey's HSD test and the statistical significance was defined as $p \leq 0.05$.

3 RESULTS AND DISCUSSION

3.1 Effects of Drying Methods on the Color Properties of Dried Apple Pomace

3.1.1 Hunter Color CIE

Apple pomace's color was examined by Hunter Color CIE L* a* b* system. Color Reader CR-10 was used to analyze all samples. The mean L*, a*, b* variables for raw apple pomace were 33.8, 13.73 and 29.2 respectively. The mean values for apple pomace after freeze drying methods, drum drying method and cabinet drying methods are shown in Figures 6, 7, 8 and 9.

The freeze dried pomace sample showed the highest average level in whiteness (L*), whereas the drum dried sample showed the lowest L* value (Figure 6). As expected, higher temperature used in drum drying affected the Hunter color L* values negatively. The drying methods used had significant impact on (L*) (p= 0.0023). On the other hand, increasing temperature from 60 ℃ to 10 0 ℃ in the cabinet dryer did not affect the L* values significantly (Figure 6). There is limited literature on apple pomace drying using the same methods used in the present study. However, Yan and Kerr (2013) also reported lower whiteness values in vacuum-belt dried apple pomace when the temperature was increased from 80°C to 110 ℃ (Yan and Kerr 2013).

The cabinet drying at 100℃ was shown to impart the highest redness or color a* values, and freeze drying showed the lowest redness or color a* values (Figure 7). Similar to the Hunter color L*, a* values did not change significantly when cabinet drying temperatur was increased from 60 ℃ to 100 ℃. The cabinet drying at 100℃ showed the highest average level in yellowness or b* value, and the drum drying sample

showed the lowest average level in yellowness b* value (Figure 8); however, the differences were not significant statistically among all samples (p=0.1174).

The total color difference (△E) values did not show any significant difference (p=0.1348) when the apple pomace was dried using different drying methods (Figure 9). This showed that although there were differences in individual color parameter L* and a*, these did not impact the overall color, as determined by △E. The study by Yan and Kerr (2013) reported similar results to my result on Hunter color L*, a*, or b* of dried apple pomace, the apple pomace was dried by freeze drying and vacuum-belt drying at 80C, 95C and 110C. freeze dried sample was the highest average level in whiteness (L*), whereas the vacuum-belt dried sample at 110C was the lowest L* value(Yan and Kerr 2013).

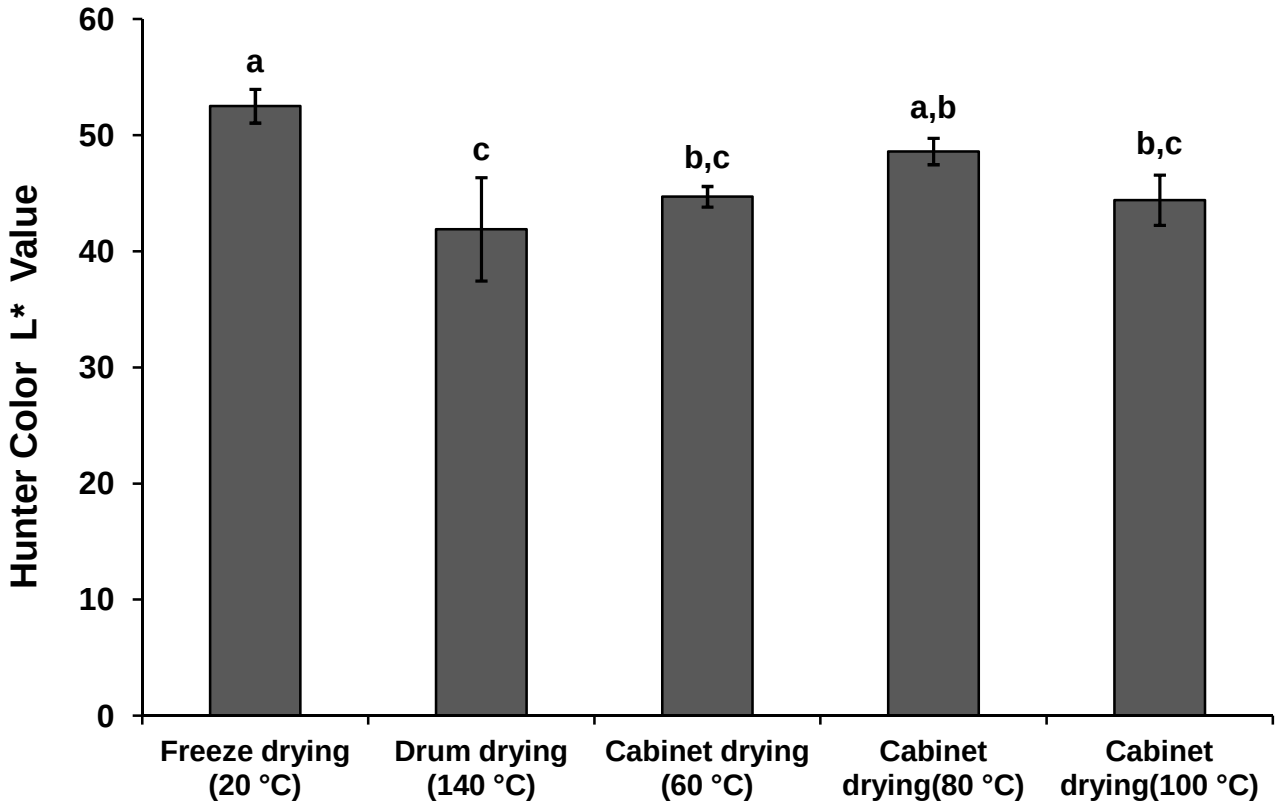

Figure 6. Effect of different drying methods on the Hunter color L* values of dried apple pomace (means sharing the same letters are not significantly different from each other at p= 0.0023, based on Tukey's HSD test)

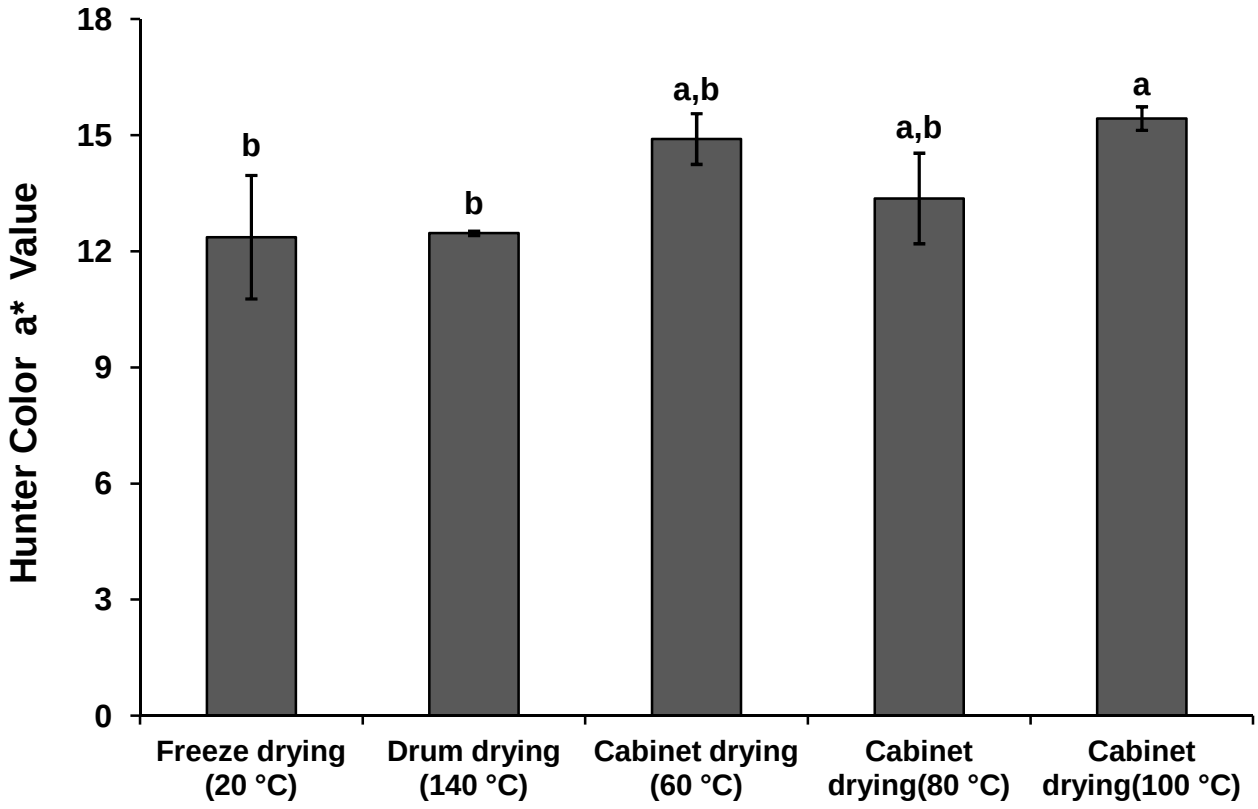

Figure 7. Effect of different drying methods on the Hunter color a* values of dried apple pomace (means sharing the same letters are not significantly different from each other at p= 0.0071 , based on Tukey's HSD test)

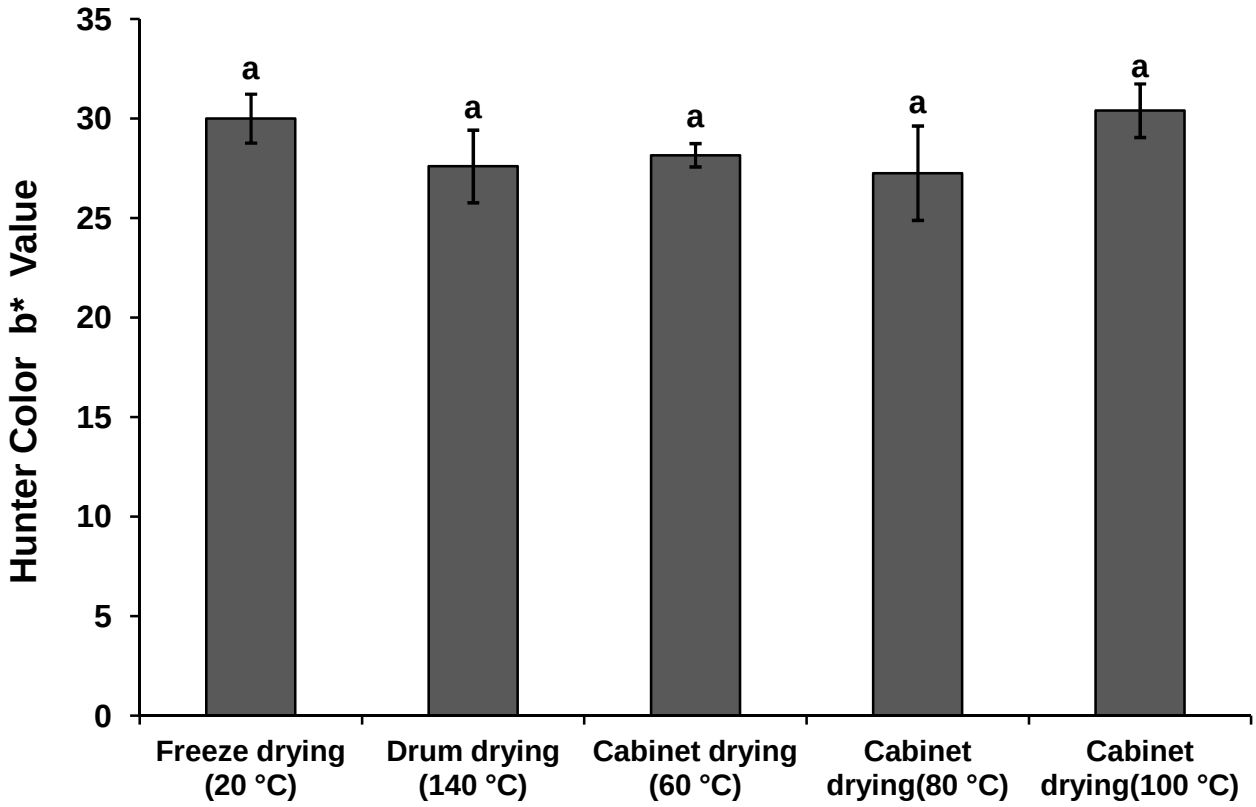

Figure 8. Effect of different drying methods on the Hunter color b* values of dried apple pomace (means sharing the same letters are not significantly different from each other at p= 0.1174, based on Tukey's HSD test).

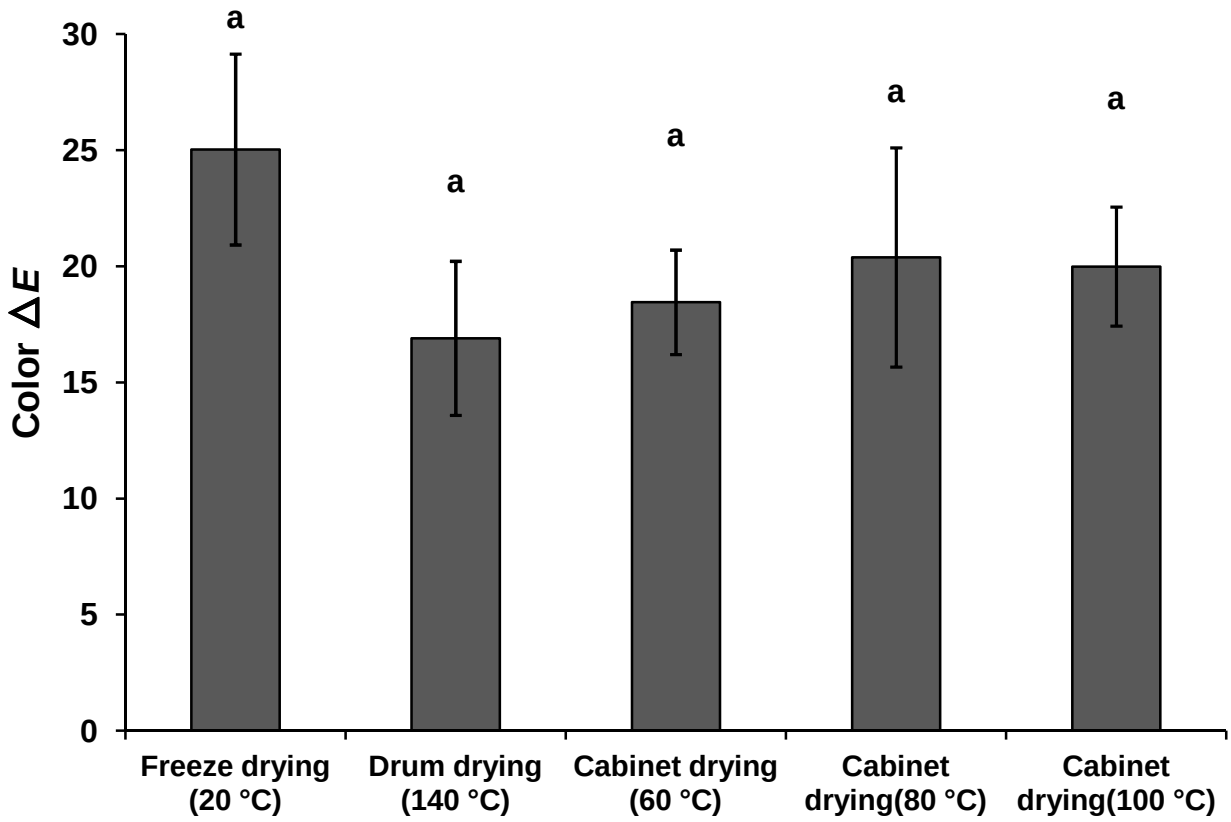

Figure 9. Effect of different drying methods on the color changes (ΔE values) of dried apple pomace (means sharing the same letters are not significantly different from each other at p= 0.1348, based on Tukey's HSD test)

3.2 Effects of Drying Methods on the Antioxidant Properties of Dried Apple Pomace

3.2.1 Total Phenolics

The mean of total phenolics in apple pomace was measured by spectrophotometer and expressed in milligrams of gallic acid equivalents (GAE) per gram of apple pomace. The mean of total phenolic in raw apple pomace was 3.56 ± 0.18 mg GAE/ g apple pomace. Total phenolic of apple pomace average for all samples are shown and Figure 10. The highest value of total phenolics was noticed in freeze drying samples and the lower value was noticed in the drum dryer at 140 ℃. The total antioxidant activity of dried apple pomace was significantly affected by temperature and drying methods (p=0.0001).

Phenolic compounds are heat-sensitive and, even cabinet drying at the lowest temperature (60 ℃) resulted in significant decreas e as compared to freeze drying method. Yan and Kerr (2013) also reported that higher temperature used during vacuum belt drying of pomace negatively impacted total phenolic content (Yan and Kerr 2013). The results of the present study are also similar to those reported by Sogi and others (2013) for mango peel and kernel drying, they have used four different drying methods; freeze drying -20C, hot air drying at 60C, vacuum drying at 60C and infra-red. The highest value of total phenolic was noticed in freeze drying samples and hot air drying at 60C was lower the freeze dried sample (Sogi and others 2013). The heat treatment decreases the total phenolic content due to the cleaving of esterified bond and glycosylated bond (Xu and others 2007).

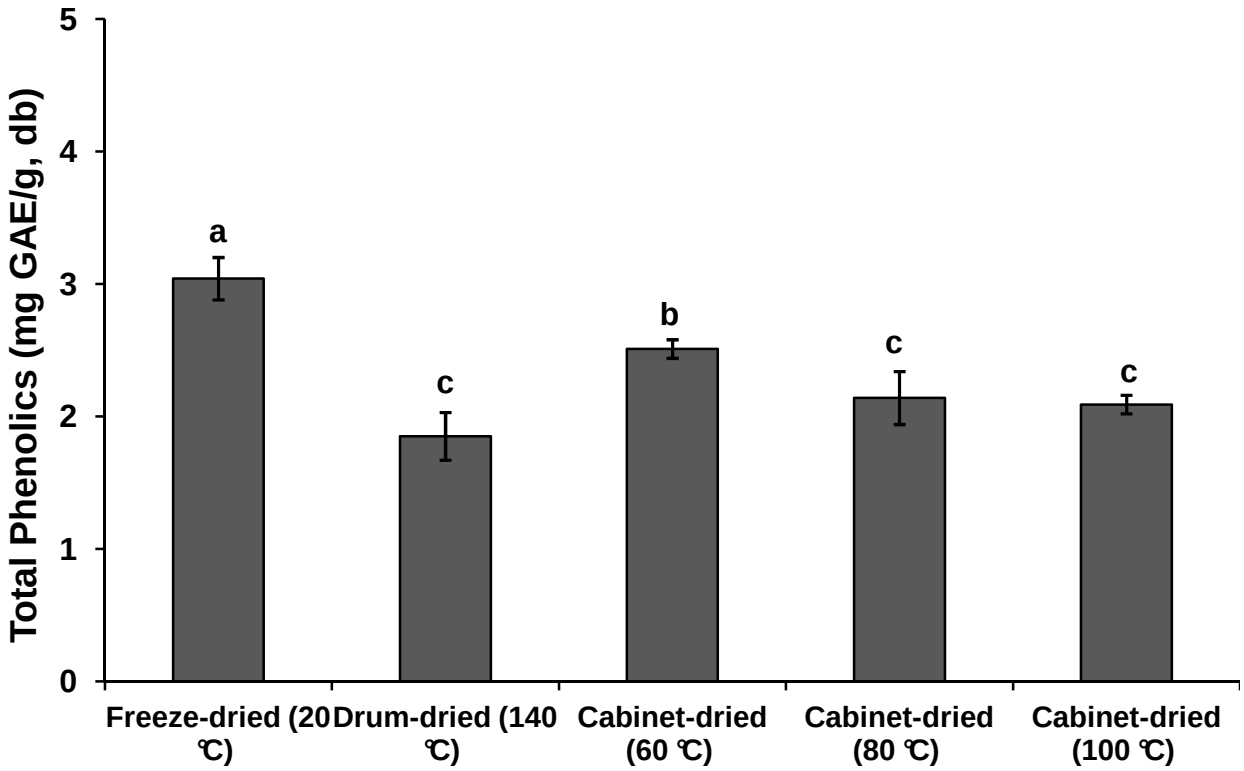

Figure 10. Effect of different drying methods on the total phenolics content of dried apple pomace (means sharing the same letters are not significantly different from each other at p= 0.0001, based on Tukey's HSD test)

3.2.2 Antioxidant Capacity assayed by ORAC

The antioxidant properties of dried apple pomace were compared using ORAC and DPPH assays. The mean ORAC in raw apple pomace was 1136.2 ± 480 µmol TE/gsample db. The mean ORAC values of dried apple pomace using different drying methods are shown in Table 6. The highest mean ORAC value of 350.275 µmol TE/g (dry basis) was observed in freeze dried apple pomace and the lowest in the drum dryer at 140 ℃ sample (Figure 11). The highest content o bserved in freeze dried samples might be due to lack of any heat used in other methods. The freeze drying method showed significantly higher (p=0.0001) ORAC values as compared to the other drying methods used. These results are similar with those reported by Sogi and other (2013),

who dried mango peel and kernel using freeze, cabinet (60 ℃) and infra-red and vacuum drying. They reported the highest value of ORAC was in freeze dried sample and hot air dried sample was lower freeze dried sample (Sogi and others 2013).

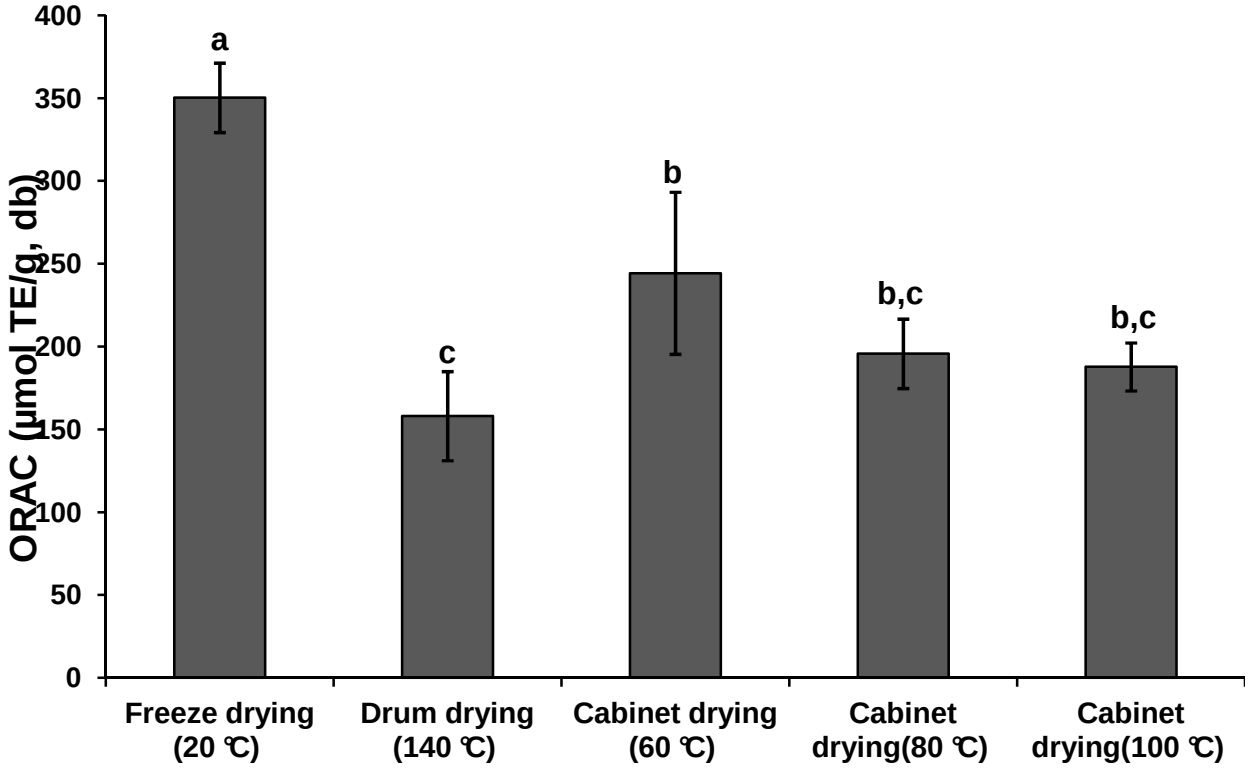

Figure 11. Effect of different drying methods on the ORAC values of dried apple pomace (means sharing the same letters are not significantly different from each other at p= 0.0001, based on Tukey's HSD test)

3.2.3 Antioxidant activity by DPPH (diphenylpicrylhydrazyl) assay

The DPPH assay was the second method used to assess effect of drying methods on antioxidant levels of dried apple pomace. The mean of DPPH in raw apple pomace was 294.839 ± 6.132 µM TE/ g (dry basis). Total phenolic of apple pomace average for all samples are shown in Table 7 and Figure 12. The highest mean of total DPPH was observed in freeze drying sample and the lower in the drum dryer at 140° C. Successively lower DPPH values were observed in cabinet dried apple pomace, as the

temperature was increased from 60 ℃ to 80 ℃ and 1 00 ℃. The DPPH of dried apple pomace was significantly affected by temperature and drying methods (p=0.0001). Sogi and others also reported similar results where freeze dried mango peel and kernels retained the highest DPPH values, as compared to cabinet drying (Sogi and others 2013).

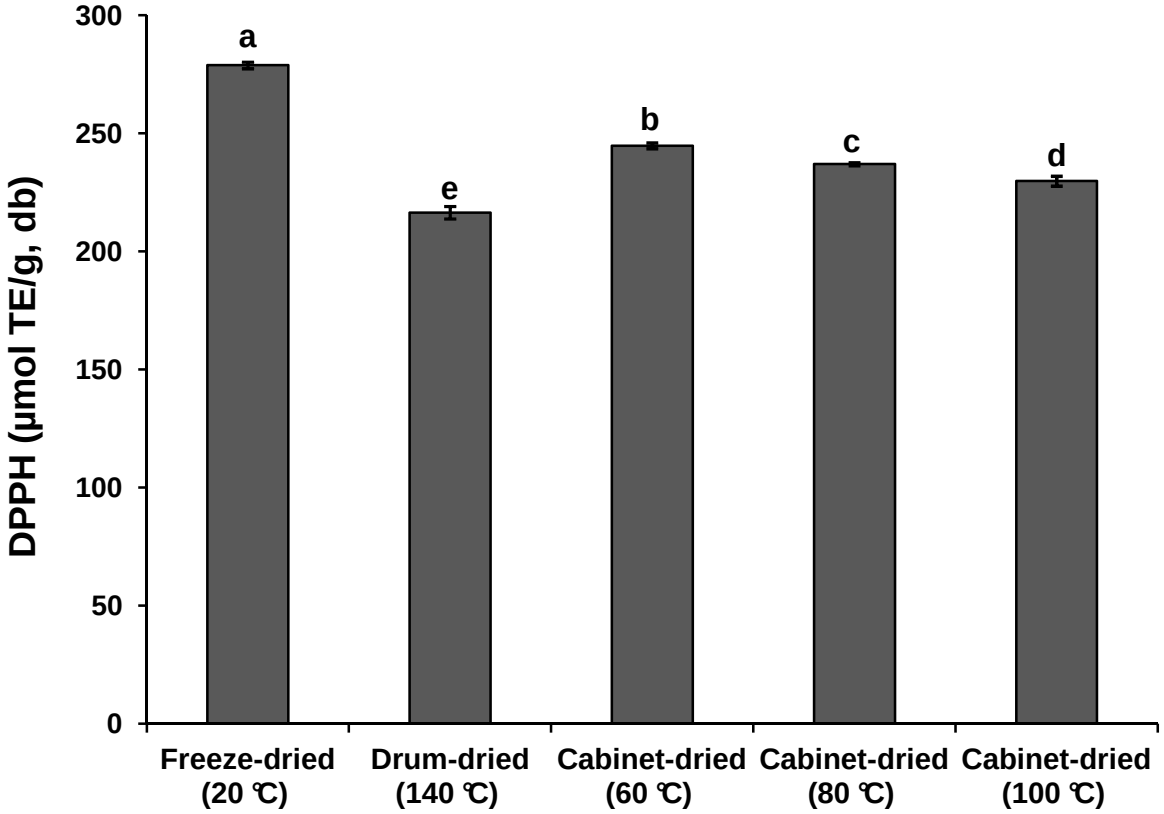

Figure 12. Effect of different drying methods on the antioxidant values of dried apple pomace using DPPH assay (means sharing the same letters are not significantly different from each other at *p*= 0.0001, based on Tukey's HSD test)

Trolox was used as standard curve for ORAC and DPPH assays. There are some differences between these assays. The ORAC assay is sensitive, more expensive and requires more time. However, it measures the degradation throughout the experiment. The DPPH assay is quicker than the ORAC assay, but it measures the degradation only at one time, rather than throughout the experiment.

4 CONCLUSIONS

4.1 Conclusions

The apple pomace color was affected by the type of drying method and temperature. Hunter Color CIE variables were used to measure affect on L*, a*, and b* values to assess differences among drying methods. The freeze dried sample was the highest in L* values. Cabinet drying at 100 ℃ was shown to result the highest average a* and b* values. The freeze dried sample showed the lowest average level in a* value, which was slightly different with drum drying sample.

The total antioxidant activity of dried apple pomace was significantly affected by the three different drying methods; freeze drying, drum drying, and cabinet drying. However, cabinet drying air temperatures of 60 ºC, 80 ºC, or 100 ºC did not significantly influence on total antioxidant in apple pomace. Type of drying method was shown to have a significant effect on total antioxidant of dried sample of apple pomace.

The total phenolics of dried apple pomace samples did change significantly; the freeze dried sample contained the highest levels of total phenolics, and the lowest levels of the total phenolics were observed in the drum dried sample.

The DPPH of dried apple pomace samples did show significant differences; the highest average of total phenolic was absorbed in the freeze dried sample, and the lowest average of the total phenolics was observed in the drum-dried sample. The results of this study demonstrated that freeze drying was the best method to process apple pomace for value-added ingredient use. The next best method was cabinet drying at lower temperatures, and finally drum drying.

Although drum drying showed the most detrimental effect on color, phenolics, and antioxidants, its significantly lower cost and faster speed of drying may offset the negative nutritional effects. For example, drum-dried apple pomace could be used as a low-percentage ingredient or blended with premium-dried apple pomace to meet both cost and nutritional requirements.

4.2 Future Research

1. Investigate other drying methods such as vacuum drying or infrared drying, which use different drying temperatures.
2. Study various packaging options for shelf life, which include microbial analysis and sensory analysis.
3. Do analysis of vitamins and minerals and dietary fiber of the pomace.

APPENDIX

Table 3 Moisture percent of apple pomace sample before drying methods

Raw apple pomace Sample	Percent Moisture %	Average Percent Moisture %
1	76.2	76.06 ± 0.4163
2	76.4	
3	75.6	

Table 4 Moisture percent of drum dried apple sample

drum dried apple sample	Percent Moisture %	Average Percent Moisture %
1	3.03	3.01 ± 0.1212
2	3.12	
3	2.88	

Table 5 Moisture percent of freeze dried apple sample

Drum dried apple sample	Percent Moisture %	Average Percent Moisture %
1	5.06	5.04 ± 0.1755
2	4.86	
3	5.21	

Table 6 Moisture percent of cabinet dried apple sample at 60 ºC

Drum dried apple sample	Percent Moisture %	Average Percent Moisture %
1	3.14	3.16 ± 0.0251
2	3.17	
3	3.19	

Table 7 Moisture percent of cabinet dried apple sample at 80 ºC

Drum dried apple sample	Percent Moisture %	Average Percent Moisture %
1	3.02	3.04 ± 0.0251
2	3.04	
3	3.07	

Table 8 Moisture percent of cabinet dried apple sample at 100 ºC

Drum dried apple sample	Percent Moisture %	Average Percent Moisture %
1	3.01	2.97 ± 0.0321
2	2.96	
3	2.95	

Table 9 The relative humidity of drum dried apple sample

Samples	Relative humidity %	Average Percent Moisture %
1	45.2	45.2 ± 2.4637
2	47.2	
3	42.3	

Table 10 The relative humidity of cabinet dried apple sample at 60 ºC

Samples	Relative humidity %	Average Percent Moisture %
1	31	30.66 ± 0.5773
2	31	
3	30	

Table11 The relative humidity of cabinet dried apple sample at 80 ºC

Samples	Relative humidity %	Average Percent Moisture %
1	35	35.33 ± 0.5773
2	36	
3	35	

Table 12 The relative humidity of cabinet dried apple pomace at 100 ºC

Samples	Relative humidity %	Average Percent Moisture %
1	39	39.00 ± 1.00
2	40	
3	38	

Table 13 Air velocity of cabinet drying

Samples	Air velocity m/s	Average Air velocity m/s
1	15.8	15.23 ± 0.5131
2	14.8	
3	15.1	

Table 14 The Hunter Color CIE data of apple pomace before drying methods

	L*	Average L*	a*	Average a*	b*	Average b*	ΔE*	Average ΔE
Sample 1	30.1	33.8 ± 5.00	13.0	13.73 ± 0.94	24.8	29.2 ± 3.96	7.44	3.96 ± 3.22
Sample 2	31.8		14.8		30.3		3.40	
Sample 3	39.5		13.4		32.5		1.06	

Table 15 Location of blank, Trolox and sample wells

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A												
B		BLK	STD1 0	STD3 50	STD5 12.5	SPL1 100	SPL2 100	SPL3 100	SPL4 100	SPL5 100	SPL6 100	
C		BLK	STD1 0	STD3 50	STD5 12.5	SPL1 100	SPL2 100	SPL3 100	SPL4 100	SPL5 100	SPL6 100	
D		BLK	STD1 0	STD3 50	STD5 12.5	SPL1 100	SPL2 100	SPL3 100	SPL4 100	SPL5 100	SPL6 100	
E		BLK	STD2 100	STD4 25	STD6 6.25	SPL1 50	SPL2 50	SPL3 50	SPL4 50	SPL5 50	SPL6 50	
F		BLK	STD2 100	STD4 25	STD6 6.25	SPL1 50	SPL2 50	SPL3 50	SPL4 50	SPL5 50	SPL6 50	

Table 15 (cont'd)

G		BLK	STD2 100	STD4 25	STD6 6.25	SPL1 50	SPL2 50	SPL3 50	SPL4 50	SPL5 50	SPL6 50	
H												

Table 16 The Hunter Color CIE data of apple pomace after drying methods

Drying Method	Tempera-ture ºC	L*	Average L*	a*	Average a*	b*	Average b*	∆E*	Average ∆E
Freeze Dried	20	52.6 51 53.9	52.5 ± 1.4525	14.2 11.3 11.6	12.36 ± 1.59	30.9 28.6 30.5	30 ± 1.22	28.77 23.9 22.42	25.03 ± 3.32
Drum Dried	140	38.8 47 39.9	41.9 ± 4.45	12.4 12.5 12.5	12.45 ± 0.05	26.4 29.7 26.7	27.6 ± 1.82	15.98 21.4 13.33	16.9 ± 4.11
Cabinet Dried	60	43.7 45 45.4	44.7 ± 0.88	14.8 14.3 15.6	14.9 ± 0.65	28.6 27.5 28.4	28.16 ± 0.58	20.77 18.32 16.26	18.45 ± 2.25
	80	47.8 49.9 48.1	48.6 ± 1.13	14.7 12.9 12.5	13.36 ± 1.17	30 25.8 26	27.26 ± 2.36	24.6 21.26 15.28	20.38 ± 4.72
	100	44.1 42.4 46.7	44.4 ± 2.16	15.1 15.7 15.5	15.43 ± 0.30	30.8 28.9 31.5	30.4 ± 1.34	22.74 17.66 19.59	19.99 ± 2.56

Table 17 Effects drying methods on the antioxidant in apple pomace before drying

ORAC (µmol TE/g apple pomace)	Average ORAC (µmol TE/g apple Pomace)
1059.2 1488.7 1498.2 2060.5 1706.2 790.6 700.4 949.2 817.3 1511.4 1869.1 707.2 321.5 913.8 666.3 1089.7 1166.7 1059.2 1488.7 1498.2 2060.5	1136.2 ± 480

Table 18 Effects drying methods on the antioxidant in apple pomace after drying

Drying Method	Air Temperature (℃)	ORAC (μmol TE/g apple Pomace)	Average ORAC (μmol TE/g apple Pomace)
Freeze Dried	20	378.8 425.8 388.8 305.9 263.6 190.1 363.2 373.4 370.3 336.5 292.6 211.2 406.1 463.5 413.8 324.1 318.2 145.2 405.1 453.4 420.5 360.4 378.8	350.275 ± 21

Table 18 (cont'd)

Drum Dried	140	152.7 159.2 154.8 168.4 143.1 115.7 126.8 139.2 153.8 160.4 164.7 125.1 210.1 285.9 280.7 154.0 136.6 102.3	158.066 ± 26.9
Cabinet Dried	60	224.7 256.1 296.3 292.4 299.4 278.6 201.5 222.6 238.1 291.1	244.450 ± 48.9

Table 18 (cont'd)

		329.5 272.5 162.0 15.3 349.2 311.9 242.3 179.2 338.2 352.3 313.7 302.9	
Cabinet Dried	80	133.9 148.5 148.3 243.4 237.2 198.7 167.8 161.0 179.5 282.3 301.5 253.2 158.0 172.6 149.2 264.8 303.8 250.1 93.7 134.8 125.4 245.7 251.3 238.8	195.700 ± 21

Table 18 (cont'd)

Cabinet Dried	100	135.2 202.1 210.7 228.1 239.1 203.7 140.3 188.4 240.8 221.3 248.6 212.2 162.2 221.3 222.8 0.0 257.9 194.5 158.3 198.3 227.6 189.1 180.8 161.7	187.775 ± 14.5

Table 19 Effects drying methods on total phenolics in apple pomace before drying

Total Phenolics (mg GAE/g apple pomace)	Average Total Phenolics (mg GAE/g apple pomace)
3.65 3.42 3.37 3.77	3.56 ± 0.18

Table 20 Effects drying methods on the total phenolics in apple pomace after drying

Drying Method	Air Temperature (℃)	Total Phenolics (mg GAE/g apple pomace)	Average Total Phenolic (mg GAE/g apple pomace)
Freeze Dried	20	2.84 2.99 3.18 3.17	3.05 ± 0.16
Drum Dried	140	1.70 2.10 1.90 1.72	1.85 ± 0.18
Cabinet Dried	60	2.58 2.55 2.40 2.51	2.51 ± 0.07
	80	2.20 1.95 1.99 2.41	2.14 ± 0.20
	100	2.01 2.09 2.18 2.07	2.09 ± 0.07

Table 21 Effects drying methods on DPPH in apple pomace before drying

DPPH µmol TE/g	Average DPPH µmol TE/g
286.502 297.618 294.442 300.793	294.839 ± 6.132

Table 22 Effects drying methods on DPPH in apple pomace after drying

Drying Method	Air Temperature (℃)	DPPH µmol TE/g	Average DPPH µmol TE/g
Freeze Dried	20	278.326 279.526 277.125 280.327	278.826 ± 1.400
Drum Dried	140	214.891 219.593 217.634 213.716	216.459 ± 2.657
Cabinet Dried	60	245.459 244.674 245.851 243.104	244.772 ± 1.214
	80	237.700 237.308 236.132 236.916	237.013 ± 0.669
	100	229.303 227.344 230.086 232.436	229.792 ± 2.106

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