MICROENCAPSULATION OF POLYPHENOLS EXTRACTED FROM POMEGRANATE PEELS AND THEIR APPLICATION IN SALAD DRESSING

by

BORAN YANG

(Under the Direction of Kevin Mis Solval)

ABSTRACT

Microencapsulation *via* spray drying may be a viable alternative to enhance the stability of pomegranate phenolic extracts (PPP) during processing and storage. The objective of this study was to develop microencapsulated polyphenols (MPP) powders using pomegranate peel pectin (PPE): maltodextrin (MD) (ratios of 1:0, 0:1, 3:1, 4:1, and/or 5:1, w/w) and utilize them in salad dressings. The powders were evaluated for moisture, water activity ($a_w$), particle size, bulk density, water-solubility, microstructure, encapsulating efficiency (EE), and antioxidant activity. The effect of PPP and MPP, and/or grape seed extract on the lipid oxidation of Italian-style homemade salad dressings homogenized at low or high shear rates and stored at accelerated or ambient conditions was also evaluated. The analysis results showed that MPP powders coated with a mixture of PPE and MD had better physical properties analyzed in this study, and MPP showed a higher protective effect on lipid oxidation of salad dressings.

INDEX WORDS: Microencapsulation, Spray-dried maltodextrin/pectin matrix, Polyphenol, Pectin, Particle agglomeration, Salad dressing, Lipid oxidation, Antioxidant, Accelerated storage, Ambient shelf-life test

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by

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# DEDICATION

This thesis is especially dedicated to my beloved parents, who have always been a constant source of support, encouragement, and unconditional love throughout my life.

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# CHAPTER 1

## INTRODUCTION AND LITERATURE REVIEW

Pomegranate (*Punica granatum*) belongs to the order *Myrtales*, *Lythraceae* family, and genus *Punica* which only has two species: *Punica granatum* and *P. protopunic*. In the early eighteenth century, Spanish sailors introduced it to the U.S. including Spanish Florida and English Georgia, and later in the 1770s, it was spread and grown on the West Coast (Caligiani, 2016). Pomegranates can be processed into jelly, juice, jam, and molasses (Oliveira *et al*., 2016). Nevertheless, pomegranate juice is the most popular value-added pomegranate product because of its nutritional value and health benefits. Moreover, pomegranate is also used in traditional medicine because of its therapeutic properties such as antioxidant, anti-inflammatory, antitumor in *vivo* and *vitro,* and anticancer (Rahmani, Alsahli, & Almatroodi, 2017). The pomegranate juice processing generates considerable amounts of by-products that may lead to various environmental problems (Hanani, Yee, & Nor-Khaizura, 2019). Qu *et al*. (2009) reported that juicing 1,000 kilograms of fresh pomegranate generated 669 kilograms of by-products (78% peels and 22% seeds). Pomegranate seeds contain 37~42% of fibers, 24~33% of carbohydrates, 14~17% of lipids (up to 90% unsaturated fatty acids), and 9~11% of proteins (Kakaei, Noshad, Nasehi, Hojjati, & Beiraghi-Toosi, 2019). Therefore, they can potentially be used to develop functional ingredients. On the other hand, pomegranate peels are a good source of fibers (11%~34%), polyphenols (4.9%~18%), and pectin (7%~10%) (Abid *et al*., 2017; Caligiani, 2016). Despite the enormous potential for developing novel food ingredients from pomegranate seeds and peels, the Georgia pomegranate industry does not produce any value−added products from those raw materials.

Several "*in vitro*" and "*in vivo*" studies have reported that phenolic compounds, including polyphenols, have a wide range of biological activities and medical benefits in terms of anti−inflammation, antioxidation, antimicrobial and anticancer properties (Beata, 2018). Phenolic compounds can reduce oxidation reactions by scavenging free radicals, break radical chain reactions, and chelate metals (Gil, Tomás-Barberán, Hess-Pierce, Holcroft, & Kader, 2000). It has been reported that pomegranate is an excellent source of phenolics (which are highly concentrated in the peels) with reported potential health benefits (Cam *et al*., 2014). The predominant phenolic compounds found in pomegranate peels are flavonoids, proanthocyanidin, and ellagitannins. Among these phenolic compounds, punicalagin ($C_{48}H_{28}O_{30}$) is the predominant polyphenol found in pomegranate peels with strong antioxidant properties and is highly soluble in water (Fischer, Carle, & Kammerer, 2011). The concentration of punicalagin in peels depends on the geographical area in which the fruits are grown, the processing and storage conditions (Lu, Ding, & Yuan, 2008). Natural polyphenols have been used in food applications; for example, a complexity of polyphenols extracted from malted grains and hops were used in beer to contribute to the mouthfeel, antioxidant properties, and stability, as well as foam retention (Lentz, 2018). Previous studies have shown that virgin olive oil phenolics might influence flavor perception and consumer perception of oil-in-water (O/W) emulsions (Genovesea, Caporasob, di Barib, Yang, & Fisk, 2019). However, bitterness and pungency associated with natural phenolics may reduce their potential applications in foods. Limited studies report the use of pomegranate polyphenols in foods to control lipid oxidation. Therefore, one of the objectives of this project is to develop polyphenols-containing ingredients produced from Georgia-grown pomegranates that can be used to control lipid oxidation in foods.

Pectin is a natural macromolecule that is composed of a group of complex polysaccharides rich in galacturonic acid (GalA). It is mainly found in the primary cell wall,

which surrounds dividing and growing cells, and in the middle lamella of fruits and vegetables. It is an essential component of the initial cell growth and ripening process of plants and it can make up nearly 40% (dry basis) of the cell wall (Abid *et al*., 2017). The linear structure of pectin (Figure 1.1) is mainly comprised of α-(1→4)-linked D-galacturonic acid polysaccharide backbone, and a part of the galacturonic acid residues of the polysaccharide backbone exists in methyl or acetyl ester form with a small proportion of L-rhamnose units (Kpodo *et al*, 2018). Pectin with different properties can be extracted from fruit peels. Based on the degree of methyl esterification (DE), pectin can be divided into two forms: high methoxyl (HM) form (DE is more than 50%), and low methoxyl (LM) form (DE is less than 50%) (Güzel, & Akpınar, 2019). Furthermore, pectin is used as a stabilizer and thickening agent in foods. The DE is an intrinsic factor that can determine the gelling properties of pectin. For instance, LM pectin can form thermally reversible gels without or with few amounts of sugars in the presence of $Ca^{2+}$ and at low pH (3~4.5). It is widely used in low sugar products. In products with high sugar concentration (more than 65% of sucrose by weight), LM pectin will form a pre-gel. On the other hand, HM pectin can form thermally irreversible gels in the presence of a high concentration of sugars (more than 70% by weight) and at low pH (≤ 3.5). It has been reported that the DE of pomegranate peel pectin is larger than 50% (Güzel *et al*., 2019), so it can potentially be used as HM pectin in food applications. Extrinsic factors such as sugar content, pH, temperature as well as concentration of $Ca^{2+}$ can affect the gel-forming properties of pectin. Güzel *et al* (2019) reported that higher gel strengths and shorter gelling times can be achieved at high sugar concentrations and low pH when pectin with higher DE was used. Because of its ideal gelling properties, DE pectin is widely used as a thickening, stabilizing, and gelling agent in jam and jelly manufacturing (Ahmadi Gavlighi, 2018). In addition, the ability of pectin to form a gel network contributes to the stability of simple emulsions through steric and electrostatic stabilization

(Ngouemazong, Christiaens, Shpigelman, Loey, & Hendrickx, 2015). Pectin has been also used as a microencapsulating agent of bioactive. Locali Pereira, Gonçalves Cattelan, & Nicoletti (2019) reported the use of pectin/soy protein isolate (SPI) double layer stabilized emulsions to prepare microcapsules containing pink pepper essential oil. Moser, Ferreira, & Nicoletti (2019) evaluated the effect of atomization and drying conditions in the microencapsulation of carotenoids using chickpea protein and HM pectin complexes. Therefore, pectin extracted from Georgia-grown pomegranate can be used as an effective microencapsulating agent for polyphenols.

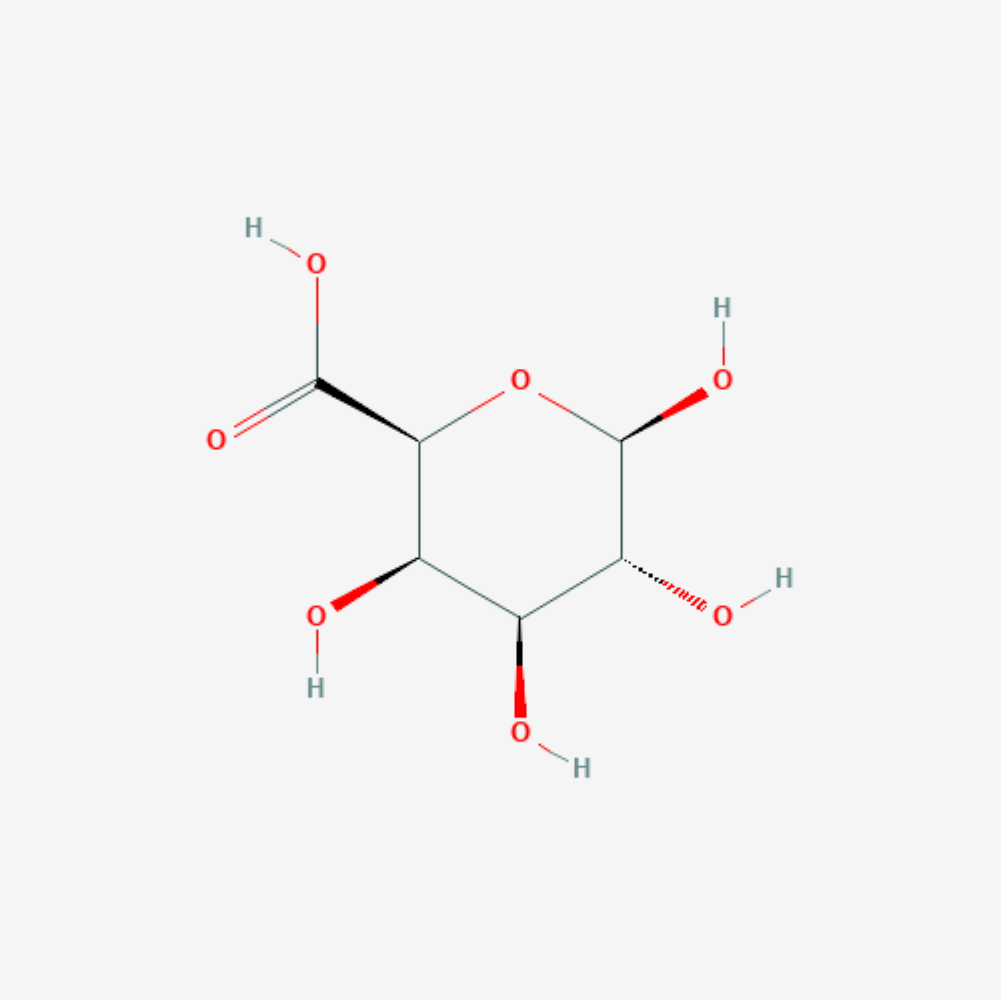


Figure 1.1 Structure of pectin (National Center for Biotechnology Information, 2021)

Microencapsulation technology is the process whereby “core materials” such as bioactive, antioxidants, fatty acids, and vitamins are embedded in a homogeneous or heterogeneous matrix or packed within “wall materials” (commonly known as microencapsulating agents) including maltodextrin, gum Arabic, sodium caseinate and other materials to produce tiny, micro-sized capsules. Microencapsulating bioactive requires three main steps: formation of a shell, avoidance of undesired leakage, and discharge of unwanted materials (Mozafari *et al*., 2008). The main advantages of microencapsulation are i) improving the physical and chemical properties of the bioactive; ii) protecting the bioactives from environmental factors including oxygen, light, and temperature to maintain the desired stability and quality; iii) allowing controlled release of the bioactive, and iv) masking the unpleasant taste and flavor produced by the bioactives. Thus, microencapsulation technology is wildly used in the food industry. It has been reported that microencapsulation of bioactives can be accomplished by spray drying (SDR), freeze dying (FDR), and supercritical fluid precipitation. However, characterized by the low cost, high stability, well continuity, and flexibility, SDR is the most common microencapsulation technique used in the food industry (Fang & Bhandari, 2011). SDR involves three fundamental steps, atomization, dehydration, and collection of dried particles (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). During SDR, liquid products are transformed into powdered products which are more resistant to oxidative degradation. Moreover, because of short particle residence times in spray drying, it can be used to microencapsulate a wide spectrum of bioactives including polyphenols (Shishir & Chen, 2017). Furthermore, microencapsulation of bioactives by SDR can extend their shelf life and improve their water-solubility properties (Arslan-Tontul and Erbas, 2017). Successful microencapsulation of bioactives requires the use of effective microencapsulating agents. The type of microencapsulating agent can affect the effectiveness of the microencapsulating process, as well as the stability and physicochemical properties of

the bioactives (Mishra, P., Mishra, S., & Lata Mahanta, 2014). Modified starches, maltodextrin, gums, whey proteins, and other microencapsulating agents can be used to microencapsulate bioactives. Several studies have reported the use of SDR to microencapsulate phenolics extracted from various fruits (Bakowska-Barczak, & Kolodziejczyk, 2011; Cam, İçyer, & Erdoğan, 2014; Ersus, & Yurdagel, 2007; Kaderides, Mourtzinos, & Goula, 2019). In addition, studies involving bioactives encapsulated in the pectin−based matrix have also been reported (Ahmadian, Niazmand, & Pourfarzad, 2019; Mohammadi, Jafari, Assadpour, & Faridi Esfanjani, 2016; Sun, Cameron, & Bai, 2019). Hence, microencapsulation by SDR using pectin can enhance the shelf stability of polyphenols extracted from pomegranate peels.

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# CHAPTER 2

# DEVELOPING MICROENCAPSULATED POWDERS CONTAINING POLYPHENOLS AND PECTIN EXTRACTED FROM GEORGIA-GROWN POMEGRANATE PEELS[1]

[1] Yang, B., Chen, J., Kealey, K.S., & Mis Solval, K. To be submitted to *Journal of Food Science*

## Abstract

Pomegranate peels are excellent sources of pectin (PPE) and polyphenols (PPP) with strong antioxidant properties and potential applications in foods. However, the direct addition of fruit phenolic extracts into foods may create unpleasant flavors and reduce their antioxidant activity. Hence, the objective of this study was to develop microencapsulated powders containing polyphenols (PPP) and pectin (PPE) extracted from Georgia-grown pomegranate peels. PPP and PPE were solvents extracted from dried pomegranate peels and were subsequently freeze-dried. Mixtures of maltodextrin (MD) : PPE (ratios of 1:0, 0:1, 3:1, 4:1, and/or 5:1, w/w) were used as microencapsulating agents. Suspensions were prepared by homogenizing microencapsulating agents (15 g/100 mL) and PPP (3 g/100 mL) in deionized water. Then, suspensions were spray-dried under mixed-flow conditions at 140 °C inlet air temperature to obtain microencapsulated powders which were evaluated for moisture, water activity ($a_w$), particle size, bulk density, water-solubility, microstructure, encapsulating efficiency (EE), and antioxidant activity. The total polyphenol content (mg GAE/mL) in PPP was 5,22.82±6.94, and the degree of methyl esterification (%) of PPE was 53.67±0.37. Powders produced with higher amounts of PPE had significantly ($P$<0.05) higher moisture, $a_w$, and lower bulk densities than the rest of the powders. Powders microencapsulated with a mixture of MD: PPE had significantly ($P$<0.05) higher EE (>89.90±0.47%), higher water solubility (>88.09±0.42%), and higher antioxidant activities compared to the powders produced with MD or PPE alone. Moreover, powders microencapsulated with MD: PPE (ratio 4:1) showed the highest antioxidant activity (% inhibition rate=57.30±0.64) and the largest mean particle sizes (9.69±0.11 µm) of all powders. All microencapsulated powders were agglomerated and quasi-spherical particles. These results suggest that microencapsulation with MD: PPE *via* spray drying effectively protects the stability of PPE.

**Key words:** Microencapsulation, Spray-dried maltodextrin/pectin matrix, Polyphenol, Pectin, Particle agglomeration

## Introduction

Pomegranate (*Punica granatum*) is an excellent source of various health-promoting phytochemicals with antioxidative, anti-inflammatory, antitumor in *vivo* and in *vitro,* and antimicrobial activities (Rahmani, Alsahli, & Almatroodi, 2017). Furthermore, pomegranates have been widely utilized in traditional medicine for curing diarrhea, worm infection, and pregnancy disorders (Bhatia & Asrey, 2019). According to Oliveira *et al*. (2016), several pomegranate-containing products can be found in the marketplace such as jellies, juices, jams, and molasses, among which, pomegranate juice and pomegranate arils are the most popular value-added pomegranate products because of their sensorial properties, nutritional value, and health benefits. It has been reported that pomegranate juice processing generates considerable large amounts of underutilized by-products that may lead to various environmental problems (Hanani, Yee, & Nor-Khaizura, 2019). Qu *et al*. (2009) reported that juicing 1,000 kilograms of fresh pomegranate generated 669 kg of by-products (78% peels and 22% seeds). Interestingly, by-products of pomegranate juice processing contain high levels of phytochemicals (bioactive compounds found in plant-based foods) that may provide health benefits including polyphenols and pectin that can be used to develop novel food ingredients for functional foods. Polyphenols are a type of phytonutrients with high antioxidant and anti-inflammatory properties (Gupta & Prakash, 2014). Pomegranates contain high amounts of polyphenols that can be classified as hydrolysable tannins (ellagitannins), condensed tannins, flavonoids (anthocyanins), and phenolic acids (ellagic acid and gallic acid) (Kandylis & Kokkinomagoulos, 2020). Surprisingly, most of the polyphenols present in pomegranates are found in their peels and the predominant type of polyphenol is a type of ellagitannin called punicalagin (Fischer, Carle, & Kammerer, 2011). According to Li *et al*. (2015), punicalagin made up approximately 77% of the total polyphenols in the peel of Chinese pomegranates. Interestingly, polyphenols can reduce oxidation reactions in foods

through scavenging free radicals, breaking radical chain reactions, and chelating metals (Gil, Tomás-Barberán, Hess-Pierce, Holcroft, & Kader, 2000). Lipid oxidation results in undesirable flavors and odors and reduces the quality and shelf life of foods. Therefore, the food industry utilizes different strategies to control lipid oxidation in foods including the use of herbal extracts, essential oils, and synthetic antioxidants (Mariutti & Bragagnolo, 2017). Nevertheless, some challenges have been associated with the direct incorporation of polyphenol-containing extracts into foods because these compounds are often prone to degradation and polymerization due to environmental factors such as oxygen, light, and adverse temperatures and pH ranges, which may cause undesirable flavors and colors as well as a loss of antioxidant activity (Đorđević *et al*., 2015). Moreover, polyphenol extracts are sticky materials with an unpleasant smell and undesired water solubility which makes them difficult to handle and use in food applications. Thus, effective alternatives to stabilize pomegranate polyphenols extracts that can facilitate their incorporation in foods are currently being investigated.

Pectin is a natural macromolecular polysaccharide that is extensively used as texturizing, stabilizing, thickening, and gelling agent in food, cosmetic and pharmaceutical applications (Zhuang *et al*., 2019). Even more, pectin can serve as an effective carrier to microencapsulate heat-sensitive bioactives with excellent antioxidant properties via spray drying (Sansone *et al*., 2011). Yang *et al*. (2018) extracted pectin from pomegranate peels and reported that the extracted pectin showed effective emulsification properties at pH 2-6, represented 8.5% (w/w) of dried pomegranate peels, and is comprised of 83.2% polysaccharides, 7.52% moisture, 3.24% protein, and 0.12% ash.

Microencapsulation technology may be a suitable option to improve the stability of pomegranate peel extracts by protecting these bioactive compounds from environmental factors including heat, light, and oxygen. It is a process where tiny particles or droplets are

embedded in a matrix or packed within microencapsulating agents to produce micro-sized capsules with various beneficial properties (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Moreover, microencapsulated products are easier to handle and have longer shelf life compared to non-encapsulated products (Bora, Ma, Li, & Liu, 2018). Freeze drying (FDR) and spray drying (SDR) are widely used microencapsulation techniques. FDR is suitable for the microencapsulation of heat-sensitive ingredients such as polyphenols, but it is a time-consuming and expensive process (Jiang, Kumar, Chen, Mishra, & Solval, 2020). Meanwhile, SDR is a continuous, versatile, and cost-effective process to produce dry microencapsulated products with high quality and stability (Kaderides, Goula, & Adamopoulos, 2015). Several polysaccharides, proteins, fats, waxes, and other encapsulating materials have been used to microencapsulate plant-based polyphenols. Maltodextrin (MD), produced from the partial hydrolysis of corn starch, has been used as an effective encapsulating agent because of its high water solubility, low viscosity, low sugar content, and the ability to reduce oxygen permeability of the wall matrix (Rai, Wahile, Mukherjee, Saha, & Mukherjee, 2006). Several studies have reported the use of MD in combination with other materials to microencapsulate polyphenols extracted from various natural products *via* SDR (Lee & Chang, 2020; Tolun, Altintas, & Artik, 2016). However, no studies have reported the feasibility of microencapsulating pomegranate peel polyphenols (PPP) with MD and pomegranate peel pectin (PPE) *via* SDR. We have hypothesized that MD combined with PPE can be used as an effective encapsulation agent for PPP. Hence, the objective of this study was to determine the feasibility of extracting pectin (PPE) and polyphenols (PPP) from pomegranate peels and to develop microencapsulated PPP powders using PPE and maltodextrin (MD) *via* SDR.

## Materials and Methods

### Materials

Fresh pomegranate peels were obtained from Alma Nursery & Berry Farms (Alma, GA, USA) and air-dried at 60 °C for 24 h. Ethanol (99.5%), Folin-Ciocalteu phenol reagent, gallic acid 2, 2-diphenyl-1-picrylhydrazyl (DPPH), citric acid, TPTZ (2,4,6-tripyridyl-s-triazine), acetate buffer (pH 3.6), methanol (99.5%), and all the other chemicals were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

### Pectin extraction

The pectin extraction from pomegranate peels was based on the method reported by Yang *et al*. (2018) with some modifications. Air-dried peels were ground into powder using a grinder (Slsy 2500 g, Shanghai Shangquan Wuliu Co., Ltd., China), then dried powders were mixed with deionized water at the ratio of 1:20 (w/w) and a 1 M citric acid solution was used to adjust the pH to ~1.7. The mixture was then stirred and heated at 86°C for 80 minutes to form a slurry. Afterward, the slurry was cooled to room temperature, filtered, and centrifuged at 4000 × g for 10 minutes at 10℃. The supernatant was collected and placed in a rotary evaporator (RE 111, Brinkmann Instruments, Inc., Westbury, NY, USA) at 50°C until reaching one-third of its original volume. Subsequently, the concentrated supernatant was mixed with 96% (v/v) ethanol at a ratio of 1:3 (v/v) at 4 °C for 12 ~ 18 hours for pectin precipitation. The mixture was then centrifuged at 8000 × g for 20 minutes using a centrifuge (Model J2-21M, Beckman Instruments Inc., Palo Alto, CA, USA), frozen at −4°C and lyophilized at − 55 °C for 5 days using a pilot-scale freeze dryer (Genesis 25 ES, The Virtis Company, Gardiner, NY, USA) to obtain freeze-dried pectin which was ground using an electric grain grinder mill (SLSY & MOONCOOL, Shanghai, China) for 1 minute to obtain pomegranate peel pectin powders (PPE) which were stored at 4℃ until needed for analysis and further steps.

**Determination of methyl esterification (DE) of PPE**

The DE value of PPE was determined according to the method reported by Muhoza *et al*. (2019). Briefly, 0.5 g of pectin powder was homogenized with 2 mL ethanol and 100 mL deionized water. Then, five droplets of phenolphthalein were added to the solution which was titrated with a 0.5M sodium hydroxide (NaOH) solution until a pink color was persisted for > 5s. The consumed volume of NaOH solution was recorded as $V_1$. Afterward, 10 mL of the same NaOH solution was added, and the mixture was shaken and kept for 15 minutes. Subsequently, ten mL of 0.5M hydrochloric acid (HCl) solution were added and mixed finely until the pink color vanished. Then the resultant mixture added with another five droplets of phenolphthalein was titrated again with 0.5M NaOH solution until a pink color was persisted for > 5s, the consumed volume of NaOH solution was recorded as $V_2$. DE was calculated using Eq. (1):

$$\text{DE}\ (\%) = \frac{\text{V2}}{\text{V1+ V2}} \times 100 \qquad (1)$$

**Polyphenol extraction**

Air-dried peel powders were mixed with 10-fold volume of 95% (v/v) ethanol in a flask, then the flask was incubated at 45℃ and stirred at 30 × g for 1hour in water bath (Model 2872, Thermo Fisher Scientific Inc., Marietta, OH, USA) at. Afterward, the mixture was homogenized at 8000 × g for 8 minutes using an ultra-high shear homogenizer (Fisherbrand 850 Homogenizer, Thermo Fisher Scientific Inc., Chicago, IL, USA). Then the mixture was centrifuged at 6000 × g and 10℃ for 20 minutes. The resulting supernatant was filtered through Whatman No.1 filter paper (Whatman International Ltd., Maidstone, England) and placed in a rotary evaporator at 50℃ to evaporate off ethanol. Finally, the resultant liquid polyphenols were frozen at −4℃ and lyophilized at − 55 ℃ for 5 days using a pilot-scale freeze dryer (Genesis 25 ES, The Virtis Company, Gardiner, NY, USA) to obtain freeze-dried polyphenols which were then ground using an electric grain grinder mill

(SLSY & MOONCOOL, Shanghai, China) to produce dried polyphenols powders (PPP), and were stored in amber-glass vials at 4℃ until needed for analysis and subsequent steps.

**Determination of total polyphenol content (TPC) of PPP**

TPC was determined according to the Folin-Ciocalteu reagent method described by Pande & Akoh (2009) with slight modifications. 1 g of PPP was dissolved in methanol and diluted with deionized water to reach the proper concentration. To each 2 mL of sample, 6.5 mL of deionized water, 0.5 mL of 6N Folin-Ciocalteu phenol reagent, and one mL of a saturated sodium carbonate solution were added. Then the samples were vortexed thoroughly and allowed to stand at room temperature in the dark for at least 40 min before reading absorbances with a Genesys 30 UV-vis spectrophotometer (Thermo Fisher Scientific Inc., Madison, WI, USA) set at λ=750 nm. Quantification was based on the standard curve (Y= 0.1126X – 0.0097, $R^2$ = 0.9989) generated with 1.6–8 μg/mL of gallic acid, and the result was reported as μg GAE/ml.

**Antioxidant activity of PPP**

**2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging assay**

The DPPH assay was carried out as described by Azarpazhooh, Sharayei, Zomorodi, & Ramaswamy (2019) with some modifications. 25 mg of PPP was dissolved in 10 mL of absolute ethanol. Then, 2 mL of 0.1mM DPPH solution and 100 μL ethanol were added into 100 μL of the sample solution in a tube. A control (containing 2 mL of 0.1mM DPPH and 200 μL ethanol) and a blank (containing 2.1 mL ethanol and 100 μL sample) were prepared. All test tubes were placed in the dark for at least 40 minutes before absorbance was measured at λ=517 nm using a spectrophotometer (Thermo Fisher Scientific Inc., Madison, WI, USA). The scavenged DPPH (%DPPH) was calculated according to Eq. (2):

$$\%\text{DPPH} = \frac{(A_{\text{control}} - A_{\text{Sample}} + A_{\text{blank}})}{A_{\text{control}}} \times 100\% \quad (2)$$

Where $A_{control}$ was the absorbance of the control, $A_{sample}$ was the absorbance of the sample, and $A_{blank}$ was the absorbance of the blank.

**Ferric reducing/antioxidant power (FRAP) assay**

FRAP assay of PPP was based on the method reported by Manasa, Padmanabhan, & Anu Appaiah (2021). 15 mL of 300mM acetate buffer (pH 3.6), 1.5 mL of 10Mm 2,4,6-tripyridyl-s-triazine solution, and 1.5 mL of 20mM $FeCl_3 \cdot 6H_2O$ were mixed as working solution and then incubated at 37℃ for 30 minutes. Then, 50 μL of the sample was mixed with 1.5 mL working solution and absorbances of readings were taken at λ=593nm against 1 mmol/L $FeSO_4$ for 5 minutes. Quantification was based on the standard curve (Y = 0.0007X + 0.1733, $R^2$ = 0.9982) generated with 0.125–1mmol/L of $FeSO_4 \cdot 7H_2O$, and the results were reported as mmol $Fe^{2+}$/ g of pomegranate peel extracts.

**Preparation of PPP suspensions**

Stable suspensions were prepared with 150 g of a mixture of MD and PPE (MD: PPE = 1:0, 3:1, 4:1, 5:1 and/or 0:1, w/w) which was dissolved in 1 L of deionized water at ambient temperature and kept in a refrigerator for 24 hours for completion of hydration. Afterward, 30 g of PPP were added to a mixture containing the encapsulating materials and homogenized at 5000 × g for 5 minutes at room temperature to produce stable PPP suspensions (Table 2.1).

Table 2.1 Variables and levels used in encapsulation of pomegranate peel extract

| **Treatment code** | **Coating materials (g/100mL)** | | **Ratio (MD:PPE)** | **Core material** |
|---|---|---|---|---|
| | **MD** | **PPE** | | **PPP** |
| SD01 | — | 15 | 0:1 | **3% (w/v)** |
| SD31 | 11.25 | 3.75 | 3:1 | |
| SD41 | 12 | 3 | 4:1 | |
| SD51 | 12.5 | 2.5 | 5:1 | |
| SD10 | 15 | — | 1:0 | |

MD=maltodextrin; PPE = pectin extracted from pomegranate peels; PPP= polyphenols from pomegranate peels.

## 2.8 Spray drying of PPP suspensions

PPP suspensions were spray-dried under mixed-flow conditions using a pilot-scale spray dryer (Anhydro, PSD 52, Denmark). The inlet and outlet air temperatures were set at 140°C and 70±5°C, respectively. The outlet air temperature was kept constant by adjusting the feed flow rate which was between 0.75–1 L/h, while the air pressure of the two-fluid atomizer was set at 30 psi. The resultant spray-dried PPP powders were collected and kept in a desiccator at room temperature until needed for analysis. The basic processing flowchart was listed in Figure 2.1.

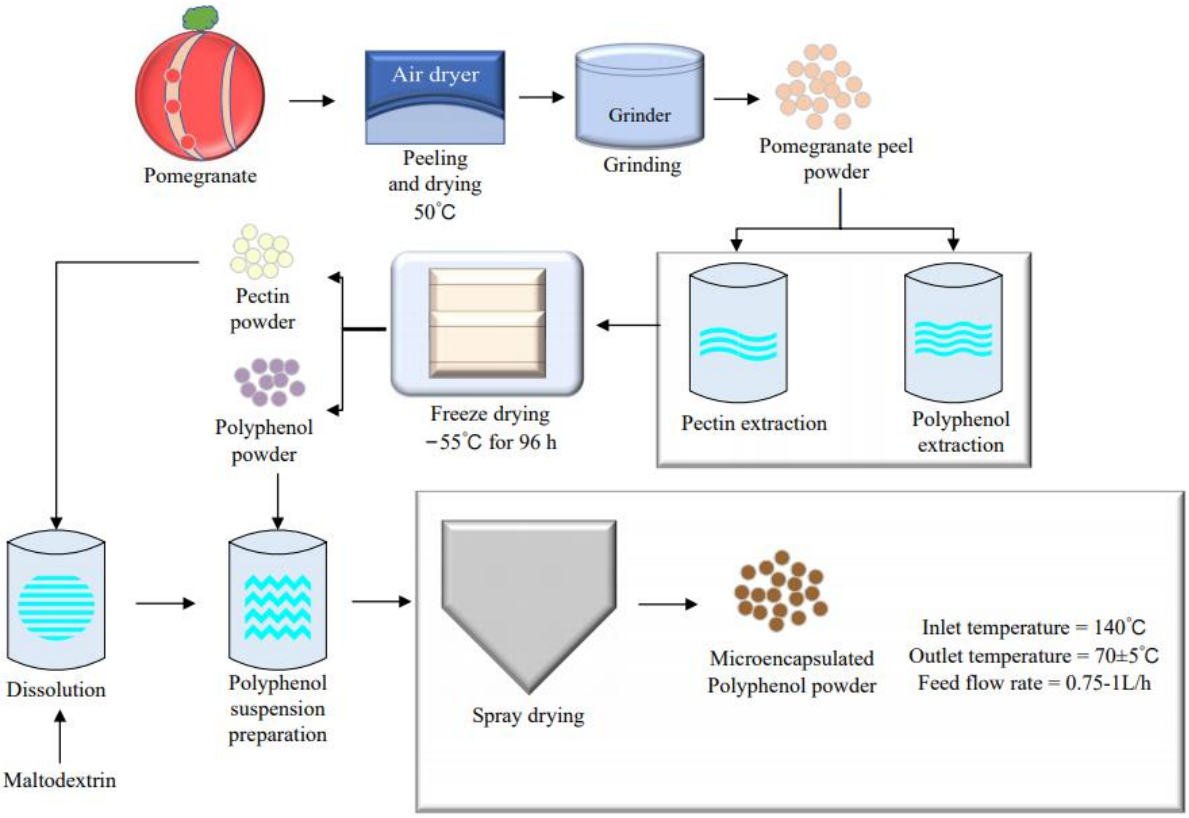


Figure 2.1 Schematic for the spray drying of Georgia-grown pomegranate polyphenol

**Physico-chemical properties of spray-dried PPP powders**

**Encapsulation efficiency (EE)**

For surface polyphenol, 2 g of spray-dried PPP powders were mixed with 10 mL absolute ethanol and 10 mL absolute methanol. The mixture was centrifuged at 8000 × g for 5 minutes and the supernatant was collected. Then, surface polyphenol content was quantified with the same method described earlier. For total polyphenol content, 2 g of spray-dried PPP powders were dissolved in a 20 mL mixture of absolute ethanol: acetic acid: deionized water (50:8:42 v/v/v). Then the same procedure described for surface polyphenol content of microencapsulated PPP was carried out (Robert *et al*., 2010). The EE was calculated according to Eq. (3):

$$\mathrm{EE}(\%) = \left(1 - \frac{\text{surface polyphenol content of encapsulated PPP}}{\text{Total polyphenol content of encapsulated PPP}}\right) \times 100\% \quad (3)$$

**Antioxidant activity**

Antioxidant activity of spray-dried PPP powders was determined using DPPH assay. One and a half g of PPP powders were dissolved in 10 mL of absolute ethanol, then the antioxidant activity was determined using the DPPH method which was described earlier.

2.9.3 Moisture content and water activity ($a_w$)

The moisture content of the spray-dried PPP powders was determined by using a moisture analyzer (HR73 Halogen Moisture Analyzer, Mettler-Toledo GmbH, Greifensee, Switzerland). Water activity ($a_w$) values were obtained using a water activity meter (AquaLabSeries 3 TE, Decagon Devices, Inc., Pullman, WA, USA).

**Bulk density**

The bulk density of the spray-dried PPP powders was determined by following the method reported by Edris, Kalemba, Adamiec, & Piątkowski (2016). The sample was freely flowed and weighed in a 50 mL graduated cylinder. The bulk density was calculated by dividing the sample mass (g) by the sample volume ($cm^3$).

**Water solubility index (WSI)**

WSI of the spray-dried PPP powders was determined using a modified method of Phoungchandang & Sertwasana (2010). Approximately 2 g of sample powder was dissolved in 25 mL of deionized water. The mixture was then placed in a water bath at 37°C for 1 hour and centrifuged at 7500 × g for10 minutes. Afterward, the supernatant was decanted into pre-weighed evaporating dishes and dried at 105°C in the oven for 24 hours. The WSI (%) was calculated according to Eq. (4):

$$\text{WSI}\% = \frac{\text{mass of dried supernatant}}{\text{mass of sample powder}} \times 100\% \quad (4)$$

**Color**

The color of the spray-dried PPP was measured using a Lab Scan XE Colorimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA) and was reported Chroma value and hue angle value. The equation of Chroma value and Hue angle value was calculated according to Eqs. (5) and (6) (Solval, Sundararajan, Alfaro, & Sathivel, 2012):

$$\text{Chroma} = [a^2 + b^2]^{1/2} \quad (5)$$

$$\text{Hue angle} = \arctan(b/a) \quad (6)$$

Where a is the degree of redness to greenness, and b is the degree of yellowness to blueness.

Total color difference (ΔE) of powders after the drying procedure was calculated using Eq. (7):

$$\Delta E = \sqrt{(L_0 - L_p)^2 + (a_0 - a_p)^2 + (b_0 - b_p)^2} \quad (7)$$

Where, $L_0$, $a_0$, and $b_0$ are the values of a reference white calibration; and $L_p$, $a_p$, and $b_p$ are the corresponding values of the microencapsulated powders.

**Particle size distribution**

The particle size distribution of powders was quantified using a particle size analyzer (Model PSA 1190, Anton Paar GmbH, Graz, Austria). The whole light scatters pattern was

collected and used to calculate the particle size distribution using the Modified Michelson Interferometer (MMI) method which quantifies the angular distribution of the backscattered light. The results were described for $D_{10}$, $D_{50}$, and $D_{90}$ which are the volume diameter of the particles at 10%, 50%, and 90% cumulative volume, respectively, and the span value (spread of particles) was calculated by following the method referred to Mis Solval, Bankston, Bechtel, and Sathivel (2016).

**Scanning electron microscopy (SEM)**

A scanning electron microscope (1450 EP, Carl Zeiss MicroImaging, Inc., Thornwood, NY, USA) with an acceleration potential of 10 kV was used to elucidate particle morphologies after powdered samples were sputter-coated with gold. The powder particles were systematically observed at a magnification between 800 and 1000x.

2.10 Statistical analysis

All the experiments were carried out in triplicate determinations. Means and standard deviations of experimental results were reported, and the data were analyzed using the statistical software SAS (SAS university edition version 3.8, SAS Institute, Cary, NC, USA). The significance of the observed differences among means of experimental results was evaluated by Analysis of Variance (ANOVA). A *P* value less than $\alpha = 0.05$ was statistically significant.

## Results and Discussion

**DE of pomegranate peel pectin (PPE)**

DE is very important as it can determine the mechanism of formation of pectin gels, their conformation, and their rheological properties (Abid *et al*., 2017). The overall mean value of DE of PPE was 53.67%± 0.37 which was considered as high-methoxyl pectin (DE > 50%) with desirable emulsifying properties. Abid *et al* (2017) have investigated the DE of four varieties of Tunisia pomegranates, and they reported the DE values of all cultivars were

less than 50% which was considered to be low-methylated. Yuliarti *et al* (2015) have reported that the maturation degree of the fruits and extraction methods affected the DE value of pectin. In addition, Alba, Laws, & Kontogiorgos (2015) have reported that DE could affect the intrinsic viscosity of pectin, and high-methoxyl pectin exhibited a higher ability to increase the viscosity. Interestingly, high methoxyl pectin could result in more spherical capsules with higher encapsulation efficiency due to its relatively high viscosity and molecular weight compared to low methoxyl pectin (Muhoza *et al*., 2019). Therefore, the pectin extracted in this study can be used in the microencapsulation technique.

**TPC of pomegranate peel extracts**

The level of phenolic compounds in pomegranate peels varies considerably from one cultivar to another, and it largely depends on the growing conditions of the trees (e.g. climate, soil type, etc.) (Gözlekçi, Saraçoğlu, Onursal, & Özgen, 2011; Lu, Ding, & Yuan, 2008). In this study, specific cultivars of pomegranates were not identified, but it is believed that the peels came from more than 10 cultivars of Georgia-grown pomegranates, and the TPC of the polyphenol containing peel extracts was 522.82±6.94 mg GAE/g. Similar findings have been reported by Kam *et al*. (2013) who have elucidated the differences in the TPC content and antioxidant activities of the methanolic extracts of pomegranate peels from three different countries. In their study, the total polyphenol contents were ranging from 201.5±5.7 to 629.5±5.2 mg GAE/g pomegranate peel extracts. Organic solvent (methanol, ethanol, and acetone) extraction and ultrasound-assisted extraction are the most commonly used methods for the extraction of polyphenols. The polar characteristics of these organic solvents and their capacity to limit polyphenol oxidase activity help to achieve high recovery yields of polyphenol extracts (Abad-García *et al*., 2007).

**Antioxidant activity of pomegranate peel extracts**

There are two main types of assays used to determine the antioxidant activity of fruit extracts. The first category includes the DPPH assay which measures the ability of fruit extracts to scavenge free radicals. The second category includes FRAP assay which measures the potential of fruit extracts to reduce ions or oxidants (Qabaha, Al-Rimawi, Nusseibeh, Abbadi, & Abu-Lafi, 2019). DPPH antioxidant activity of pomegranate peel extract was 64.10±1.10 %. These results are between the range (31.16%–66.82%) reported by Hmid, Elothmani, Hanine, Oukabli, & Mehinagic (2017) on ten cultivars of pomegranate from Morocco, and those (60.1%–83.5%) reported by Tabaraki, Heidarizadi, & Benvidi (2012) on pomegranate peels from Iran. Furthermore, the FRAP value of pomegranate peel extracts was 9.77 ±0.01 mmol $Fe^{2+/}$g. Qabaha *et al*. (2019) reported a solvent extraction of polyphenols from pomegranate peels from Palestine with a FRAP value of 12.4 ±0.4 mmol $Fe^{2+/}$g. Kam *et al*. (2013) reported that the FRAP values of pomegranate peel extracts (cultivars from Australia, China, and the USA) varied from 1.51 ± 0.28 to 4.63 ± 0.22 mmol $Fe^{2+/}$g dry weight. They also demonstrated that the total antioxidant activity measured by DPPH and FRAP assays was significantly higher in the pomegranate cultivars from China than those from Australia and the USA. Thus, the geographical location influences the antioxidant activities of pomegranate cultivars. Nevertheless, these results suggest that pomegranate peel extracts used in this study have high antioxidant activity presumably due to their phenolic content. According to Gupta and Prakash (2014), polyphenols are phytochemicals with antioxidant and anti-inflammatory activities. Interestingly, pomegranates contain high amounts of polyphenols that can be classified into hydrolyzable tannins (ellagitannins), condensed tannins, flavonoids (anthocyanins), and phenolic acids (ellagic acid and gallic acid) (Kandylis & Kokkinomagoulos, 2020). Notably, most of the polyphenols present in pomegranates are found in their peels and the predominant type of polyphenol is a type of ellagitannin called punicalagin (Fischer *et al*., 2011). According to Li *et al*. (2015),

punicalagin made up approximately 77% of the total polyphenols in the peel of Chinese pomegranates.

**EE and antioxidant activity of encapsulated PPP powders**

The EE of spray-dried PPP powders prepared with different combinations of MD and PPE were presented in Table 2.2. The EE of SD41 and SD51 powders was 91.87%±0.68, and 91.87%±0.26, respectively. These values were significantly ($P < 0.05$) higher than those of the rest of the powders, while the lowest EE of 66.49%±0.25 was observed in SD01 (powders containing only PPE). These results suggested that the ratios of MD to PPE had a significant effect on the EE of the microencapsulated PPP powders. Tukey-HSD test revealed that powders microencapsulated with a mixture of MD: PPE had higher EE compared to the powders produced with MD or PPE alone. As EE is an indicator of how much TPC is encapsulated in the PPP powders, hence higher EE is achieved with fewer polyphenols on the surface of microcapsules to maintain higher stability of the encapsulated materials (Çam, İçyer, & Erdoğan, 2014). As shown in Table 2.2, the surface TPC of SD01 was 25.96±0.28 mg GAE/g spray-dried powder, which was significantly higher ($P < 0.05$) than the other four powders (the surface TPC was ranging from 5.12±0.18 to 7.37±0.42 mg GAE/g spray-dried powder). This result might be due to the insufficient ability of PPE to completely encapsulate PPP compared to the combination of MD and PPE, thus, a considerable amount of polyphenol content was detected on the surface of microcapsules. Higher EE observed in the powders produced with a mixture of MD: PPE could be explained by the fact that the combination of MD and PPE quickly creates a coating around the PPP and increases the thickness of the microencapsulation wall during the microencapsulation process. It has been reported that microencapsulation improves the stability of phenolic extracts by protecting them from environmental factors such as light and oxygen that can promote their quick degradation (Ezhilarasi, Indrani, Jena, & Anandharamakrishnan, 2014).

The antioxidant activity (measured as DPPH, %) of SD41 (57.30±0.64) and SD31 (56.21±1.13) was significantly ($P$<0.05) higher than the rest of the powders (Table 2.2). Meanwhile, the lowest antioxidant activity was observed in SD10. Furthermore, the results suggested that using a combination of MD: PPE in the coating materials produced PPP powders with enhanced antioxidant activities (compared to PPP powders produced only with either MD or PPE. Lim, Cabajar, Migallos, Lobarbio, & Taboada (2019) reported that different encapsulating materials may produce powders with different antioxidant properties. Numerous studies have reported that there was a strong positive relationship between antioxidant activity and polyphenol content (Ersus & Yurdagel, 2007; Tolun *et al*., 2016; Xu, Zhang, Cao, & Lu, 2010). However, our results analyzed by the Pearson correlation test revealed that both values were moderately correlated ($\rho = 0.36$). This could be expounded by the fact that TPC does not account for all antioxidants and that some compounds might exhibit a synergistic antioxidant effect which is likely subjected to their chemical structures (Lim *et al*., 2019). The results obtained in this study suggested that PPP powders produced with a mixture of MD: PPE showed higher EE and antioxidant activities, and the optimal ratio of MD: PPE was 4:1 (SD41).

Table 2.2 TPC, EE, and antioxidant activity of microencapsulated PPP[†]

| Treatment | TPC (mg GAE/g powder) | | EE (%) | DPPH (%) |
|---|---|---|---|---|
| | Surface TPC | Encapsulated TPC | | |
| SD01 | 25.96±0.28[a] | 77.48±0.57[a] | 66.49±0.25[c] | 52.09±1.29[cd] |
| SD31 | 7.37±0.42[b] | 71.70±0.68[b] | 89.72±0.49[b] | 56.21±1.13[ab] |
| SD41 | 5.39±0.47[d] | 66.31±0.46[c] | 91.87±0.68[a] | 57.30±0.64[a] |
| SD51 | 5.12±0.18[d] | 63.06±0.22[d] | 91.87±0.26[a] | 53.45±1.26[bc] |
| SD10 | 6.49±0.10[c] | 57.11±0.72[e] | 88.64±0.11[b] | 49.80±1.27[d] |

[†]Values are the mean ± standard deviation of triplicate determinations.

[a-d]Means with the same letter in the same column are not significantly different ($P$<0.05).

See Table 2.1 for description of SD01, SD31, SD41, SD51 and SD10

TPC = total phenolic content; EE = encapsulation efficiency; DPPH = 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging assay

**Moisture content and water activity of encapsulated PPP**

The effect of different ratios of coating materials on moisture content and water activity ($a_w$) of PPP powders was presented in Table 2.3.The results revealed that the moisture content of PPP powders was significantly ($P < 0.05$) affected by the type of encapsulating agents. Interestingly, the moisture content of PPP powders decreased as the amount of maltodextrin in coating materials increased. This suggests a higher water holding capacity of PPE compared to MD. Also, the high drying rate and the low resistance towards the mass transfer of MD helps to produce spray-dried powders with reduced moisture (Ezhilarasi *et al*., 2014). Similar results have been reported by Mishra, Mishra, & Mahanta (2014) for the production of dried amla juice powder via SDR; the authors demonstrated that increasing the concentration of MD decreased the moisture content of powders considerably. A moisture content below ~5% is expected to reduce the chance of lipid oxidation and microbial contamination (Sun, Cameron, & Bai, 2019). The moisture content of spray-dried samples in this study was around or below this value. Therefore, the PPP powders with low moisture could be incorporated into food products while retaining their stability and viability. A similar trend was also found in the $a_w$ values of PPP powders (Table 2.3). PPP powders produced with PPE alone (SD01) had significant ($P < 0.05$) higher $a_w$ values than the rest of the treatments. Increasing the amounts of MD in the encapsulating materials reduced the $a_w$ values of the resultant PPP powders. The $a_w$ values of PPP powders were between 0.17 and 0.26 which were within a safe $a_w$ range for the inhibition of microbial growth and enzymatic and non-enzymatic degradation (Beuchat *et al*., 2013). Hence, the resultant powders guaranteed microbiological safety and high stability during processing and storage.

Table 2.3 Moisture content, $a_w$, bulk density, and water solubility index of microencapsulated PPP powders.

| Treatment | Moisture (g/100g, w.b.) | Water activity($a_w$) | Bulk density($g/cm^3$) | Water solubility index(%) |
|---|---|---|---|---|
| SD01 | 5.39±0.04[a] | 0.26±0.01[a] | 0.30±0.01[c] | 78.79±2.66[b] |
| SD31 | 5.13±0.16[a] | 0.25±0.00[b] | 0.31±0.01[bc] | 88.15±0.81[a] |
| SD41 | 4.48±0.10[b] | 0.25±0.00[b] | 0.33±0.00[ab] | 88.38±0.97[a] |
| SD51 | 4.26±0.27[b] | 0.19±0.01[c] | 0.33±0.01[a] | 88.50±0.87[a] |
| SD10 | 4.15±0.01[b] | 0.18±0.00[d] | 0.34±0.00[a] | 88.86±0.21[a] |

[†]Values are the mean ± standard deviation of triplicate determinations.

[a-d]Means with the same letter in the same column are not significantly different ($P<0.05$).

See Table 2.1 for the description of SD01, SD31, SD41, SD51, and SD10

**Bulk density and water solubility index of PPP powders**

The bulk densities of PPP powders ranged from 0.30 to 0.34 g/cm$^3$ (Table 2.3). In general, PPP powders produced with higher amounts of MD showed significantly ($P$<0.05) higher bulk densities. This suggests that MD (0.42−0.49 g/cm$^3$, commercially) has a higher bulk density than PPE (Takeiti, Kieckbusch, & Collares-Queiroz, 2010). Also, these results may be due to the lower viscosities of the homogenized suspension containing MD compared to those containing PPE. Lower feed viscosity results in an increased bulk density of the resultant powders (Lim *et al*., 2019). Azarpazhooh *et al*. (2019) reported that particle size of spray-dried powders might also influence the bulk density. Bulk density is a strong indicator of how well powders can be handled, stored, and processed (Lim *et al*., 2019). Powders with higher bulk densities require less space (volume) for storage per unit of mass. A higher bulk density also implies a reduced presence of air in the powder which contributes to higher protection against degradation/oxidation during storage (Edris *et al*., 2016). Similar results have been reported by Lim *et al* (2019) who investigated the spray drying of phenolic compounds extracted from mango seed kernel, and they reported the bulk densities of their microcapsules were between 0.30−0.43 g/cm$^3$.

Water solubility index (WSI) is a crucial property for food powders in their proper reconstitution in aqueous matrices. This property is affected by the composition and drying process of the resultant powders. In this study, the WSI of powders ranged from 78.79 to 88.86% (Table 2.3). Interestingly, MD significantly ($P$<0.05) increased the WSI of PPP powders. These results may indicate a higher solubility of MD than PPE in water (Ahmadian, Niazmand, & Pourfarzad, 2019). Furthermore, our results were in agreement with the results of Maia *et al*. (2020) who evaluated the effect of different concentrations of MD on the water solubility of microencapsulated craft beer. Ahmadian *et al*. (2019) reported a negative correlation between water solubility and particle size of maltodextrin−pectin powders. In our

study, the Pearson correlation test revealed that both values were negatively correlated (ρ = -0.31).

**Particle size distribution**

According to Tonon, Brabet, Pallet, Brat, & Hubinger (2009), the particle size distribution of food powders is an important factor that affects handling, processing, and storage. Particle size distribution values of PPP powders were listed in Table 2.4. Furthermore, the $D_{50}$ (μm) values of the PPP powders ranged from 6.51±0.03 (SD10) to 8.09±0.08 (SD41). PPP powders produced with only MD (SD10) showed significantly ($P$<0.05) smaller particle sizes than the rest of the powders. Moreover, it is believed that increasing the amounts of PPE increased the thickness of the coating wall of powder particles. The particle size of spray-dried powders is affected by the viscosity of the feeding solutions (Ahmadian *et al*., 2019). Therefore, it is suggested that the PPE-containing suspensions may have had higher viscosities than the suspension prepared with MD alone. Thus, the powders containing PPE with higher viscosity had larger particle sizes than those contained with MD alone. The span values of powders were ranging from 2.14 to 2.88. Spray-dried powders with span values above two indicate the presence of particle agglomerations (Jiang *et al*., 2020). Interestingly, higher particle agglomeration was observed in PPP powders prepared with higher amounts of PPE. The results suggested that small particles were sticking together because of the sticky properties of PPE, thus forming particle agglomerates. In addition, Pearson's correlation test revelated that there was a moderate relationship between particle size and antioxidant activity (DPPH%) of microencapsulated powders (ρ = 0.58). SD41 had the highest antioxidant activity and particle size while SD10 had the lowest values of the corresponding parameters. Similar findings have been reported by Cai, Qin, Ketnawa, & Ogawa (2020) that citrus peel tissue with larger particle size tended to show not significantly but comparatively higher antioxidant activity than the smaller

fractions. Zaiter, Becker, Karam, & Dicko (2016) have also found that antioxidant activities of green tea powder with smaller particles (< 50 μm) were significantly lower ($P$<0.05) than those of green tea powders with larger particle sizes (50−180 μm).

Table 2.4 Particle size distribution values of microencapsulated PPP powders[†]

| Treatment | Particle Size [µm] | | | | Span |
|---|---|---|---|---|---|
| | $D_{10}$ | $D_{50}$ | $D_{90}$ | Mean size | |
| SD01 | 0.59±0.03$^{bc}$ | 6.58±0.05$^{d}$ | 20.14±0.11$^{a}$ | 9.26±0.05$^{b}$ | 2.98±0.02$^{a}$ |
| SD31 | 1.02±0.29$^{ab}$ | 7.65±0.05$^{b}$ | 17.74±0.16$^{c}$ | 9.15±0.05$^{b}$ | 2.18±0.05$^{c}$ |
| SD41 | 1.21±0.23$^{a}$ | 8.09±0.08$^{a}$ | 18.97±0.06$^{b}$ | 9.69±0.11$^{a}$ | 2.20±0.04$^{c}$ |
| SD51 | 0.64±0.09$^{bc}$ | 7.22±0.03$^{c}$ | 16.06±0.07$^{d}$ | 8.43±0.04$^{c}$ | 2.14±0.02$^{c}$ |
| SD10 | 0.28±0.01$^{c}$ | 6.51±0.03$^{d}$ | 15.36±0.06$^{e}$ | 7.74±0.04$^{d}$ | 2.32±0.02$^{b}$ |

[†]Values are the mean ± standard deviation of triplicate determinations.

[a-d]Means with the same letter in the same column are not significantly different ($P$<0.05).

See Table 2.1 for the description of SD01, SD31, SD41, SD51, and SD10

**Color**

Table 2.5 showed the color values of PPP powders. It was observed that PPP powders containing higher amounts of MD had significantly ($P < 0.05$) higher lightness (L*) values and lower redness/greenness (a*) values than those containing higher amounts of PPE. Meanwhile, there was no significant difference ($P > 0.05$) among PPP powders regarding blueness/yellowness (b*) values. All of the powders were yellowish-green in color (hue angles between 79 and 87) with similar color saturation (Chroma value). According to the PPE concentrations used in the mixtures of wall materials, there was a change in the color of the final product. Reduction in whiteness ($\Delta E$) indicated that powders containing higher percentages of PPE had a darker appearance. The results could be due to the inherent color property of PPE that affected the color characteristics of final products. According to de Souza, Thomazini, de Carvalho Balieiro, & Fávaro-Trindade (2015) the color of spray-dried powders is affected by the properties of microencapsulating materials (composition, concentration) and drying conditions (inlet air temperature, atomization).

Table 2.5 Color values of microencapsulated PPP powders[†]

| Treatment | L* | a* | b* | Hue angle | Chroma | Δ*E* |
|---|---|---|---|---|---|---|
| SD01 | 58.63±1.09[b] | 3.50±0.19[a] | 22.78±0.74[a] | 81.27±0.24[c] | 23.04±0.76[a] | 41.79±0.75[a] |
| SD31 | 59.93±0.87[ab] | 3.96±0.44[a] | 22.40±0.53[a] | 79.98±0.89[c] | 22.75±0.60[a] | 41.82±0.42[a] |
| SD41 | 59.92±0.54[ab] | 2.90±0.13[b] | 22.21±0.21[a] | 82.53±0.27[bc] | 22.31±0.23[a] | 39.62±0.63[ab] |
| SD51 | 62.19±2.17[a] | 2.29±0.78[b] | 22.05±0.37[a] | 84.08±1.93[b] | 22.18±0.43[a] | 38.28±1.97[b] |
| SD10 | 62.46±0.72[a] | 1.26±0.13[c] | 22.00±0.50[a] | 86.72±0.27[a] | 22.03±0.51[a] | 37.91±0.33[b] |

[†]Values are the mean ± standard deviation of triplicate determinations.

[a-d]Means with the same letter in the same column are not significantly different ($P<0.05$).

See Table 2.1 for the description of SD01, SD31, SD41, SD51, and SD10

**Powder morphology**

The microstructure of PPP powders obtained via SDR could be affected by numerous factors including composition and properties of coating materials, the core to coating ratio, as well as drying and storage conditions (Maia *et al*., 2020). The three-dimensional characterization of the powders through SEM indicated that all powders were irregularly spherical with extensively dented/ wrinkled surfaces (Figure 2.2). Higher particle agglomeration was observed in PPP powders produced with higher amounts of PPE (Figure 2.2A); while powders produced with higher amounts of MD showed less particle agglomeration (Figure 2.2 B-E), this confirms the obtained results for particle size distribution. According to Tolun *et al*. (2016), the composition of coating materials might be responsible for dented characteristics of the spray-dried powders. Moreover, wrinkled surfaces of powder particles may be due to rapid evaporation of the feeding suspension in the early stage of SDR, which results in the formation of crust on the surface of the liquid droplets (Wilkowska, Ambroziak, Adamiec, & Czyżowska, 2017). Although deep hollows were observed, pores or cracks on the surface of powders were almost absent, which was important for preventing the oxidation inside coating walls, and hence for better protection of the microencapsulated agent (El-Messery, El-Said, Demircan, & Ozçelik, 2019). The results obtained in this study suggested that PPE promotes particle agglomeration which may be a desirable characteristic in some food applications. Agglomerated particles may be easier to handle and could have higher storage stability (Sun *et al*., 2019).

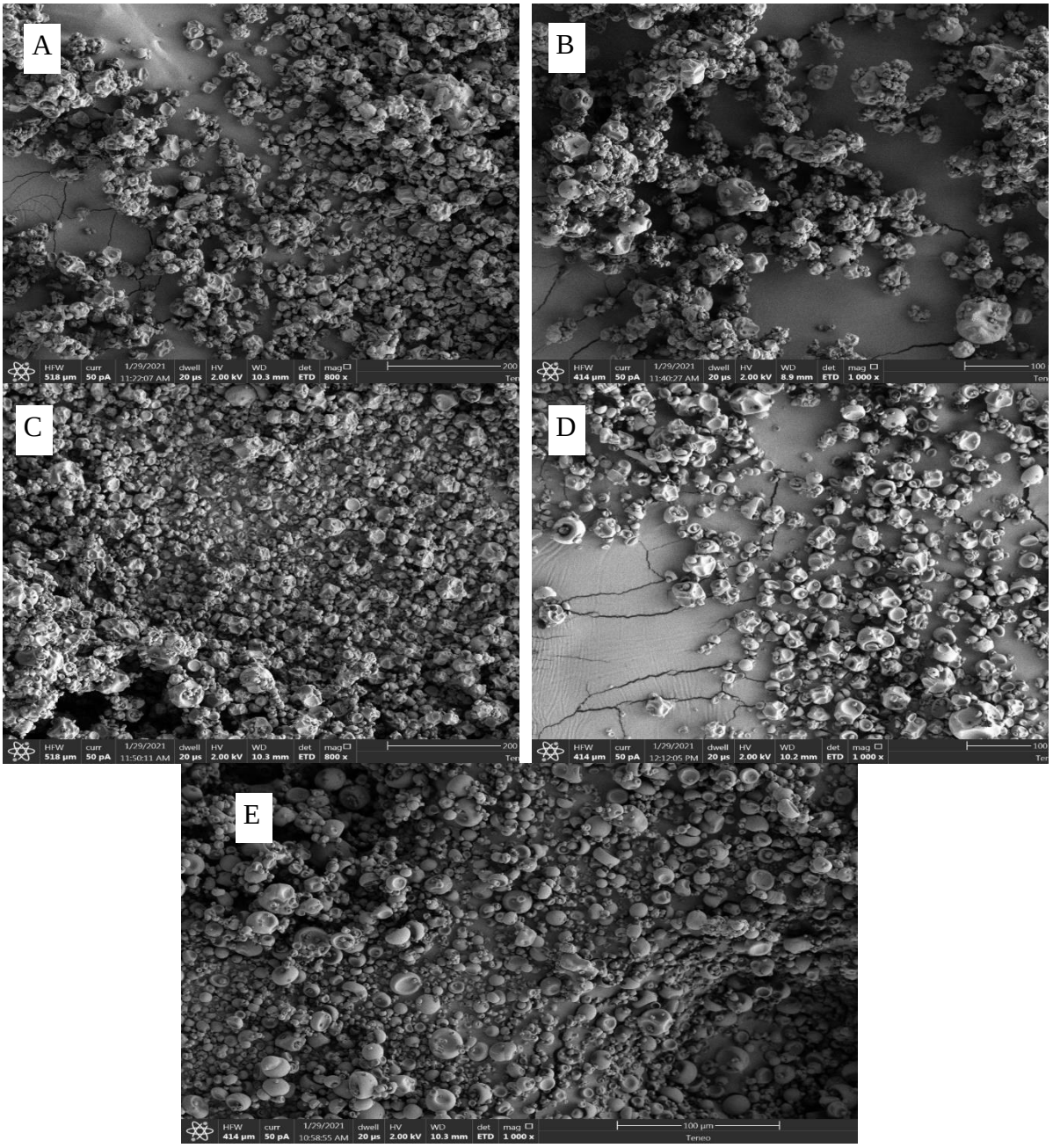


Figure 2.2 Scanning electron microscope of microencapsulated PPP powders. A) SD01, B) SD31; C) SD41; D) SD51; and E) SD10

## Conclusion

The study demonstrated that polyphenols from pomegranate peels can be successfully microencapsulated with pectin (PPE) and maltodextrin (MD) *via* spray drying (SDR). Also, it was demonstrated that extracts from Georgia-grown pomegranates peels had high polyphenol content and antioxidant activities. The use of maltodextrin with pectin as encapsulation agents was an effective strategy to microencapsulate pomegranate peel polyphenols. Furthermore, powders containing MD and/or mixtures of MD: PPE were whiter and had higher EE, bulk density, and water solubility than powders prepared with PPE only. Moreover, microencapsulated powders with MD: PPE at a ratio of 4:1 had the highest antioxidant activity. All types of powders showed an irregularly spherical shape with 7-9 μm mean particle sizes. The information presented in this study indicated that natural antioxidants could be microencapsulated in a matrix containing MD and/or natural pectin extracted from plants by-products. Also, the study demonstrated that pomegranate peels are good sources of phytochemicals including polyphenols and pectin. Microencapsulation via spray drying provides the interesting potential to increase the stability of pomegranate peel polyphenols. The resultant microencapsulated powders with high antioxidant activity may be incorporated into functional foods as a novel ingredient.

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# CHAPTER 3

# INFLUENCE OF FREE AND MICROENCAPSULATED POLYPHENOLS – CONTAINING EXTRACTS FROM GEORGIA−GROWN POMEGRANATE PEELS ON THE STORAGE STABILITY OF SALAD DRESSINGS[2]

[2] Yang, B., Chen, J., Kealey, K.S., & Mis Solval, K. To be submitted to *Journal of Food Science*

**Abstract**

Lipid oxidation is a major cause of quality deterioration in salad dressings. Polyphenols are phytochemicals with strong antioxidant properties. In this study, the effect of natural antioxidants (free or microencapsulated polyphenols from pomegranate peels, and/or grape seed extract) on the lipid oxidation of Italian salad dressings homogenized at low or high shear rates and stored at accelerated or ambient conditions was evaluated. Emulsion capacity and stability were evaluated for fresh salad dressings. Color and pH changes, as well as lipid oxidation (Iodine values, Peroxide values, and Thiobarbituric acid reactive substances values) of salad dressings were determined over 21 days of storage at accelerated conditions, and 8 weeks under ambient storage, respectively. Salad dressings prepared at a high shear rate (5,000 × g) had significantly higher ($P$<0.05) emulsion stability than those homogenized at a low shear rate (250 × g). It was found that shear rates had a minor effect on the oxidative stability of salad dressings. Salad dressing stored under accelerated storage had higher lipid oxidation after 21 days compared to the salad dressings stored under ambient conditions for 8 weeks. The study demonstrated that microencapsulated pomegranate peel polyphenols were more effective at controlling lipid oxidation in salad dressings than the free pomegranate peel polyphenols in both accelerated and ambient storage conditions. Microencapsulated polyphenols from pomegranate peels may be used as an effective functional food ingredient for controlling lipid oxidation in high lipid and acidified foods.

**Keywords**: Salad dressing, Lipid oxidation, Antioxidant, Accelerated storage, Ambient shelf-life test

## Introduction

Salad dressings are emulsified semisolid foods prepared with vegetable oils, acidifying ingredients, spices, and other additives. Moreover, salad dressings are popular foods worldwide prepared with different formulations and unique styles such as Italian, Thousand Island, and French dressings (Mizani, Yaghoti Moghaddam, Alimi, & Salehifar, 2015). Some salad dressings are oil-in-water (o/w) or water-in-oil (w/o) emulsions in which small droplets of oil or water are dispersed in an aqueous phase; while other salad dressing styles are considered suspensions (Arancibia, Bayarri, & Castell, 2013). It has been reported that the physical stability of salad dressings is associated with the capacity to maintain their structural integrity over time and is determined by several factors including interfacial composition, emulsion droplet size, flocculation, and final phase separation (Kiokias, Gordon, & Oreopoulou, 2016; Zhang, Quek, Lam, & Easteal, 2008). Commercial salad dressings are offered as emulsified products (one phase) or separated mixtures (two-phase). One-phase salad dressings usually contain emulsifiers and are finely homogenized (using high shear rates) which results in the size reduction of micelles and creamy consistency that prevents phase separation. Meanwhile, two-phase salad dressings have a distinct layer of oil on top of the water phase (Perrechil, Santana, Fasolin, Sodre da Silva, & da Cunha, 2010). During the mixing and homogenization of ingredients, some processing conditions such as shear rate, temperature, and mixing time are extremely important for the final formation of stable salad dressings with desirable organoleptic properties (Bengoechea, Lopez, Cordobes, & Guerrero, 2019; Kim, Oh, & Lee, 2020).

According to the U.S. Food and Drug Administration (FDA), salad dressings must contain a minimum of 30 % (w/w) of vegetable oil (USFDA, 2012). Olive oil, peanut oil, and sunflower oil are widely used vegetable oils in salad dressings because of their great flavor, unsaturated fatty acid profile, and/or health benefits (Kaltsa, Yanniotis, Polissiou, &

Mandala, 2018). However, using vegetable oils with a high content of unsaturated fatty acids may reduce the shelf life of salad dressings due to lipid oxidation that may lead to the formation of undesirable compounds including lipid hydroperoxides, aldehydes, ketones, and lactones (Sainsbury, Grypa, Ellingworth, Duodu, & De Kock, 2016; Tseng, & Zhao, 2013). According to Kiokias *et al*. (2016), physicochemical properties (pH, particle size, and electrical charge of micelles), as well as processing parameters (storage temperature, homogenization conditions, oxygen, and light levels) may affect the oxidative stability of salad dressings. Hence, synthetic, and natural antioxidants are widely used to minimize or delay lipid oxidation as well as the formation of oxidation products that may alter the physicochemical, taste, and nutritional value of salad dressings. Natural antioxidants such as fruit polyphenols and tocopherols are considered as safe and effective alternatives to control lipid in foods; therefore, they are often preferred over synthetic antioxidants like butylated hydroxyanisole (BHA) and *tert*-butyl hydroquinone (TBHQ) (Phisut, Nuttanapat, & Peimika, 2018). Recently, the utilization of fruit processing by-products (peels, seeds, etc.) to develop functional foods has been investigated due to their high contents of phytochemicals with antioxidant and antimicrobial properties (Rosales Soto, Brown, & Ross, 2012). Utilizing by-products of the fruit industry to develop novel food ingredients is in line with today's waste reduction and sustainability initiatives (Pande, & Akoh, 2009; Tseng *et al.*, 2013). Interestingly, it has been reported that polyphenols obtained from pomegranates peels show strong antioxidant activities (Hooks, Niu, Masabni, Sun, & Ganjegunte, 2021; Pateiro, Gómez-Salazar, Jaime-Patlán, Sosa Morales, & Lorenzo, 2021; Shahkoomahally, Khadivi, Brecht, & Sarkhosh, 2021). Nevertheless, the direct addition of polyphenols-containing fruit extracts into foods is technologically challenging due to the low stability of polyphenols during the processing and storage of foods (Santos, & Meireles, 2011). Bitterness, astringency, and unpleasant flavors are often reported when polyphenol extracts are directly

added to foods. Hence, the development of novel strategies to improve the stability and compatibility of polyphenol-containing extracts in foods has recently been investigated. To this extent, microencapsulation is an effective technique to stabilize plant-based bioactives with antioxidant properties (Jolayemi, Stranges, Flamminii, Casiraghi, & Alamprese, 2021). According to Corrigan, Hedderley, & Harvey (2012) accelerated storage (ACSL) is a cost-effective alternative to determine the shelf life of food products. Normally, ACSL exposes foods to higher storage temperatures, stronger UV light intensities, and/or pro-oxidants that accelerate deterioration. Salad dressings evaluated under ACSL conditions are often exposed to temperatures between 50 – 60 ℃ (Berton, Ropers, & Genot, 2014).

Several studies have reported the feasibility of improving the oxidative stability and nutritional quality of salad dressings during storage by incorporating phytochemicals with antioxidant properties extracted from plant by-products (Jolayemi *et al*., 2021; Tseng *et al*., 2013). Nonetheless, no studies have reported the effect of shear rates and the addition of microencapsulated polyphenol−containing extracts from pomegranate peels on the oxidative stability of a salad dressing with high oil content. Hence, this study aimed to evaluate the influence of microencapsulated polyphenol extracts from Georgia-grown pomegranate peels on the physicochemical and oxidative stability of Italian-style salad dressings homogenized at different shear rates during accelerated and ambient storage (AMSL) conditions.

## Materials and methods

### Materials

Polyphenol containing extracts (PPP) with a 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition = 64.10% ±1.10 isolated from Georgia-grown pomegranate peels were microencapsulated following the procedure described in Chapter 2 using a mixture of maltodextrin: pomegranate peel pectin (ratio 3:1, w/w), and a commercial grape seed extract (GSE, DPPH inhibition = 67.53%±0.30) (Grape seed extract, Zazzee, Montebello, NY, USA)

was purchased from a local store in Griffin, GA. Peanut oil, white wine vinegar, salt, red pepper flakes, garlic powder, basil leaves, and oregano leaves were obtained from a local supermarket in Griffin, GA, USA. Iodine monochloride Wijs solution, chloroform, potassium iodide, sodium thiosulfate, starch indicator, glacial acetic acid, iso-octane, 1, 1, 3, 3-tetraethoxtpropane, trichloroacetic acid, and 2-thiobarbituric acid were obtained from Fisher Scientific (Fair Lawn, NJ, USA).

**Methods**

**Preparation of Italian-style salad dressing (ISD)**

Fresh ISDs were prepared by mixing 50 (g/100g) peanut oil, 30 (g/100g) white wine vinegar, 4 (g/100g) table salt, 2 (g/100g) garlic powder, 2 (g/100g) red pepper flakes, 1 (g/100g) basil leaves, and 1 (g/100g) oregano leaves. Afterwards, either 0.5 (g/100g) of PPP, 3 (g/100g) microencapsulated polyphenol powder (MPP) (equivalent to 0.5 g/100g free polyphenol containing extracts), or 0.5 (g/100g) GSE were added as natural antioxidants. Also, an ISD without natural antioxidants was prepared as a control. Then, the mixtures were homogenized at low shear rates (250 × g, LOW) or high shear rate (5000 × g, HIGH) using an ultra-high shear homogenizer (Fisherbrand 850 Homogenizer, Thermo Fisher Scientific Inc., Chicago, IL, USA) for 10 minutes. In total, 8 different ISDs were prepared (Table 3.1) which were immediately characterized after production and stored under ACSL and AMSL conditions.

Table 3.1 Description of Italian salad dressings (ISDs) developed in this study

| ISD | Mixing conditions | Natural antioxidant (g/100g) | | |
|---|---|---|---|---|
| | | PPP | MPP | GSE |
| LC | LOW | — | — | — |
| LPPP | LOW | 0.5 | — | — |
| LMPP | LOW | — | 3 | — |
| LGSE | LOW | — | — | 0.5 |
| HC | HIGH | — | — | — |
| HPPP | HIGH | 0.5 | — | — |
| HMPP | HIGH | — | 3 | — |
| HGSE | HIGH | — | — | 0.5 |

Abbreviations: LOW (low shear rate, 250 rpm), HIGH (high shear rate, 5000 rpm), PPP = Polyphenol containing extracts isolated from Georgia-grown pomegranate peels, MPP = microencapsulated polyphenol powder, GSE =grape seed extract, ISD = Italian salad dressing.

**Emulsifying Properties of ISDs**

Emulsifying capacity (EC) and emulsion stability (ES) were evaluated according to the method of Yang *et al*. (2018). Samples were centrifuged at 8,000 ×*g* for 12 minutes using a centrifuge (Model J2-21M, Beckman Instruments Inc., Palo Alto, CA, USA) and EC was calculated using Eq. (1):

$$EC / ES (\%) = (EL/FE) \times 100 \quad (1)$$

Where EL (g) is the mass of the resulting emulsified layer, FE is the whole mass (g) of the fresh emulsion.

Regarding the ES, the fresh emulsions were held in a hot water bath (Model 2872, Thermo Fisher Scientific Inc., Marietta, OH, USA) at 80°C for at least 1hour, then the emulsions were centrifuged at 3,000 ×*g* for 12 minutes. Afterward, the ES value was calculated using Eq. (1).

**Storage stability**

Approximately, 100 mL of ISDs were placed in 4 oz. regular mouth mason glass jars with metal lids (Verones Direct, Shenzhen, Guangdong, China), and stored at 55°C in an air-forced oven (MO 1440SC, Lindberg/ Blum M, Asheville, NC, USA) for 21 days for ACSL and/or at room temperature (~25°C) in light-proof cabinets for 8 weeks for AMSL, respectively. All ISDs were evaluated for pH, color, peroxide value (PV), iodine value (IV), and thiobarbituric acid reactive substances (TBARS). Analyses were conducted every three days for 21 days and every two weeks for 8 weeks for samples stored under ACSL and AMSL conditions, respectively.

**pH of ISDs**

Approximately, 20 mL of sample were placed in a beaker and the pH value was measured using a previously calibrated pH benchtop meter (accumet AE150, Fisher Scientific Inc., Chicago, IL, USA).

**Color**

The color of the ISDs was measured using a Lab Scan XE Colorimeter (Hunter Associates Laboratory, Inc. Reston, VA) and the results were reported as CIE (L*, a*, and b* value). The total color difference (⊿E) of salad dressings was calculated using Eq. (2):

$$\Delta E = \sqrt{(L_0 - L_d)^2 + (a_0 - a_d)^2 + (b_0 - b_d)^2} \quad (2)$$

Where $L_0$, $a_0$, and $b_0$ are the values of freshly made ISDs (day 0); $L_d$, $a_d$, and $b_d$ are the corresponding values of the ISDs after storage for certain time intervals (day 3, day 6, day 9, day 12, day 15, day 18, and day 21 for ACSL; week 2, week 4, week 6, and week 8 for AMSL).

**Oxidation stability**

**Peroxide value (PV)**

The PV of ISDs was determined based on AOAC official method 965.33 (2016). 20 g of salad dressings were centrifuged at 7500 ×g for 5 minutes, then the top layer was collected and filtered through Whatman No.4 filter paper (Whatman International Ltd., Maidstone, England). Afterward, approximately five grams of sample were dissolved in 30 mL of glacial acetic acid−isooctane (3:2, v/v). Upon addition of 0.5 mL of saturated potassium iodide solution and 30 mL of deionized water, the solution then was titrated against a 0.01 M standardized sodium thiosulfate ($Na_2S_2O_3$) solution using 0.5 mL of 1% starch indicator until the blue color was just disappeared. Peroxide value was calculated as shown in Eq. (3).

$$\text{PV} = \frac{(S-B)\times C\times 1000}{2\times W} \quad (3)$$

Where PV is reported as the millimolar peroxide per kg of the sample, S is the volume of titrant (mL) for sample, B is the volume of titrant (mL) for blank, C is the concentration of $Na_2S_2O_3$ solution (mol/L), W is the mass of the sample (g), and 1,000 is the conversion of units (g/kg)

**Iodine value (IV)**

The iodine value of ISDs was calculated by following the AOAC official method 993.20 (2016). 10 gram of salad dressings were centrifuged at 7500 ×g for 5 minutes, then the supernatant was collected and filtered through Whatman No.4 filter paper. Afterward, 0.3 g of filtered sample was dissolved in 10 mL of chloroform. Next, 25 mL of Wijs solution was added and the mixture was then placed in the dark at room temperature for 1 hour. Thereafter, 15 mL of a 15% (w/v) potassium iodide solution and 110 mL of deionized water were added to the flask. The resultant solution was gradually titrated against a 0.1 M standardized sodium thiosulfate solution using 1 mL of 1% starch indicator until the blue color was disappeared. The iodine value was calculated based on the eq. (4).

$$\text{Iodine Value} = \frac{(B-S)\times N\times 126.9}{W\times 1000} \times 100 \quad (4)$$

Where Iodine value equals to g iodine absorbed per 100 g of sample, B is the volume of titrant (mL) for blank, S is the volume of titrant (mL) for sample, N is the normality of $Na_2S_2O_3$ (mol/L), 126.9 is the molecular mass of iodine (g/mol), W is the mass of the sample (g), and 1,000 is the conversion of units (mL/L).

**Thiobarbituric acid reactive substances (TBARS)**

TBARS value of ISDs was determined by following the method reported by Nielsen (2017). Approximately 2 g of sample was dissolved in 10 mL of 10% trichloroacetic acid solution and centrifuged at 4000 ×g for 5 minutes to collect the supernatant. Afterward, 4 mL of 0.5% 2−thiobarbituric acid solution was added to the supernatant, a blank (4 mL deionized water mixed with 4 mL of 0.5% 2-thiobarbituric acid solution) was also prepared. Then all the samples were heated in boiling water for 40 minutes. After cooling to room temperature, the absorbance of samples was recorded using a Genesys 30 UV-vis spectrophotometer (Thermo Fisher Scientific Inc., Madison, WI, USA) set at λ=532 nm. Quantification was

based on the standard curve generated with 1, 1, 3, 3-tetraethoxtpropane (TEP), and the result was reported as mg TEP/kg.

**Statistical analysis**

All the experiments and analyses were carried out in triplicate determinations. Means and standard deviations of experimental results were reported, and the data were analyzed using the statistical software SAS (SAS university edition version 3.8, SAS Institute, Cary, NC, USA). The significance of the observed differences among means of experimental results was evaluated by Analysis of Variance (ANOVA). A *P* value less than alpha = 0.05 was statistically significant.

## Results and discussion

**Emulsifying capacity and emulsion stability of ISDs**

Emulsifying capacity refers to the ability of surfactants and other ingredients to facilitate the formation of food emulsions (Liang, Wang, Chen, Liu, & Liu, 2015). ISDs homogenized at high shear rates had significantly higher ($P<0.05$) emulsion capacity (%) values than those homogenized at a lower shear rate (Table 3.2). At higher shear rates, the particle-particle interactions were higher which might result in smaller micelles and suspended solids. Therefore, only suspended and small micelles and particles remained in the salad dressings, which resulted in higher emulsion capacity (Brewer, Franco, & Garcia-Zapateiro, 2016). Interestingly, LMPP and HMPP which contained microencapsulated polyphenol powders had significantly ($P<0.05$) higher EC than the other ISDs prepared with other antioxidants and homogenized at low and high shear rates, respectively (Table 3.2). This effect may be due to the higher viscosities and emulsification properties of the maltodextrin−pectin found in the MPP powders.

Emulsion stability measures the ability of food emulsions to stabilize the fine droplets during and after the emulsification process (Liang et al., 2015). The results obtained in this

study showed that ES of ISDs ranged from 55.45% to 63.17%. As in the previous case of EC, ISDs homogenized at high shear rates had significantly ($P$<0.05) higher ES values than those homogenized at lower shear rates (Table 3.2). Moreover, LMPP had a significantly ($P$<0.05) higher ES compared to LC, LPPP, and LGSE; while HMPP showed an ES (%) of 63.17 which was significantly ($P$<0.05) higher than those of HC (60.06), HPPP (60.22), and HGSE (60.21) (Table 3.2). The higher ES values of LMPP and HMPP may be explained by the presence of maltodextrin: pectin in MPP which may have created more stable suspensions with higher viscosities. Similar findings have been reported by Perrechil *et al*. (2010) for ES of commercial Italian salad dressings during 6 days of storage (50% to 65%). However, our results were lower than the ES values (81.8% to 88.2%) reported by Mohamad, Agus, & Hussain (2019) who utilized cocoa butter as a stabilizer for salad dressings. According to Lozano-Gendreau, & Vélez-Ruiz (2019), food emulsions and suspensions with high oil content (>50% w/w) may show lower values of EC and ES.

Table 3.2 Emulsifying capacity (EC) and emulsion stability (ES) of ISDs

| ISD | Emulsifying capacity (%) | Emulsion stability (%) |
|---|---|---|
| LC | 53.54±0.06[d] | 55.45±0.26[d] |
| LPPP | 53.96±0.08[d] | 55.61±0.05[d] |
| LMPP | 57.50±0.13[b] | 58.82±0.13[c] |
| LGSE | 53.84±0.07[d] | 55.68±0.28[d] |
| HC | 56.80±0.14[c] | 60.06±0.06[b] |
| HPPP | 57.40±0.29[b] | 60.22±0.05[b] |
| HMPP | 60.41±0.32[a] | 63.17±0.05[a] |
| HGSE | 57.44±0.31[b] | 60.21±0.02[b] |

[†]Values are the mean ± standard deviation of triplicate determinations.

[a-d]Means with the same letter in the same column are not significantly different ($P<0.05$).

See Table 3.1 for the description of LC, LPPP, LMPP, LGSE, HC, HPPP, HMPP, and HGSE

**Effect of accelerated storage**

**Changes in pH and color**

Fresh ISDs had an initial pH of ~3.08−3.16, which decreased over time and showed an average value of 3.05 at 21 days of accelerated storage (Figure 3.1). It has been reported that the slight reduction in pH in salad dressing during accelerated storage may be due to increased vibrations of molecules at higher temperatures and the formation of secondary products such as acetic and propanoic acids from lipid oxidation (Sotirios, Michael, & Vassiliki, 2017). According to Tseng *et al*., (2013), the relatively stable acidic environment of salad dressings may help to stabilize polyphenols which may be able to control lipid oxidation for longer periods.

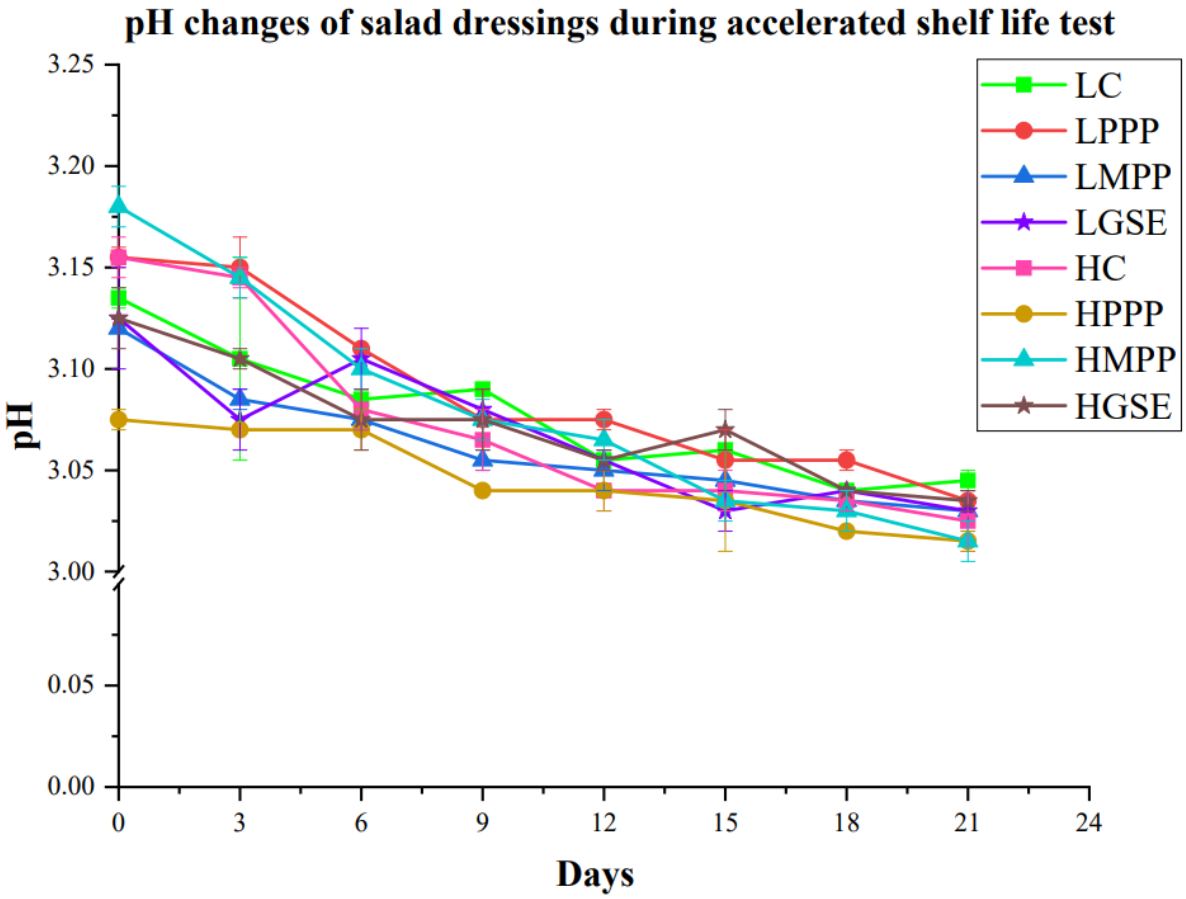


Figure 3.1 pH changes of Italian−style homemade salad dressings (ISDs) during accelerated storage (ACSL). LC = ISD prepared with low shear without antioxidant; LPPP = ISD prepared with low shear with free polyphenol extracts; LMPP= ISD prepared with low shear with microencapsulated polyphenols; LGSE = ISD prepared with low shear with grape seed extract; HC = ISD prepared with high shear without antioxidant; HPPP = ISD prepared with high shear with free polyphenol extracts; HMPP= ISD prepared with high shear with microencapsulated polyphenols; HGSE = ISD prepared with high shear with grape seed extract.

The color (L*, a*, and b* values) of ISDs dressings during ACSL were listed in Table 3.3. On day 0, all of the salad dressings had a lemon-yellow color (hue angles between 58 and 71) with color saturation ranging from 23.29 to 32.61. An analysis of variance revealed that the antioxidants and the shear rate of homogenization had a significant effect ($P<0.05$) on the color parameters of salad dressings. After 21 days of accelerated storage, the lightness (L*) and yellowness (b*) of all ISDs were significantly ($P<0.05$) reduced and resulted in darker ISDs. It has been suggested that these color changes in food emulsions /suspensions with high oil concentrations may be due to flocculation which is accelerated by the lower viscosities of the continuous phase at higher storage temperatures (Lozano-Gendreau et al., 2019). Interestingly, the changes in a* values (redness) of ISDs were less noticeable than the changes in L* and b* values. Furthermore, not all ISDs had a significant reduction in redness (a*) and the minor decrease in a* values could be explained by the degradation of functional ingredients due to oxidative reactions observed at high storage temperatures (Phisut *et al*., 2018). Moreover, the total color difference ($\Delta$E) of ISDs was presented in Figure 3.2. It was observed that the $\Delta$E of all salad dressings was greater than 10. LMPP had the most dramatic $\Delta$E values while HGSE had the lowest value of $\Delta$E. In general, salad dressings homogenized at high shear rates had lower $\Delta$E than those prepared at low shear rates. The significant color differences through storage could be attributed to a) the flocculation of the oil droplets and suspended solids (Lozano-Gendreau et al., 2019); and b) the presence of weak acids (vinegar) could lead to the extraction of more and different pigments from the ingredients at elevated temperatures which may have increased the diffusion rate and solubility of pigments in salad dressings (Mohamed, Gibriel, Rasmy, & Abu-Salem, 2016; Oancea, Stoia, & Coman, 2012).

Table 3.3 Color values (L* a* b*) of Italian salad dressings (ISDs) during accelerated storage (ACSL)[†]

| Color parameter | ISD | Day 0 | Day 3 | Day 6 | Day 9 | Day 12 | Day 15 | Day 18 | Day 21 |
|---|---|---|---|---|---|---|---|---|---|
| L* | LC | 21.55±0.56$^{a}$ | 20.67±0.3$^{a}$ | 17.9±0.25$^{b}$ | 16.58±0.08$^{c}$ | 14.12±0.56$^{d}$ | 13.07±0.15$^{e}$ | 11.84±0.32$^{f}$ | 8.21±0.30$^{g}$ |
| | LPPP | 21.59±0.41$^{a}$ | 19.97±0.49$^{b}$ | 18.46±0.52$^{c}$ | 18.60±0.58$^{c}$ | 16.94±0.58$^{d}$ | 13.97±0.31$^{e}$ | 8.76±0.16$^{f}$ | 5.33±0.25$^{g}$ |
| | LMPP | 26.07±0.10$^{a}$ | 25.86±0.39$^{a}$ | 22.28±0.38$^{c}$ | 23.66±0.54$^{b}$ | 17.41±0.39$^{d}$ | 16.05±0.02$^{e}$ | 13.75±0.37$^{f}$ | 9.66±0.54$^{g}$ |
| | LGSE | 29.94±0.39 | 24.79±0.19$^{b}$ | 19.72±0.44$^{c}$ | 16.91±0.48$^{d}$ | 16.05±0.33$^{d}$ | 12.30±0.09$^{e}$ | 9.97±0.39$^{f}$ | 7.25±0.08$^{g}$ |
| | HC | 32.02±0.14$^{a}$ | 24.50±0.57$^{b}$ | 21.45±0.32$^{c}$ | 21.23±0.26$^{c}$ | 17.43±0.08$^{d}$ | 12.54±0.53$^{f}$ | 10.61±0.24$^{g}$ | 13.65±0.36$^{e}$ |
| | HPPP | 30.33±0.24 | 23.50±0.19$^{b}$ | 24.49±0.48$^{b}$ | 15.07±0.55$^{d}$ | 20.00±0.41$^{c}$ | 13.77±0.15$^{e}$ | 11.70±0.36$^{f}$ | 11.67±0.35$^{f}$ |
| | HMPP | 35.85±0.36$^{a}$ | 24.14±0.44$^{c}$ | 26.35±0.23$^{b}$ | 23.90±0.28$^{c}$ | 16.93±0.24$^{d}$ | 11.47±0.20$^{e}$ | 11.44±0.55$^{e}$ | 11.07±0.27$^{e}$ |
| | HGSE | 27.54±0.43$^{a}$ | 26.12±0.37$^{b}$ | 22.61±0.53$^{c}$ | 21.12±0.34$^{d}$ | 19.07±0.19$^{e}$ | 14.65±0.25$^{f}$ | 15.04±0.12$^{f}$ | 11.93±0.15$^{g}$ |
| a* | LC | 15.25±0.53$^{a}$ | 11.06±0.35$^{c}$ | 9.10±0.23$^{d}$ | 12.2±0.30$^{b}$ | 9.33±0.12$^{d}$ | 14.75±0.38$^{a}$ | 15.32±0.40$^{a}$ | 12.51±0.27$^{b}$ |
| | LPPP | 15.26±0.19$^{a}$ | 13.61±0.43$^{c}$ | 14.76±0.33$^{ab}$ | 14.28±0.46$^{b}$ | 15.72±0.15$^{a}$ | 8.93±0.16$^{f}$ | 12.92±0.42$^{d}$ | 10.59±0.30$^{e}$ |
| | LMPP | 15.26±0.19$^{a}$ | 15.61±0.22$^{a}$ | 15.49±0.25$^{a}$ | 15.56±0.28$^{a}$ | 12.92±0.08$^{b}$ | 9.38±0.28$^{c}$ | 8.29±0.22$^{d}$ | 7.56±0.57$^{de}$ |
| | LGSE | 16.16±0.37$^{a}$ | 15.92±0.08$^{a}$ | 12.60±0.15$^{c}$ | 13.99±0.55$^{b}$ | 16.56±0.05$^{a}$ | 13.17±0.39$^{b}$ | 16.48±0.42$^{a}$ | 8.65±0.29$^{d}$ |
| | HC | 9.23±0.16$^{d}$ | 12.75±0.42$^{a}$ | 11.19±0.14$^{b}$ | 10.20±0.18$^{c}$ | 6.53±0.19$^{f}$ | 8.86±0.59$^{e}$ | 9.31±0.43$^{d}$ | 9.63±0.25$^{d}$ |
| | HPPP | 11.44±0.12$^{b}$ | 11.01±0.57$^{b}$ | 7.72±0.34$^{e}$ | 11.68±0.12$^{b}$ | 8.46±0.31$^{d}$ | 10.64±0.32$^{c}$ | 12.31±0.10$^{a}$ | 7.97±0.17$^{e}$ |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | HMPP | 9.19±0.19$^{b}$ | 8.43±0.29$^{bc}$ | 8.99±0.06$^{b}$ | 7.54±0.53$^{c}$ | 5.44±0.34$^{d}$ | 11.94±0.24$^{a}$ | 7.06±0.26$^{c}$ | 8.88±0.49$^{b}$ |
| | HGSE | 10.38±0.40$^{b}$ | 7.11±0.53$^{d}$ | 8.62±0.24$^{c}$ | 11.50±0.22$^{a}$ | 9.82±0.20$^{b}$ | 7.55±0.31$^{d}$ | 10.63±0.18$^{ab}$ | 10.35±0.26$^{b}$ |
| b* | LC | 28.6±0.41$^{a}$ | 22.72±0.49$^{b}$ | 15.07±0.19$^{f}$ | 20.78±0.39$^{c}$ | 16.89±0.14$^{e}$ | 22.68±0.59$^{b}$ | 18.97±0.64$^{d}$ | 12.27±0.54$^{g}$ |
| | LPPP | 32.69±0.46$^{a}$ | 19.90±0.56$^{c}$ | 21.28±0.44$^{b}$ | 19.93±0.21$^{c}$ | 13.22±0.26$^{f}$ | 17.06±0.09$^{d}$ | 14.90±0.25$^{e}$ | 8.67±0.33$^{g}$ |
| | LMPP | 32.69±0.46$^{a}$ | 29.38±0.45$^{b}$ | 23.70±0.35$^{c}$ | 20.41±0.15$^{d}$ | 12.49±0.30$^{h}$ | 17.58±0.51$^{e}$ | 15.86±0.22$^{f}$ | 13.32±0.47$^{g}$ |
| | LGSE | 15.50±0.53$^{b}$ | 15.20±0.07$^{b}$ | 14.67±0.32$^{bc}$ | 17.02±0.40$^{a}$ | 9.86±0.48$^{g}$ | 13.30±0.13$^{d}$ | 12.44±0.58$^{e}$ | 10.53±0.47$^{f}$ |
| | HC | 27.18±0.03$^{b}$ | 29.74±0.40$^{a}$ | 23.59±0.32$^{c}$ | 20.07±0.41$^{e}$ | 16.71±0.48$^{g}$ | 18.77±0.45$^{f}$ | 15.96±0.36$^{g}$ | 21.36±0.51$^{d}$ |
| | HPPP | 27.10±0.20$^{a}$ | 20.85±0.46$^{c}$ | 22.75±0.38$^{b}$ | 22.02±0.45$^{b}$ | 16.69±0.42$^{d}$ | 22.06±0.18$^{b}$ | 19.31±0.36$^{c}$ | 17.54±0.35$^{d}$ |
| | HMPP | 23.64±0.40$^{a}$ | 20.87±0.16$^{b}$ | 17.16±0.09$^{d}$ | 15.79±0.45$^{e}$ | 13.68±0.29$^{f}$ | 18.82±0.29$^{c}$ | 23.37±0.49$^{a}$ | 17.48±0.51$^{d}$ |
| | HGSE | 20.05±0.42$^{b}$ | 13.61±0.28$^{f}$ | 18.79±0.11$^{c}$ | 25.94±0.57$^{a}$ | 20.46±0.27$^{b}$ | 15.12±0.49$^{e}$ | 16.15±0.32$^{d}$ | 18.75±0.26$^{c}$ |

$^{\dagger}$Values are the mean ± standard deviation of triplicate determinations. $^{a\text{-}f}$Means with the same letter in the same row are not significantly different ($P$<0.05). See Table 3.1 for the description of LC, LPPP, LMPP, LGSE, HC, HPPP, HMPP, and HGSE

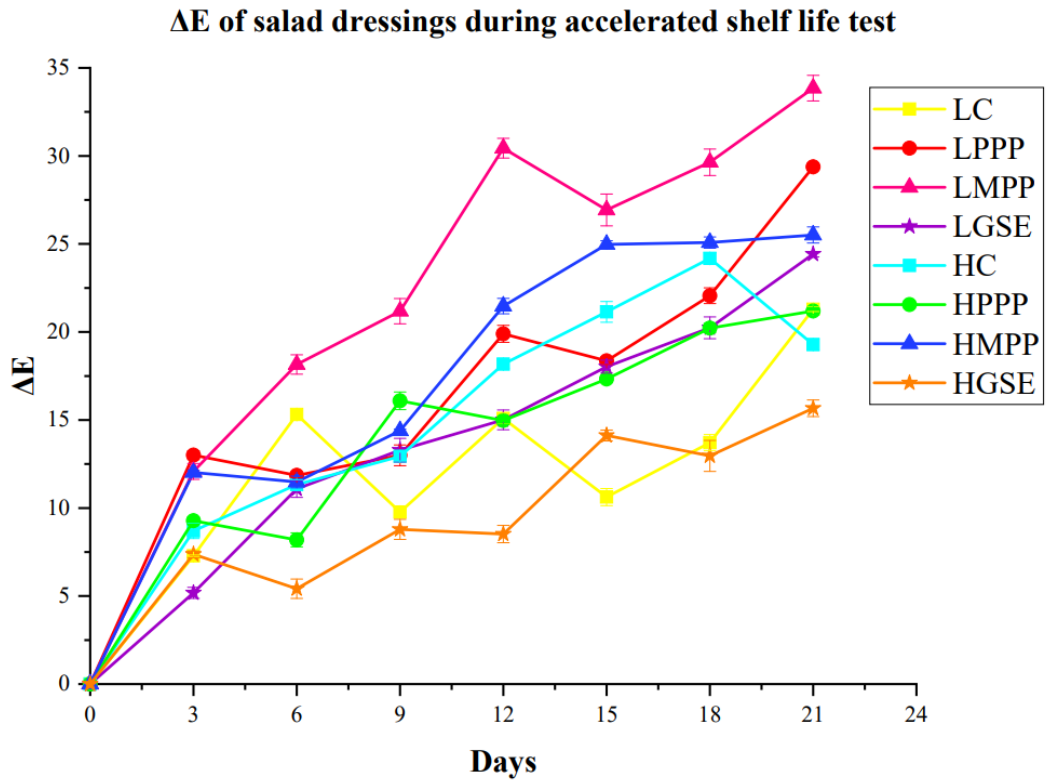


Figure 3.2 Color changes of Italian−style homemade salad dressings (ISDs) during accelerated storage (ACSL). See Figure 3.1 for the description of LC; LPPP; LMPP; LGSE; HC; HPPP; HMPP; and HGSE.

**Lipid oxidation**

Lipid oxidation is one of the major concerns in food quality deterioration. The oxidative process of lipids may be catalyzed by light, heat, enzymes, metals, and microorganisms (Tseng *et al*., 2013). PV, IV, and TBARS values are three common indicators of lipid oxidation. Furthermore, PV indicates the quantity of peroxides and hydroperoxides formed in the initiation stage of lipid oxidation. As shown in Figure 3.3, the PV of all ISDs significantly ($P$<0.05) increased during storage, especially for those without antioxidants (LC and HC). Peroxides were detected after 6 days in LC and HC, and after 9 days in antioxidant-containing ISDs. Moreover, LC and HC had significantly ($P$<0.05) higher PVs (approximately 50 %) than the rest of ISDs after 21 days of storage. Curiously, it was observed that shear rates did not affect the PV of ISDs (Figure 3.3). All antioxidant-containing ISDs showed similar PVs at the end of 21 days of storage. The resultant data may also indicate that high storage temperature could accelerate the oxidation of oils. Interestingly, it seemed that antioxidants were able to delay lipid oxidation in ISDs to some extent. It has been reported that the PV of commercial salad dressings should not exceed 10 mmol/kg oil (Lozano-Gendreau *et al*., 2019). In our study, all ISDs were under the maximum limit for PV; however, they all showed signs of lipid oxidation as we observed in PV and TBARS. Vegetable oils with a high content of polyunsaturated fatty acid (PUFA) are more vulnerable to lipid oxidation, while the presence of saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) could improve their oxidative stability (Cao *et al*., 2015). The main fatty acids in peanut oil are oleic acid (45–53%, MUFA), linoleic acid (27–32%, PUFA), and palmitic acid (11–14%, SFA) (Ghazani & Marangoni, 2016). PV can only measure initial products of lipid oxidation; meanwhile, hydroperoxides are unstable molecules that can decompose quickly into secondary oxidation products such as aldehydes during storage at

elevated temperatures (Eidhin, & O'Beirne, 2010). This may have occurred in LH and HC after day 18 (the PVs of LC and HC at day 18 were higher than those of corresponding samples at day 21). Given the possibility of decomposition of hydroperoxides, PV only was not enough to assess the quality of edible oils.

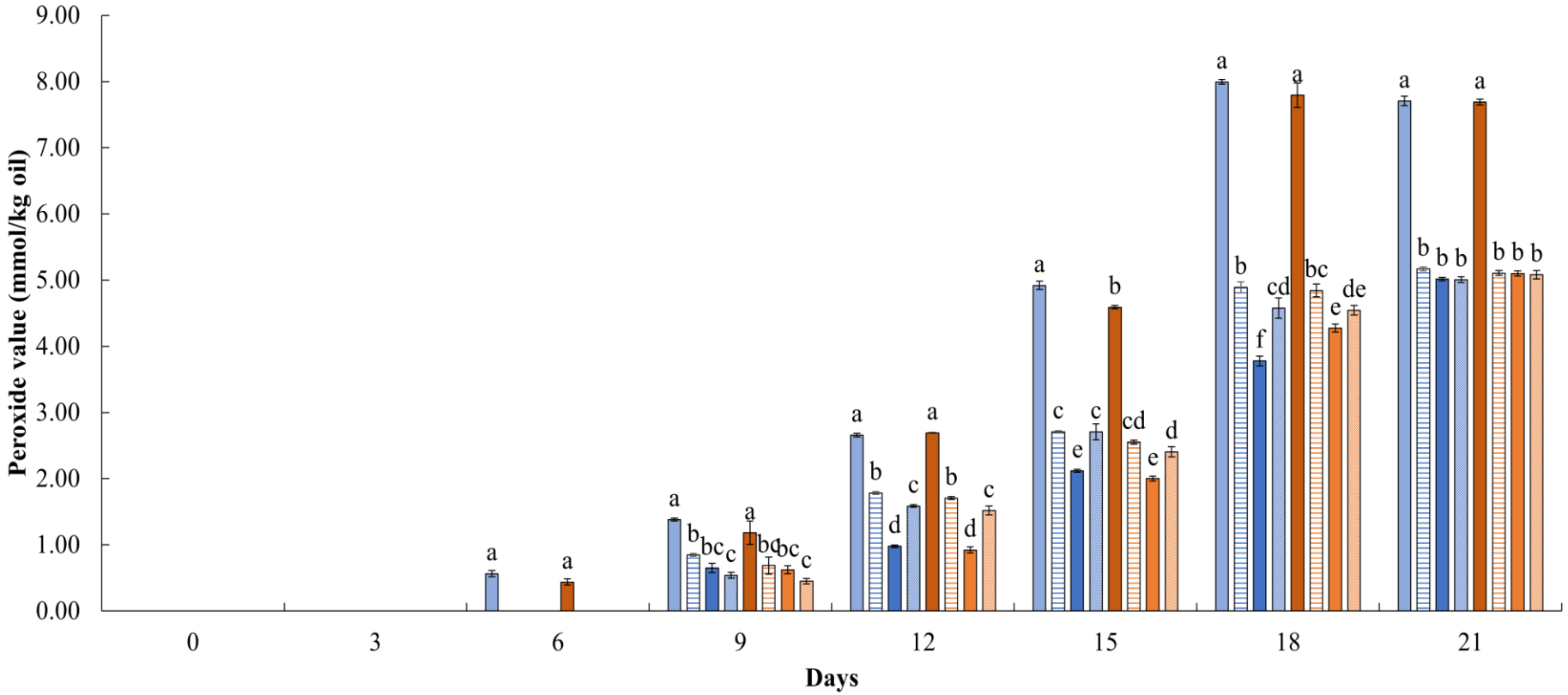


Figure 3.3 Peroxide value (PV) of Italian−style homemade salad dressings (ISDs) during accelerated storage (ACSL). ( = LC; = LPPP; = LMPP; = LGSE; =HC; = HPPP; = HMPP; = HGSE). [abcd]Means with the same letter in the same day are not significantly different ($P$<0.05). See Figure 3.1 for description of LC; LPPP; LMPP; LGSE; HC; HPPP; HMPP; and HGSE.

Iodine value measures the degree of unsaturation of fatty acids. A decrease in IV indicates an increase in the degree of saturation of fatty acids (Ayodeji, & Ganiyu, 2015). Changes in IV of ISDs are presented in Figure 3.4. During the first 12 days of storage, the IV of all ISDs was within the standard range (84−107) which was close to the IV of fresh peanut oil (Karl, 2017). Not surprisingly, the IV of LC and HC reduced at higher rates compared to those of antioxidant-containing ISDs. After 21 days of storage, the IV of LC and HC were significantly ($P$<0.05) lower than the rest of the ISDs. Interestingly, LMPP and HMPP had significantly ($P$<0.05) higher IV (~5%) than those of LC and HC after 21 days of storage. Even more, shear rates did not have any effect on the IV of the ISDs during ACSL. These results suggested that ISDs experienced a reduction in unsaturation of their fatty acids due to the breakdown of carbon chain bindings, thus forming saturated carbon chains (Mohamad et al., 2019). Antioxidants containing ISDs, especially those containing microencapsulated antioxidants, have shown lower lipid oxidation than ISDs without antioxidants (especially in the first 15 days of storage). Similar trends for IV have been reported by Guo et al. (2016) in palm oil with rosemary ethanol extract during frying and accelerated storage, and Jahurul et al. (2017) in mango seed fat and palm oil mid-fraction blends as cocoa butter replacers under accelerated storage conditions.

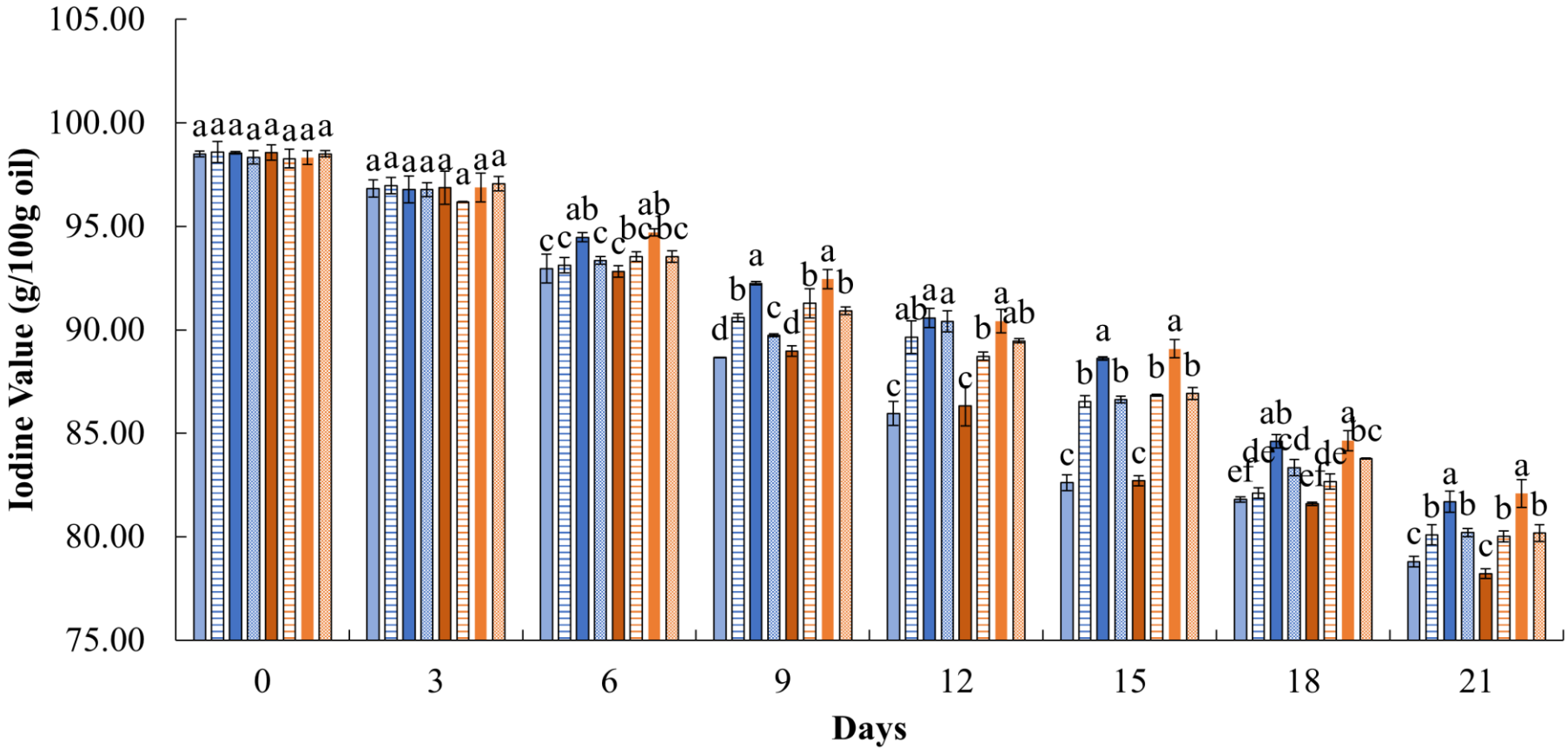


Figure 3.4 Iodine value (IV) of Italian−style homemade salad dressings (ISDs) during accelerated storage (ACSL). ( = LC; = LPPP; = LMPP; = LGSE; =HC; = HPPP; = HMPP; = HGSE). [abcd]Means with the same letter in the same day are not significantly different ($P$<0.05). See Figure 3.1 for description of LC; LPPP; LMPP; LGSE; HC; HPPP; HMPP; and HGSE.

The TBARS is a parameter used to monitor the production of secondary products of lipid oxidation, mainly malondialdehyde (MDA). It was noted that the TBARS of all ISDs significantly ($P$<0.05) increased after 21 days of storage (Figure 3.5). Surprisingly, LMPP and HMPP showed significantly ($P$<0.05) lower TBARS than the rest of the treatments after 21 of storage. Furthermore, there was no apparent effect of the shear rate on the TBARS of ISDs. Food products with TBARS values lower than 0.576 mg MDA/kg dry weight (DW) of the sample are considered fresh, those with TBARS values between 0.65−1.44 mg MDA/kg DW are considered rancid but still acceptable, and those with TBARS values higher than 1.5 mg MDA/kg DW are considered unacceptable for consumption (Cong *et al*., 2020). Using those classification criteria, all treatments were considered fresh after 9 days of storage, and after 18 days of storage, all ISDs, but LMPP and HMPP could have been classified as unacceptable for consumption. In addition, the results confirmed that antioxidants effectively delayed the formation of MDA in salad dressings (Phisut *et al*., 2018). Also, these findings suggest that MPP was an effective antioxidant for delaying lipid oxidation in a salad dressing system after 21 days of accelerated storage and that the breakdown of peroxides to carbonyl and aldehyde compounds such as MDA was accelerated by high storage temperatures (Ayodeji et al., 2015).

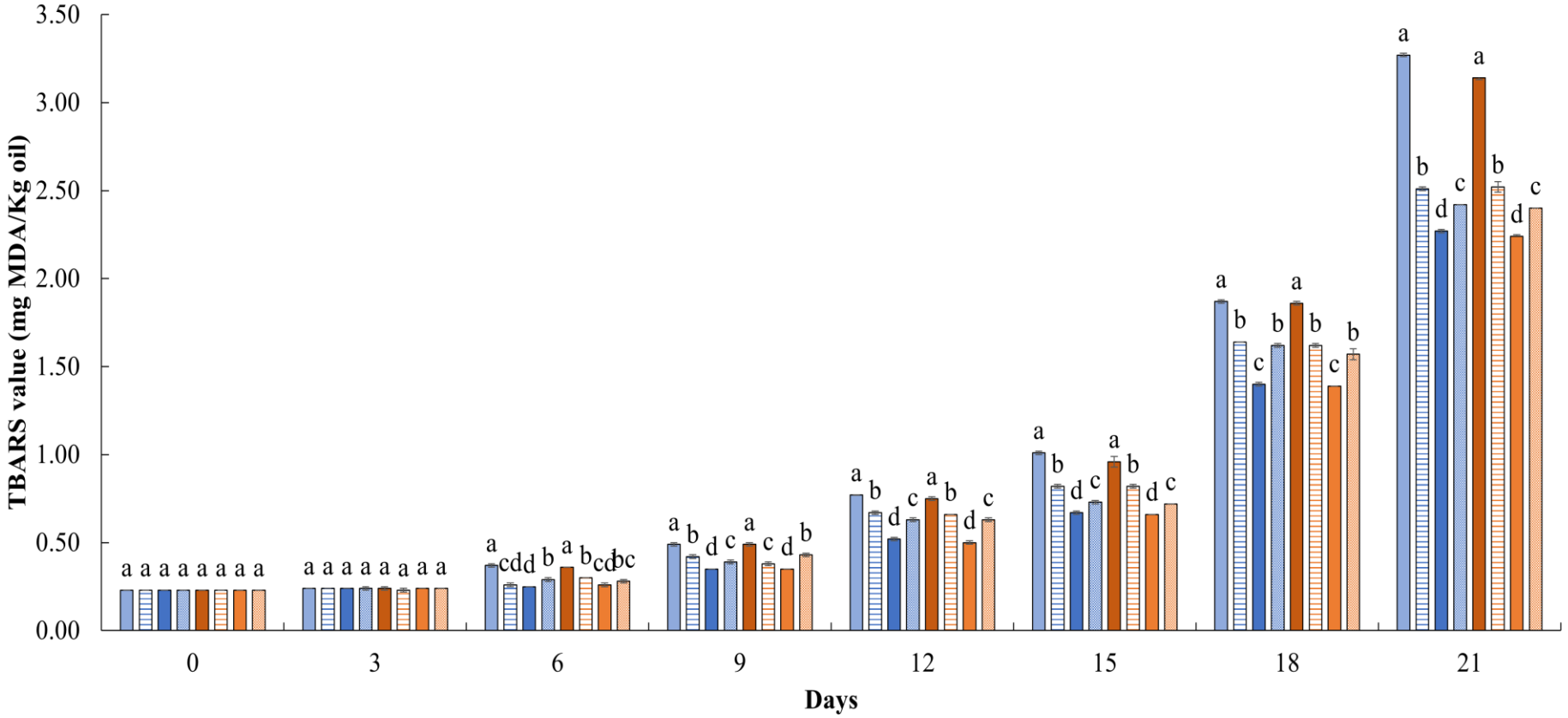


Figure 3.5 TBARS values of Italian−style homemade salad dressings (ISDs) during accelerated storage (ACSL). ( = LC; = LPPP; = LMPP; = LGSE; =HC; = HPPP; = HMPP; = HGSE). [abcd]Means with the same letter in the same day are not significantly different ($P$<0.05). See Figure 3.1 for description of LC; LPPP; LMPP; LGSE; HC; HPPP; HMPP; and HGSE.

When predicting the shelf life of foods, accelerated storage is always a cost-effective approach (Feng, 2011). The estimated shelf life can be calculated based on the eq. (5) (Joseph, 2016):

Estimated shelf life = $Q_{10}{}^{(T1-T2/10)}$ × days of ACSL (5)

Where, $Q_{10}$ is a typical value (2.0) to estimate reaction rates in food, $T_1$ is the temperature (55°C) of accelerated conditions, and $T_2$ is the room temperature (25°C).

In this study, 21 days of ACSL was equivalent to 168 days (5.6 months) of ambient storage. Using PV as an indicator for the determination of the shelf life of ISDs, all treatments were within the normal range after 21 days of ACSL. Furthermore, if the results for IV were to be used to calculate shelf life, the IVs of LC and HC were below the normal range after 15 days of accelerated storage (equivalent to 120 days of ambient storage), while MPP-containing salad dressings were still acceptable after 18 days of ACSL. Using TBARS, LC, LPPP, LGSE, HC, HPPP, and HGSE were considered rancid and unacceptable after 15 days of accelerated storage (equivalent to 120 days of ambient storage), while LMPP and HMPP were still considered acceptable after 18 days (equivalent to 144 days of ambient storage). These results suggest that MPP may extend the shelf life of ISDs by 24 days by delaying lipid oxidation. However, the results obtained under accelerated conditions must be interpreted with care when predicting the shelf life. Because the mechanisms of oxidation could change with temperature and samples could exhibit excessive rancidity, which is not associated with normal storage conditions. Depending on the type of oil, these predictions may lead to an overestimation or underestimation of the actual shelf life (Farhoosh, 2007). Therefore, it is recommended to confirm accelerated storage with ambient storage conditions. Nevertheless, when time is constrained, accelerated

storage studies provide an interesting approach to evaluate the preliminary effectiveness of natural antioxidants.

**Effect of ambient storage**

**Changes in pH and color**

The pH of the aqueous phase has been reported as a critical factor in controlling the microstructural stability of food emulsions and suspensions (Seo, Lee, & Kim, 2013). The pH changes of salad dressings during ambient storage conditions were presented in Figure 3.6. The initial pH values of ISDs were ~ 3.2 of and significantly ($P$<0.05) increased to 3.32−3.38 after storage (25℃, a relative humidity of 40 to 60% in the dark) for 8 weeks. The increased pH might be explained by the slight decomposition of acetic acid and other ingredients in the salad dressings during storage in a warm and humid environment (Ahmad, 2020). Generally, the pH of salad dressings is less than 4.6 (pH of acidified foods) which limits microbial growth during storage at ambient temperature (Breidt, Kay, Osborne, Ingham, & Arritt, 2014).

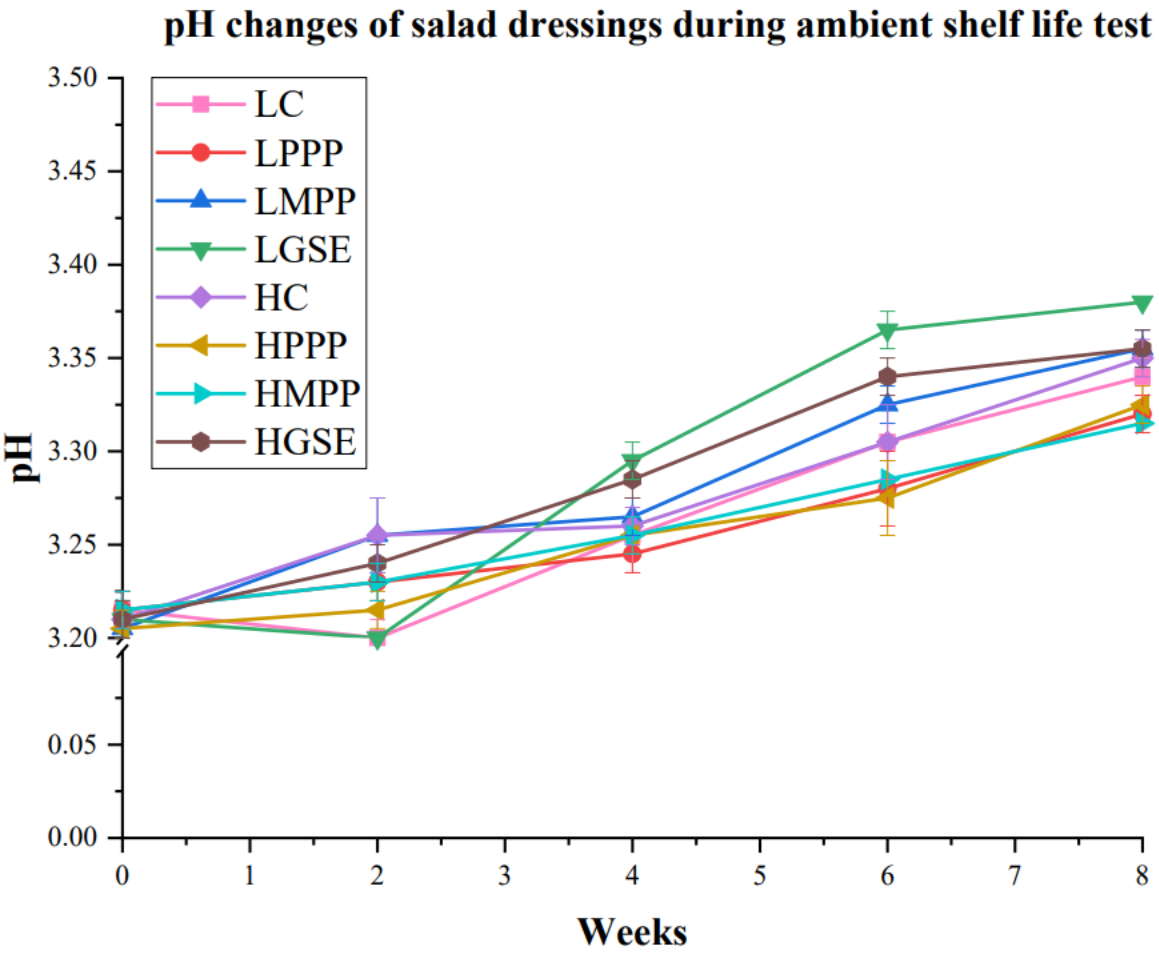


Figure 3.6 pH changes of Italian−style homemade salad dressings (ISDs) during ambient storage (AMSL). See Figure 3.1 for the description of LC; LPPP; LMPP; LGSE; HC; HPPP; HMPP; and HGSE.

The color (L*, a*, and b* values) of salad dressings during ambient storage are presented in Table 3.4. Fresh ISDs had a different lightness, redness, and yellowness because of different homogenization conditions and different types of ingredients used. After 8 weeks of storage under AMSL conditions, the degree of lightness of all salad dressings was significantly ($P$<0.05) lower than that of the corresponding fresh samples while there were no obvious patterns of change in the values of a* and b* during storage. These results confirmed the results observed in ACSL, where darker ISDs were observed after 21 days of storage.

Total color differences ($\Delta$E) of ISDs during AMSL were shown in Figure 3.7. In general, ISDs prepared at high shear rates showed lower changes in color compared to the ISDs prepared at low shear rates. Furthermore, compared with $\Delta$E of salad dressing during accelerated storage, the $\Delta$E of all treatments was smaller under ambient storage. Moreover, the $\Delta$E of HC and HMPP were relatively low and stable (6−8) during the 8-week storage period, which indicated that their color did not change as much as in the case of the other ISDs. It has been hypothesized that the different ΔE values could be explained by the extrinsic color changes of ingredients as well as the homogenization conditions such as shear rates and homogenization time (Eissa *et al*., 2016). Overall, these color parameters should be taken into account when formulating various salad dressings because consumers have a preconceived prospect of the appearance of the different products (Chung, Sher, Rousset, Decker, & McClements, 2017). The appearance of all salad dressings after ambient storage was shown in Figure 3.8.

Table 3.4 Color values (L*a*b*) of Italian salad dressings (ISDs) stored under ambient storage (AMSL) conditions[†]

| Color value | ISD | Week 0 | Week 2 | Week 4 | Week 6 | Week 8 |
|---|---|---|---|---|---|---|
| L* | LC | 18.65±0.13$^{a}$ | 10.72±0.24$^{d}$ | 11.43±0.20$^{b}$ | 14.38±0.31$^{b}$ | 9.50±0.07$^{e}$ |
| | LPPP | 18.95±0.08$^{a}$ | 13.56±0.23$^{e}$ | 14.82±0.37$^{d}$ | 16.05±0.11$^{c}$ | 17.50±0.13$^{b}$ |
| | LMPP | 25.03±0.12$^{a}$ | 17.58±0.14$^{b}$ | 14.75±0.22$^{e}$ | 19.65±0.15$^{b}$ | 15.84±0.31$^{d}$ |
| | LGSE | 20.95±0.43$^{a}$ | 17.00±0.14$^{b}$ | 15.10±0.23$^{c}$ | 17.01±0.06$^{b}$ | 16.62±0.30$^{b}$ |
| | HC | 18.95±0.13$^{a}$ | 14.60±0.36$^{c}$ | 17.53±0.16$^{b}$ | 17.32±0.19$^{b}$ | 15.01±0.13$^{c}$ |
| | HPPP | 19.83±0.10$^{a}$ | 17.49±0.18$^{c}$ | 16.82±0.33$^{d}$ | 18.55±0.03$^{b}$ | 18.32±0.09$^{b}$ |
| | HMPP | 23.23±0.11$^{a}$ | 19.46±0.36$^{b}$ | 16.82±0.42$^{d}$ | 19.51±0.19$^{b}$ | 18.58±0.22$^{c}$ |
| | HGSE | 25.91±0.15$^{a}$ | 15.93±0.03$^{c}$ | 14.62±0.04$^{d}$ | 17.34±0.06$^{b}$ | 16.19±0.07$^{c}$ |
| a* | LC | 12.63±0.34$^{c}$ | 15.93±0.21$^{a}$ | 14.25±0.20$^{b}$ | 9.42±0.22$^{d}$ | 14.37±0.36$^{b}$ |
| | LPPP | 12.07±0.26$^{a}$ | 12.69±0.23$^{a}$ | 9.58±0.28$^{b}$ | 9.28±0.11$^{b}$ | 9.40±0.17$^{b}$ |
| | LMPP | 9.06±0.29$^{b}$ | 9.24±0.31$^{b}$ | 8.16±0.13$^{c}$ | 6.61±0.24$^{d}$ | 9.97±0.31$^{a}$ |
| | LGSE | 13.77±0.27$^{a}$ | 9.51±0.27$^{c}$ | 9.33±0.10$^{c}$ | 10.94±0.30$^{b}$ | 13.22±0.51$^{a}$ |
| | HC | 9.40±0.14$^{ab}$ | 8.89±0.57$^{b}$ | 7.62±0.04$^{c}$ | 7.08±0.12$^{c}$ | 9.66±0.10$^{a}$ |
| | HPPP | 9.88±0.11$^{a}$ | 8.30±0.01$^{b}$ | 9.43±0.32$^{a}$ | 7.94±0.16$^{b}$ | 8.05±0.10$^{b}$ |
| | HMPP | 12.39±0.12$^{a}$ | 11.03±0.14$^{b}$ | 11.53±0.28$^{b}$ | 10.00±0.37$^{c}$ | 12.51±0.21$^{a}$ |
| | HGSE | 12.64±0.21$^{b}$ | 13.94±0.25$^{a}$ | 12.11±0.27$^{b}$ | 12.86±0.24$^{b}$ | 13.99±0.11$^{a}$ |
| b* | LC | 30.07±0.71$^{a}$ | 18.23±0.40$^{b}$ | 18.21±0.19$^{b}$ | 18.98±0.17$^{b}$ | 14.64±0.33$^{c}$ |
| | LPPP | 27.95±0.19$^{a}$ | 21.83±0.33$^{b}$ | 18.51±0.20$^{c}$ | 18.86±0.24$^{c}$ | 14.29±0.53$^{d}$ |
| | LMPP | 22.02±0.92$^{a}$ | 18.10±0.64$^{b}$ | 16.27±0.17$^{c}$ | 13.07±0.45$^{d}$ | 18.91±0.49$^{b}$ |
| | LGSE | 25.37±0.60$^{a}$ | 15.55±0.52$^{d}$ | 14.43±0.25$^{e}$ | 19.40±0.58$^{b}$ | 18.04±0.35$^{c}$ |

| | | | | | |
|---|---|---|---|---|---|
| HC | 26.44±0.28[a] | 22.07±0.10[b] | 19.75±0.18[d] | 20.01±0.16[c] | 19.82±0.53[cd] |
| HPPP | 28.14±0.82[a] | 20.66±0.49[c] | 24.49±0.76[b] | 18.89±0.21[d] | 15.65±0.64[e] |
| **HMPP** | 27.94±0.48[a] | 22.79±0.40[bc] | 23.44±0.44[b] | 21.29±0.38[c] | 23.15±0.59[b] |
| **HGSE** | 20.41±0.47[c] | 23.71±0.26[a] | 20.43±0.47[c] | 23.95±0.28[a] | 21.44±0.46[b] |

[†]Values are the mean ± standard deviation of triplicate determinations.

[a-f]Means with the same letter in the same row are not significantly different ($P<0.05$).

See Table 3.1 for the description of LC, LPPP, LMPP, LGSE, HC, HPPP, HMPP, and HGSE.

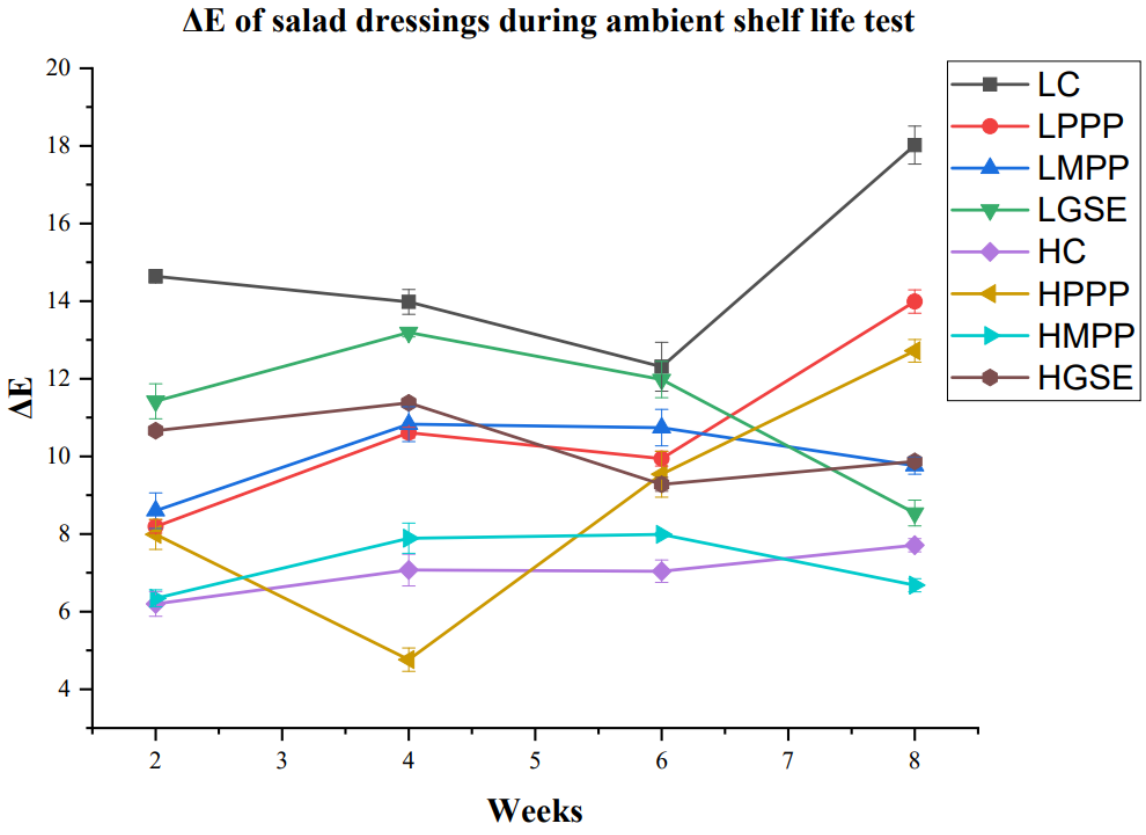


Figure 3.7 Color changes (ΔE) of Italian−style homemade salad dressings (ISDs) during ambient storage (AMSL). See Figure 3.1 for the description of LC; LPPP; LMPP; LGSE; HC; HPPP; HMPP; and HGSE.

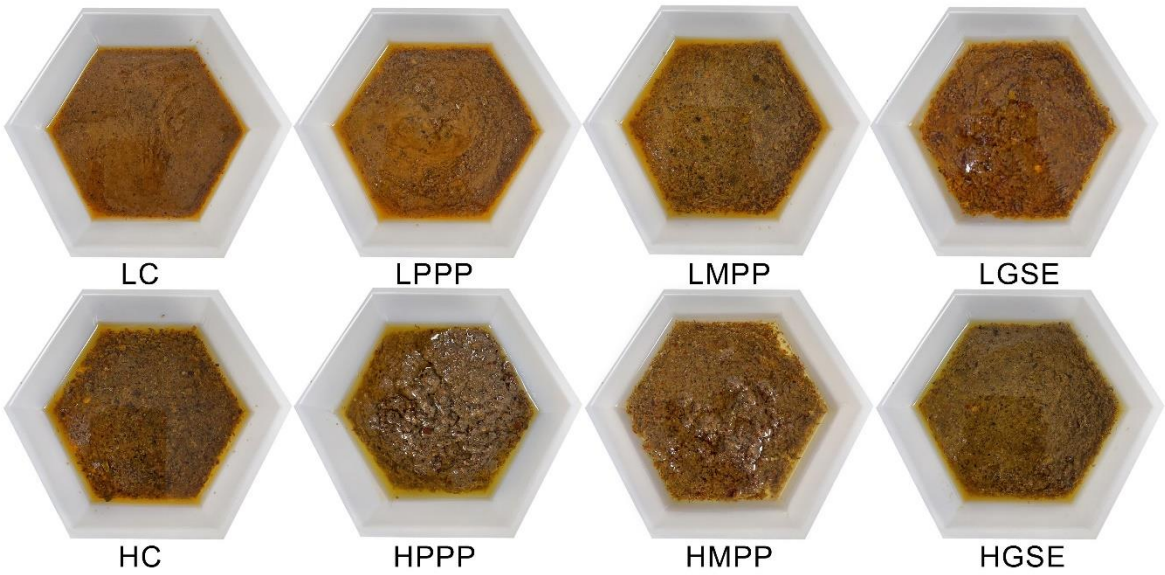


Figure 3.8 Pictures of Italian−style homemade salad dressings (ISDs) after storage at ambient conditions for 8 weeks. See Figure 3.1 for the description of LC; LPPP; LMPP; LGSE; HC; HPPP; HMPP; and HGSE.

**Lipid oxidation**

Auto-oxidation of oil is a major problem in salad dressings and the primary products from lipid oxidation can be measured as PV (Kishk, & Elsheshetawy, 2013). The PV of ISDs under AMSL was shown in Figure 3.9. It was observed that all ISDs started to show signs of oxidation within the first two weeks of storage. Also, all samples showed PV lower than 5.5 mmol/kg oil after 8 weeks of storage. Interestingly, LMPP and HMPP had significantly ($P$<0.05) lower PVs than the rest of the ISDs at the end of the storage time. The results also revealed that ISDs prepared with MPP had the lowest PV followed by those prepared with GSE and PPP, respectively. Shear rates did not affect the PV of ISDs under AMSL. Upon comparison of PV in ACSL (Figure 3.3) with PV in AMSL (Figure 3.9), interesting findings were made. PVs obtained in ACSL (3 weeks) were higher than those obtained under AMSL (8 weeks). This finding was consistent with the previous findings that PVs under ambient storage were lower than those obtained under ACSL (Branco, Rodrigues, Gioielli, & Castro, 2011). Similar results have been reported by Mohammadi, Jafari, Esfanjani, & Akhavan (2016), who demonstrated that microencapsulation of phenolic compounds in double emulsion systems can increase antioxidant capacity due to a controlled release. The relative effectiveness of an antioxidant was dependent on the lipid substrate, physical state (emulsion), oxidation time, and temperature (Lee *et al.*, 2014).

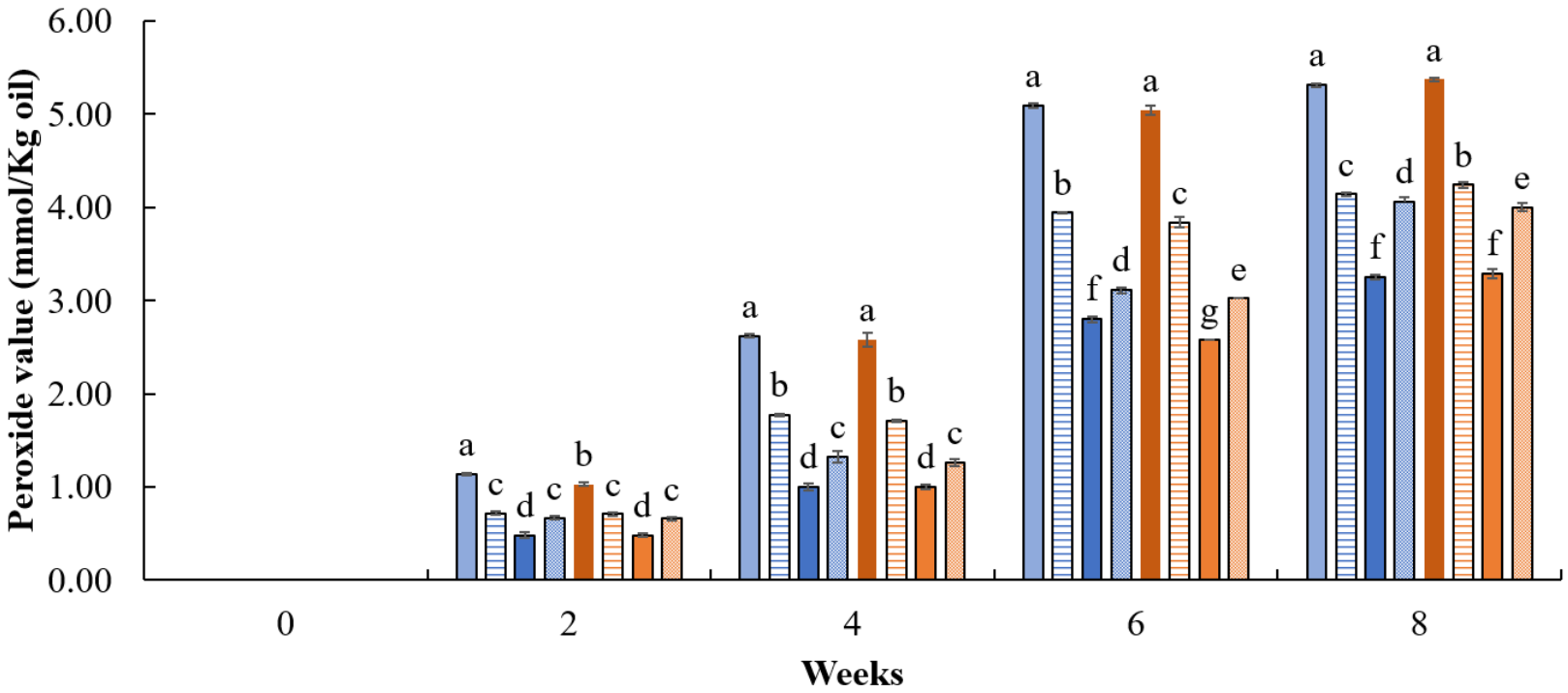


Figure 3.9 Peroxide value (PV) of Italian−style homemade salad dressings (ISDs) during ambient storage (AMSL). ( = LC; = LPPP; = LMPP; = LGSE; =HC; = HPPP; = HMPP; = HGSE). [abcd]Means with the same letter in the same day are not significantly different ($P$<0.05). See Figure 3.1 for description of LC; LPPP; LMPP; LGSE; HC; HPPP; HMPP; and HGSE.

The IVs of all salad dressings during ambient storage were presented in Figure 3.10. Initially, all ISDs had IVs higher than 96 g iodine/100 g oil. Then, the IV of all ISDs was significantly ($P$<0.05) reduced during ambient storage. At the end of the 8 weeks, LMPP and HMPP showed significantly ($P$<0.05) higher IVs compared to the rest of the treatments. Moreover, the IV of LMPP and HMPP was ~8.1% higher than those of LC and HC, respectively. Meanwhile, LC and HC showed the lowest IV which may have indicated the highest decrease in unsaturation (presumably due to oxidation). As in the case of PV, there was no apparent effect of shear rates on the IV of ISDs. The results suggested that antioxidants could help to inhibit /delay the destruction of fatty acid double bonds, thus delaying lipid oxidation. In addition, the controlled release of MPP helped to sustain their antioxidant activities for a longer time compared to unencapsulated /free antioxidants.

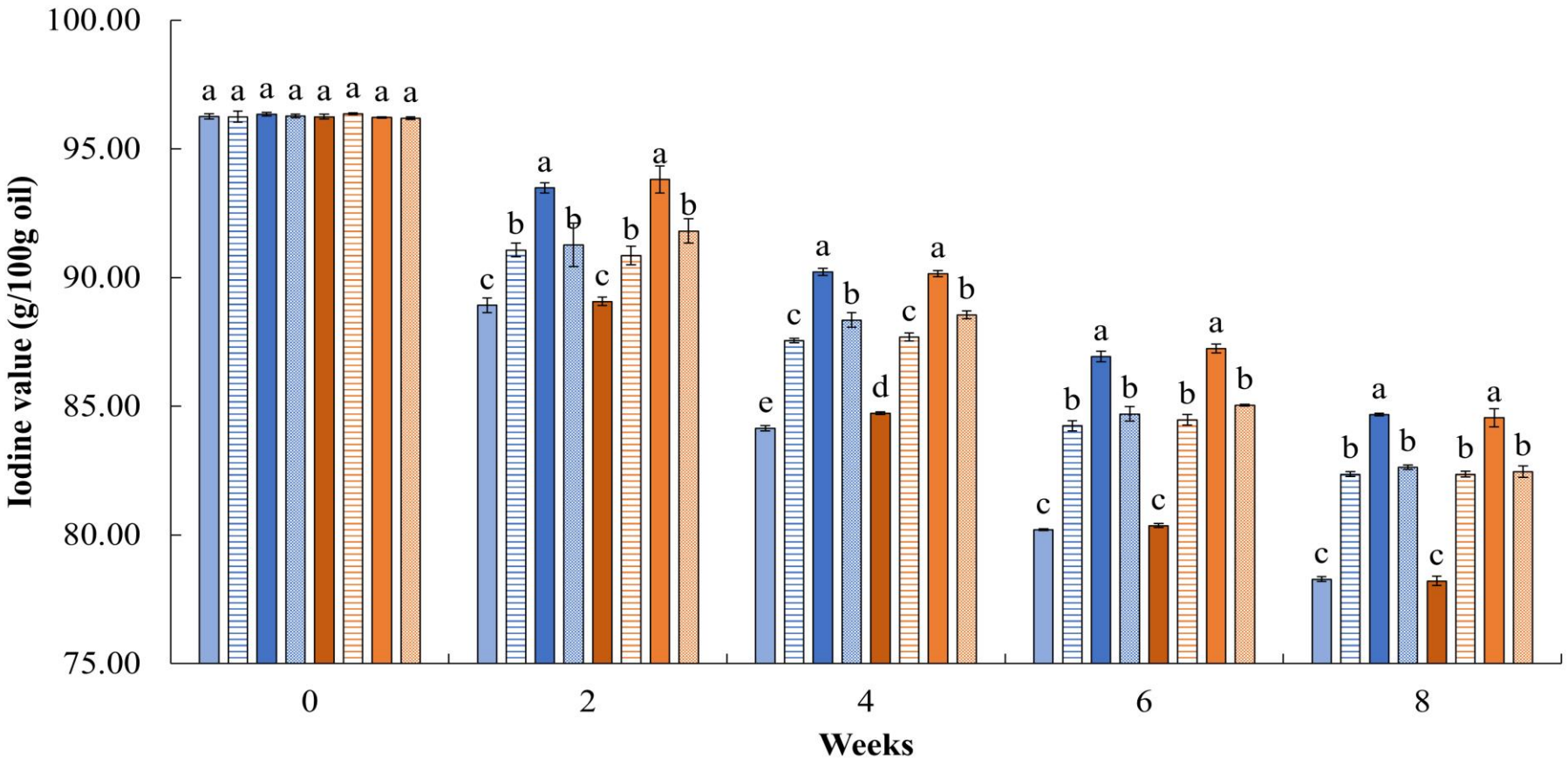


Figure 3.10 Iodine value (IV) of Italian−style homemade salad dressings (ISDs) during ambient storage (AMSL). ( = LC; = LPPP; = LMPP; = LGSE; =HC; = HPPP; = HMPP; = HGSE). [abcd]Means with the same letter in the same day are not significantly different ($P$<0.05). See Figure 3 for description of LC; LPPP; LMPP; LGSE; HC; HPPP; HMPP; and HGSE.

Similarly, TBARS values of ISDs under AMSL are shown in Figure 3.11. The initial TBARS value of all samples was 0.23mg MDA/kg oil. At the end of 8 weeks, LC and HC showed a significantly ($P$<0.05) higher TBARS (~3.02−3.04 mg MDA/kg oil) than the rest of ISDs. Moreover, LMPP and HMPP showed significantly ($P$<0.05) lower TBARS than the rest of the treatments. As in the previous cases of PV and IV, there was not a clear effect of shear rates on TBARS of ISDs. As we mentioned previously, LC, LPPP, LGSE, HC, HPPP, HGSE may have been classified as rancid but still acceptable; while LMPP and HMPP could have been classified as fresh (TBARS <0.6 mg MDA/Kg oil) after 4 weeks of storage. After 6 weeks of storage, all ISDs, but LMPP and HMPP could have been classified as unacceptable for consumption. It has been reported that MDA is one of the many reactive electrophile species that cause oxidative stress in cells and the formation of advanced glycation end-products which are associated with several degenerative diseases such as cancer, diabetes mellitus, and kidney dysfunction (Oboh, Falade, & Ademiluyi, 2014).

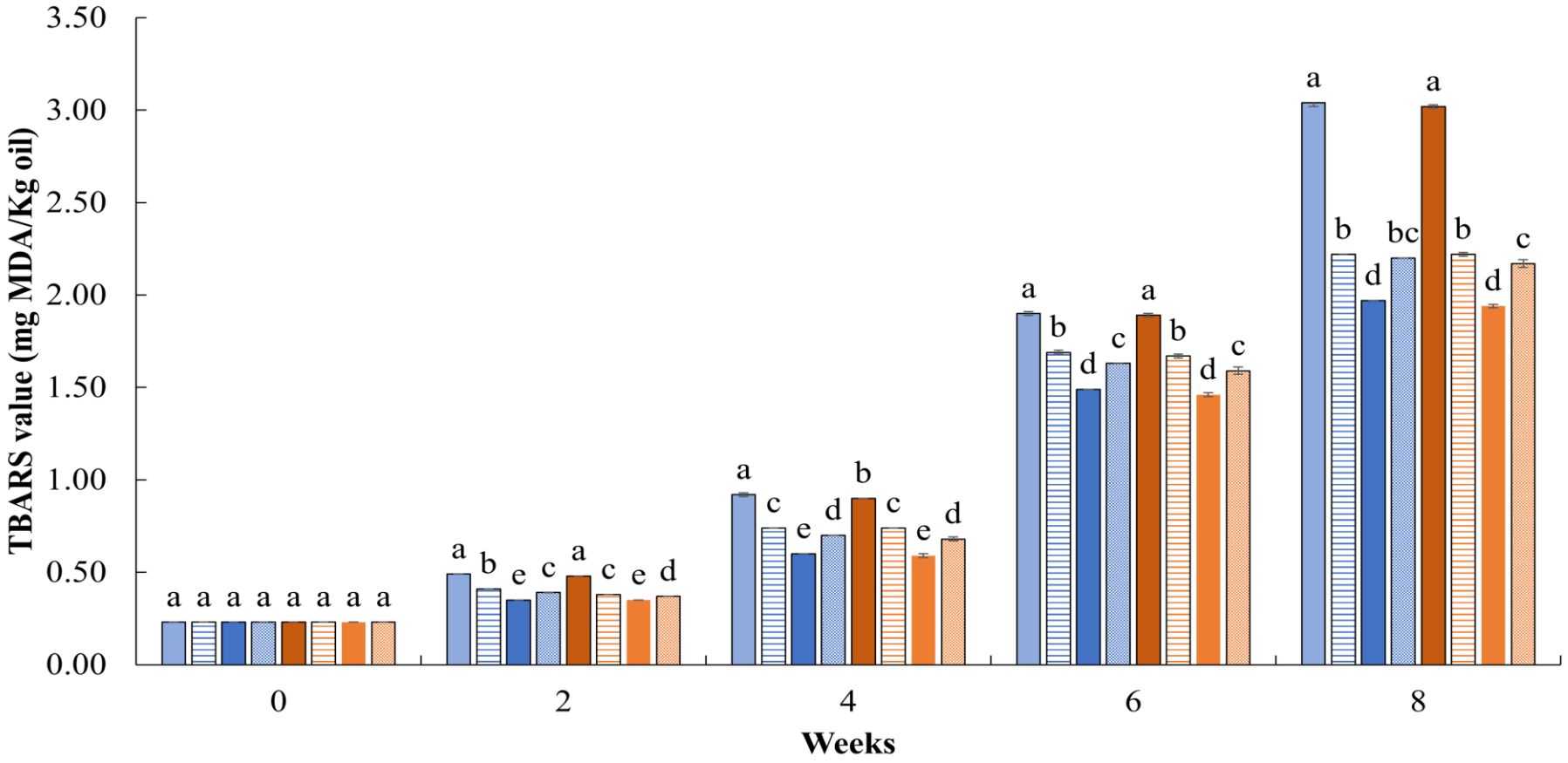


Figure 3.11 TBARS values of Italian−style homemade salad dressings (ISDs) during ambient storage (AMSL). ( = LC; = LPPP; = LMPP; = LGSE; =HC; = HPPP; = HMPP; = HGSE). [abcd]Means with the same letter in the same day are not significantly different ($P$<0.05). See Figure 3 for description of LC; LPPP; LMPP; LGSE; HC; HPPP; HMPP; and HGSE.

Normally, polyphenols can act as chain-breaking antioxidants, hydroperoxide destroyers, and metal chelators (Chong, Chang, Sia, & Yim, 2015). The phenolic hydroxyl groups could donate hydrogen atoms to scavenge free radicals such as hydroxyl, peroxyl, superoxide, and nitric oxide which were produced from the mixtures of secondary oxidation products and transition metals in the aqueous phase of salad dressings, resulting in retardation of the initiation or propagation stage of lipid oxidation. Therefore, these antioxidants can interfere with further lipid oxidation in salad dressings.

## Conclusion

The study demonstrated the effectiveness of using microencapsulated polyphenol from pomegranate peels (MPP) in the Italian salad dressings system to control lipid oxidation and quality degradation. All fresh salad dressings had a lemon-yellow color, and those prepared at high shear rates had significantly higher emulsion stability than those prepared at low shear rates. During 21 days of accelerated storage, pH values of salad dressings dropped from 3.13 to 3.05. However, the pH values of salad dressings slightly increased after 8 weeks of ambient storage. All salad dressings became darker after 21 days and 8 weeks of accelerated and ambient storage, respectively. Shear rates neither accelerated nor delayed lipid oxidation and quality deterioration in the salad dressings during storage. Accelerated storage suggested that MPP could have extended the shelf life of salad dressings by 24 days compared to free polyphenols. Moreover, MPP containing salad dressings stored at both accelerated and ambient conditions showed less indication of lipid oxidation compared to those salad dressings prepared with non−encapsulated antioxidants. Microencapsulation provides an interesting potential to improve the stability of natural antioxidants when they are added to high lipid content and acidified foods to control lipid oxidation.

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# CHAPTER 4

# CONCLUSIONS

Polyphenols extracted from pomegranate fruits have strong antioxidant activity. Natural antioxidants are more appealing to health-conscious consumers than synthetic antioxidants. Natural polyphenols from pomegranate fruits can be used to control lipid oxidation in foods. Microencapsulation is an ideal approach to protect pomegranate's polyphenols from undesired environmental factors such as light and oxygen. Moreover, pomegranate peels are an excellent source of pectin, a food-grade ingredient that is used as food thickeners in several food applications, as well as a microencapsulating agent of bioactives such as polyphenols.

In our study, polyphenols (PPP) from pomegranate peels can be successfully microencapsulated with pectin and maltodextrin via spray drying (SDR). The use of maltodextrin (MD) with pectin (PPE) as encapsulation agents was an effective strategy to microencapsulate pomegranate peel polyphenols and increase their stability. The resultant microencapsulated powders with high antioxidant activity may be incorporated into functional foods as a novel ingredient.

The antioxidant activity (AA) of SD was the highest, while AA of SD31 and SD41 was not significantly different ($P>0.05$), and based on our preliminary study, SD31 had better viscosity than SD41 when being incorporated in the salad dressings. Therefore, we used SD31 as microencapsulated antioxidants to test their effectiveness in the Italian salad dressings system to control lipid oxidation and quality degradation. Different shear rates and storage conditions were applied. The results suggested that shear rates neither accelerated nor delayed lipid oxidation and quality deterioration in the salad dressings during storage.

Accelerated storage suggested that microencapsulated pomegranate polyphenols (MPP) could have extended the shelf life of salad dressings by 24 days compared to free polyphenols. Moreover, MPP−containing salad dressings stored at both accelerated and ambient conditions showed less indication of lipid oxidation compared to those salad dressings prepared with non-encapsulated antioxidants.

Several limitations underlie this research; however, they provide opportunities for further research. First, the processing conditions of homemade salad dressings were not comparable with those of commercial products, thus, our results suggested that MPP could extend the shelf life of salad dressing even longer when being applied in the food industry. Second, due to time constrain, we did not conduct the sensory analysis of our samples, which is important to be considered as safe and appealing food products. However, overall, this research provided useful preliminary data of shelf life for the development of functional foods containing microencapsulated antioxidants.