Stability of phenolic compounds and antioxidant capacity of concentrated mulberry juice-enriched dried-minced pork slices during preparation and storage

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**Abstract**

Functional foods have been of increasing demand due to the growing consumer awareness of the relationship between diet and health. Addition of healthy ingredients to meat products is an important method for development of functional meat products. In this contribution, functional dried-minced pork slices incorporated with concentrated mulberry juice (CMJ) were developed and stability of phenolic compounds and antioxidant capacity during product preparation and storage were evaluated. The CMJ contained high amounts of total phenolics, anthocyanin and flavonoids (19.13±0.64 mg GAE/g d.m., 4.91±0.18 C3GE mg/g d.m. and 18.39±1.21 mg CE/g d.m., respectively) and showed excellent antioxidant activities in all assays used. CMJ incorporation drastically minimized lipid and protein oxidation of dried-minced pork slices by decreasing thiobarbituric acid-reactive substances (TBARS) value and carbonyls content during processing and storage. Thermal treatment significantly destroyed the main antioxidant ingredients including anthocyanins, flavonoids, and phenolics in dried-minced pork slices with added CMJ; however, this deterioration could be effectively counteracted by using β-cyclodextrin. The redness increased but lightness decreased with CMJ added, and color of the product remained stable during storage. Therefore, CMJ rich in phenolic compounds could be used as a natural antioxidant and pigment in dried-minced pork slices with suitable protective strategy.

**Keywords:** dried-minced pork slice; concentrated mulberry juice; phenolic compounds; lipid and protein oxidation; β-cyclodextrin

**Abbreviations:**

ANOVA  analysis of variance

C3G cyanidin-3-glucoside
C3GE  cyanidin-3-glucoside equivalent
C3R  cyanidin-3-rutinoside
CD  cyclodextrins
CE  catechin equivalent
CMJ  concentrated mulberry juice
DF  dilution factor
d.m.  dry matter
DPPH  1,1-diphenyl-2-picrylhydrazyl
fw  fresh weight
GAE  gallic acid equivalent
HPLC  High performance liquid chromatography
MDA  malondialdehyde
MQA  metal chelating activity
ROS  reactive oxygen species
RP  reducing power
RSA  radical scavenging activity
TBARS  thiobarbituric acid-reactive substances
TAC  total anthocyanin content
TFC  total flavonoid content
TPC  total phenolic content
1. Introduction

Meat and meat products occupy a prominent position in the human diet for their high quality protein content, essential amino acids and excellent source of B vitamins, minerals and other nutrients (Zhang, Xiao, Samaraweera, Lee, & Ahn, 2010). Nevertheless, lower antioxidant and high quality nutrients leads to the problem of perishability of the meat and meat products (Das, Ranjan, Nath, & Laskar, 2013). The inherent antioxidant capacity of meat products is very low leading to concern about the quality and shelf life of meat and meat products (Kumar, Kumar, Tripathi, Mehta, Ranjan, Bhat, et al., 2013). Many consumers also believe that meat products are unhealthy due to their high animal fat, synthetic pigment, antioxidant, and antimicrobial contents which may be associated with several degenerative diseases (Cross, Leitzmann, Gail, Hollenbeck, Schatzkin, & Sinha, 2007). With the consumer’s growing awareness of a link between diet and health, there have been increasing demands for foods with healthy characteristics, such as meat products with bioactive or functional components. The effective approach of developing healthy food products is decreasing the undesired substances and/or increasing desired healthier components. Low molecular weight polyphenolic compounds such as flavonoids can protect the cells by scavenging and inhibiting the production and initiation of free radicals, including superoxide anions and lipid peroxyl radicals, and hence are useful ingredients for developing functional meat products (Kumar, Kumar, Tripathi, Mehta, Ranjan, Bhat, et al., 2013).

Generally, meat products when subjected to processing (heat) and storage undergo changes in their physical and chemical characteristic that leads to the development of oxygen free radicals which initiate the oxidation of polyunsaturated fatty acids. Besides, keeping attractive color is also important for food quality which affects consumer acceptance (Wang, Cao, & Prior, 1997).
Synthetic antioxidants and artificial colorants have been reported to have potential toxicity to human health, especially when they are excessively consumed daily (Ito, Hirose, Fukushima, Tsuda, Shirai, & Tatematsu, 1986). Presently, researchers are interested in the utilization of plant based derivatives that have both coloring and antioxidant properties (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009; Wang & Lin, 2000). Contrary to the artificial additives, natural colorants have attracted considerable interest due to their presumed safety, as well as potential health effects. However, incorporation of natural colorants to food systems remains to be a challenge due to their low stability when subject to processing (heat) and storage.

The use of naturally-occurring substances instead of synthetic additives in meat and meat products is an effective means of developing healthier and novel meat products. A number of publications have focused on the utilization of herbs, spices, fruits and vegetable extracts, and have shown that the addition of these extracts to raw and cooked meat products decrease lipid and protein oxidation (Estevez, Ventanas, & Cava, 2006), and improve color stability and antioxidant capacities of meat products. For example, Estévez et al. (2008) reported that adding 0.1% sage extract significantly inhibited the increase of protein carbonyls in porcine liver pâté during refrigerated storage. Rosemary extract was found to be effective in inhibiting protein oxidation in beef patties (Lund, Hviid, & Skibsted, 2007). Black currant extract delayed thiobarbituric acid-reactive substances (TBARS) value increasing in pork patties under chilled storage conditions (Jia, Kong, Liu, Diao, & Xia, 2012). The major active components responsible for the pigmentation and antioxidant activity of plant derivatives are polyphenols, flavonoids, phenolic diterpenes and tannins (Zhang, Xiao, Samaraweera, Lee, & Ahn, 2010). Nevertheless, information
concerning changes of such active ingredients in meat products during processing and storage and their contribution to product quality are still limited.

Mulberry has been found to have large amounts of phenolic compounds and its antioxidant potential has been demonstrated in vitro (Cheng, Liu, Chen, Zhang, & Zhang, 2016; Liu, Xiao, Chen, Xu, & Wu, 2004). As a potential artificial additives substitute, mulberry juice has superior properties in term of color, anthocyanin content, and beneficial health effects compared to the commonly used vegetable or herbaceous plant extracts, such as red cabbage, eggplant, and citronella, among others. (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009; Wang, Cao, & Prior, 1997; Wang & Lin, 2000). Theoretically speaking, mulberry juice could be used as an alternative colorant as well as antioxidant in improving meat product quality. However, literature suggests that phenolic compounds, especially anthocyanins, are particularly unstable during heat processing (Cheng, Liu, Chen, Zhang, & Zhang, 2016; Slavin, Lu, Kaplan, & Yu, 2013).

Dried-minced meat slices are snack meat products and have gained much popularity and acceptance in the world. Presently, to develop healthy dried-minced meat slices is one of the hot projects in this area. Natural plant extracts rich in phenolic compounds are becoming important ingredients in the production of dried-minced meat slices. In this study, a new healthy dried-minced pork slices was developed by incorporating concentrated mulberry juice (CMJ). The stability of phenolic compounds and antioxidant capacities of CMJ-enriched dried-minced pork slices were investigated.

Since meat products must be thermally processed prior to consumption, the thermal degradation of phenolic compounds is a major problem in the application of mulberry juice as
natural pigment in the meat industry. Therefore, to retain more bioactive compounds in the end products is one of the key techniques waiting for solving in the production of healthy dried-minced pork slices. Cyclodextrins (CDs) are inexpensive cyclic oligosaccharides produced from enzyme-degradation of starch, which are non-toxic ingredients, are not absorbed in the upper gastrointestinal tract, and are completely metabolized by the colon microflora (Szente & Szejtli, 2004). There are several types of cyclodextrin, including α-, β-, γ-, and δ-cyclodextrins named according to their number of glucopyranose unit. As the purification of α- and γ-CDs increases considerably the cost of production, 97% of the CDs used in the market are β-CDs. β-CD comprises seven glucose units, and has been on the GRAS list since 1998, as a flavor carrier and protectant, at a level of 2% in many food products. CDs can be considered as empty capsules of a certain molecular size that can include a variety of molecules in this cavity. Thus, CDs are capable of improving the physical, chemical and biological properties of bioactive molecules (Astray, Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gándara, 2009). Hence, CDs are widely used in the food industry as food additives, for stabilization of flavors, elimination of undesired tastes or other undesired compounds and to avoid microbiological contamination and browning reactions. Many reports have shown that CDs can protect active ingredients against oxidation, light-induced reactions, heat-promoted decomposition, loss due to volatility and sublimation, among others (Pinho, Grootveld, Soares, & Henriques, 2014). A wide range of reports have been published regarding the encapsulation of natural polyphenolic agents such as chlorogenic, ferulic and gallic acids by CDs (Zhao, Wang, Yang, & Tao, 2010; Olga, Styliani, & Ioannis, 2015), and for food and drug delivery proposes. Thermal stability of anthocyanin extract of Hibiscus sabdariffa L. was significantly improved in the presence of β-CD (Mourtzinos, Makris, Yannakopoulou,
Kalogeropoulos, Michall, & Karathanos, 2008). Phenolic compounds are the most important bioactive compounds in CMJ, therefore, effects of β-CD on the stability of phenolic compounds and antioxidant capacity were also evaluated.

2. Materials and methods

2.1 Materials and chemicals

Trimmed pork was obtained from a commercial meat processing company (Guangzhou, Guangdong, China). Concentrated mulberry juice, produced by vacuum concentration, was kindly provided by Guangdong Bosun Health Food Company (Guangdong, China) that is involved in mulberry fruit processing. All other chemicals and reagents were purchased from Qiyun Company (Guangzhou, China) and Sigma Chemicals (Sigma-Aldrich, Steinheim, Germany).

2.2 Dried-minced pork slice preparation

The trimmed pork (*longissimus thoracis*) was minced twice (10 mm plate followed by 4 mm plate) using a meat grinder. The dried-minced pork slices were prepared as follows: 850 g lean meat, 150 g pork fat, 20 g sodium chloride, 3 g phosphate, and 4 g papain (2500 u/g). Ground meat and other ingredients were mixed by blending for 30 min in a Kitchen Aid mixer (Suihua, Guangdong, China). After blending, CMJ and β-cyclodextrin were added, followed by another 10 min blending. Minced pork was shaped by hand into square with approximately 4 cm side length and 4 mm thickness. After that, two steps of heat treatment were conducted for the dehydration: firstly, heat pump drying (temperature 55°C, humidity 60%) was employed until the sample moisture content was close to 18%; secondly, all samples were baked for 3 min at 180 °C. After cooling to room temperature, dried-minced pork slices were vacuum-packed in plastic bags and stored at room temperature (25±3 °C) under fluorescent lights to simulate supermarket retail.
display conditions. Three experimental groups were designed to study the antioxidant activity and polyphenolic compound stability: 1% CMJ (10.0 g CMJ per kg of meat stuffing) was added; 1% CMJ and 1% β-cyclodextrin (10.0 g CMJ and 10.0 g β-cyclodextrin per kg of meat stuffing) were added. A control was prepared in the absence of mulberry juice and β-cyclodextrin. A strict sanitation procedure was followed to avoid microbial contamination. Three independent experimental trials (replications) were conducted.

2.3 TBARS and carbonyls analysis

Before experiments, dried minced pork slices were chopped up with a knife. TBARS value and protein carbonyls were measured according to our previous study (Cheng, Liu, Zhang, Zhang, Chen, Tang, et al., 2017).

2.4 Extraction of anthocyanins and phenolic compounds from samples

Anthocyanins and phenolic compounds in the dried-minced pork slice samples were extracted according to the procedure described by Žilić et al. (2016). Total phenolic in 500 mg of samples were released by alkaline hydrolysis for 4 h at room temperature using 10 mL 4 mol/L NaOH. After the pH was adjusted to 2.0 by 6 mol/L HCl, 5 mL of all the hydrolyzates were extracted with 5 mL of ethyl acetate and diethyl ether (1:1, v/v) four times. Five milliliters of combined extracts were evaporated under N₂ stream at 30 °C to dryness. Final residues were redissolved in 1.5 mL of methanol. The methanolic solutions so prepared were used for the analyses of total phenolic compounds, flavonoids and anthocyanins. The extracts were kept at -70 °C prior to analyses. All extractions were performed in triplicate for each replications of baking experiment.

2.5 Total phenolic content and flavonoid content analysis
The total phenolic content (TFC) of CMJ and extract was determined by using the Folin-Ciocalteu assay as described by Shi et al. (2005) and expressed as mg of gallic acid equivalent (GAE) per kg of dry matter (d.m.). A hundred milliliters of CMJ or extract was transferred into graduated cylinders and their volume made up to 500 mL with distilled water. After addition of Folin-Ciocalteu reagent (2.50 mL) and 20% Na$_2$CO$_3$ solution (1.25 mL), tubes were vortexed. The absorbance of the mixture was read at 750 nm after 40 min.

Total flavonoid content (TFC) of CMJ and dried-minced pork slice was determined as described by Eberhardt, Lee, and Liu (2000) and expressed as mg catechin equivalent (CE) per kg of d.m. Briefly, 250 μL of CMJ or extract was mixed with 1 mL distilled water and subsequently with 150 μL of 15% (w/v) sodium nitrite solution. After 6 min incubation, 75 μL of a 100 g/L aluminum chloride solution was added, and the mixture was allowed to stand for an additional 5 min before 1 mL of 4% (w/v) NaOH solution was added. The mixture was immediately made up to 2.5 mL with distilled water and mixed well. The absorbance of the mixture was then measured at 510 nm. The total flavonoid content was expressed as mg CE per kg of d.m.

2.6 Anthocyanin content analysis

The total anthocyanin content (TAC) was determined using the pH differential method (Cheng et al., 2017). Briefly, 0.25 mL of CMJ or extract was diluted 20 times with a pH 1.0 potassium chloride buffer (0.025 M) and a pH 4.5 sodium acetate buffer (0.4 M), respectively. The absorbance at 520 nm was compared with a distilled water blank after incubation in darkness for 15 min. Anthocyanin content, expressed as cyanidin-3-glucoside (C3G) equivalents, was calculated as: anthocyanin content (mg/L) = (A$_{\text{pH 1.0}}$ - A$_{\text{pH 4.5}}$) × 449.29 × DF × 1000 / (26,900×1)

where A$_{\text{pH 1.0}}$ and A$_{\text{pH 4.5}}$ are the absorbance of the sample in a pH 1.0 and a pH 4.5 buffer,
respectively; 449.29 is the molecular weight of C3G (g/mol); DF is the dilution factor (20); 1000 is the factor for conversion from g to mg; 26,900 is the molar extinction coefficient of C3G (M$^{-1}$•cm$^{-1}$); and 1 is the pathlength (cm).

C3G and cyanidin-3-rutinoside (C3R) are the primary anthocyanins present in mulberry juice and are analyzed by high performance liquid chromatography (HPLC) according to the method reported by Liu, Xiao, Chen, Xu, and Wu (2004).

2.7 Antioxidant activity determination

The antioxidant activity of the juice based on the scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by the method described by Wu et al. (2003). The Fe$^{2+}$-chelating ability of the extracts was estimated according to Andres et al. (2017). The reducing power was determined based on the method described by Wu et al. (2003) with ascorbic acid as a positive control.

2.8 Color evaluation

Surface color measurements of dried mince pork slice were performed using a colorimeter (UltraScan VIS, Chroma Meter, US) according to our previous study (Cheng et al., 2017). Before each session the colorimeter was calibrated on the CIE color space system using a white tile ($L^* = 93.16, a^* = -0.79, b^* = 1.28$). Color measurements, $L^*$ value (lightness), $a^*$ value (redness) and $b^*$ value (yellowness), were made on the surface of each slice in triplicate at three randomly selected locations. Color measurements were made at room temperature (25 °C) with illuminant D65 and a 0° angle observer.

2.9 Statistical analyses

Three dried-minced pork slices per group and per sampling time (the initial, before baking,
after first heat treatment, after second heat treatment, storage for 1 days, storage for 7 days and
storage for 14 days) were sampled and three independent experimental trials (replications) were
conducted. The results are presented as means ± standard deviation of three replicates of
independent experiments, each analyzed three times (n = 3 x 3 → n = 9 per group and
sampling time). The analysis of variance was conducted and the differences between variables
were tested for significance by one-way ANOVA with a SPSS 11 (Statistical Package for the
Social Sciences) program. Differences at P<0.05 were considered statistically significant.

3. Results and discussion

3.1 Antioxidant characteristics of CMJ

Total phenolic, flavonoid and anthocyanin contents, and antioxidant capacity of CMJ are
summarized in Table 1. There were 19.13±0.64 mg GAE/g d.m. phenolic, 18.39±1.21 mg CE/g
d.m. flavonoid and 4.91±0.18 mg C3GE/g d.m. anthocyanin in CMJ, respectively. Although no
data are found in the literature about total phenolic, flavonoid and anthocyanin contents in
concentrated mulberry juice, there are many reports about their contents in fresh mulberry fruits
and mulberry juice. We tested total anthocyanin content in fruit juice of 31 cultivars of mulberry
(Morus atropurpurea Roxb., Morus alba, etc.), and these ranged from 0.15 to 2.73 mg C3GE/mL
(Liu, Xiao, Chen, Xu, & Wu, 2004). Koca et al. (2008) reported that total phenolic and
anthocyanin contents in juice of wild purple mulberry (Morus rubra) grown naturally were 1.31
mg GAE/g and 0.19 mg C3GE/g. Özgen et al. (2009) found that the average total phenolic
contents of Morus nigra and Morus rubra were 2.74 and 1.60 mg GAE/g fw, and Morus nigra had
the highest amount of anthocyanin with an average of 0.57 mg C3GE/g fw. Kamiologlu et al.
(2013) showed that TPC, TFC and TAC in fresh black mulberry (Morus nigra L.) fruits were
14.51 mg GAE/g dw, 7.69 mg CE/g dw and 12.21 mg C3GE/g dw, respectively. Yang et al. (2016) found values of 11.23 mg GAE/g, 15.1 mg RE /g fw and 11.77 mg C3GE /g fw, respectively, in fresh fully matured mulberry (Morus atropurpurea Roxb.) fruits. It could be concluded that TPC, TFC and TAC in mulberry fruits are highly diversified, and affected by many factors such as mulberry species and cultivar, cultivation place, plant administration, growing climate, harvest season and weather, and processing method, among others. (Liu, Xiao, Chen, Xu, & Wu, 2004; Koca, Ustun, Koca, & Karadeniz, 2008; Özgen, Serçe, & Kaya, 2009; Kamiloglu, Serali, Unal, & Capanoglu, 2013). Phenolic compounds, especially anthocyanins, are sensitive to heat treatment and may be destroyed to some extent depending on heating method and conditions (Fazaeli, Hojjatpanah, & Emam-Djomeh, 2013). CMJ used in this experiment was produced by vacuum concentration and still contained high amounts of phenolic compounds even after the concentration process.

Phenolic contents were highly correlated with the antioxidant capacity. The DPPH scavenging activity and Fe$^{2+}$-chelating ability of CMJ, expressed by IC$_{50}$, were 0.67±0.08 mg/ml and 0.31±0.07 mg/ml, respectively; the reducing power of 1 mg/ml CMJ expressed by A$_{700}$ was 0.26. Nevertheless, in spite of higher phenolic content (134.79±0.8 mg GAE/g) in pomegranate extract, lower antioxidant capacities (DPPH scavenging activity of 0.16±0.02 mg/ml and reducing power of 0.03) were detected (Andres, Petron, Adamez, Lopez, & Timon, 2017). The conflicting results may be ascribed to the existing differences in phenolic types and extract concentrations.

Phenolic compounds in mulberry fruits comprise anthocyanins and non-anthocyanin phenolics. The former ones are mainly C3G, C3R and pelargonidin-3-O-glucoside (Liu, Xiao, Chen, Xu, & Wu, 2004), while the latter ones are mainly rutin, chlorogenic acid, 4-caffeolyquinic acid,
quercetin, and procatechuic acid (Zhang, Han, He, & Duan, 2008). Not all phenolic compounds inhibit oxidation via the same mechanism and some may be more effective than others (Brewer, 2011). Flavonoids with multiple hydroxyl groups exhibit stronger antioxidant property than those with a lower degree of hydroxylation (Brewer, 2011). Thus, the antioxidant activities cannot be attributed solely to the phenolic contents, but also to the actions of different antioxidant compounds and their interactions with each other (Ahn, Grün, & Fernando, 2002).

3.2 Effects of CMJ addition on oxidation of dried-minced pork slices

Due to their rich nutritional composition, meat and meat products are susceptible to quality deterioration which is mainly attributed to chemical and microbial changes. The most common form of chemical deterioration is the oxidation of meat lipids and proteins. Lipid and protein oxidations are complex processes, dependent on chemical composition of meat, light and oxygen availability and storage temperature, and are also affected by some processing technological procedures (Shah, Bosco, & Mir, 2014). They lead to the formation of several other compounds which have negative effects on the quality of meat and meat products causing changes in sensory (color, texture and flavor) and nutritional quality (Karakaya, Bayrak, & Ulusoy, 2011). Natural plant extracts are believed to play an important role in ameliorating oxidation processes by quenching free radicals, chelating catalytic metals and scavenging oxygen in food and biological systems (Ahn, Grün, & Fernando, 2002; Andres, Petron, Adamez, Lopez, & Timon, 2017), and they are widely used as antioxidants in the meat industry (Karakaya, Bayrak, & Ulusoy, 2011; Shah, Bosco, & Mir, 2014; Kumar, Yadav, Ahmad, & Narsaiah, 2015). In this study, the oxidation was assessed by comparing both TBARS and carbonyls formation in dried-minced pork slices formulated with and without CMJ during heat processing and storage. Besides, the protective
effect of β-CD against oxidation was also studied, since it has been reported that the stability of
anthocyanins could be enhanced by CD (Flores, Ruiz del Castillo, Costabile, Klee, Bigetti-Guergoletto, & Gibson, 2015).

3.2.1 TBARS

Lipid oxidation, expressed as TBARS, in control dried-minced pork slice which prepared
without CMJ, increased rapidly throughout heat processing and storage, reaching 2.34±0.10
mg/kg at the end of the storage (Fig.1 A). The levels of malonaldehyde (MDA) equivalents for all
samples are lower than 5 mg MDA equivalents /kg meat which is the detectable concentration for
rancidity (Insausti et al., 2001). The analysis of variance with the data indicated that the TBARS
level was significantly affected \( P<0.05 \) by CMJ. After heat processing, TBARS values for all
samples treated with CMJ were significantly lower than the control \( P<0.05 \), indicating a
protective effect against lipid oxidation during processing. Interestingly, this protective effect was
maintained during the entire storage period. At the end of storage (Day 14), samples treated with
CMJ had significantly lower TBARS value than control. Addition of 1% CMJ with and without β-
CD inhibited TBARS in the dried-minced pork slices by 39.3% and 31.6%, respectively. These
results are consistent with the antioxidant characteristics of CMJ (Table 1), which contained
19.13±0.64 mg GAE/g d.m. phenolic and 18.39±1.21 mg CE/g d.m. flavonoids. The inhibitory
efficacy of CMJ on lipid oxidation of dried-minced pork slices (39.3%) was lower than that of
black currant extract on pork patties (90.7%) during chilled storage (Jia, Kong, Liu, Diao, & Xia,
2012). The main cause for this difference may be attributed to different systems in addition to the
different extracts. In the study of Jia et al. (2012), the pork patties incorporating black currant
extract were not heat-treated, and only stored at 4 °C up to 9 days. It is noteworthy that β-CD
facilitates the protective effect since the TBARS values of all samples were significantly lower than that contained CMJ alone ($P<0.05$). This result was identical to that of other researchers who also demonstrated that cyclodextrin facilitated the stability and bioavailability of anthocyanins (Flores, Ruiz del Castillo, Costabile, Klee, Bigetti Guergoletto, & Gibson, 2015). Thus, CMJ is a good source of natural antioxidants that protects meat products against lipid oxidation.

Natural plants and their extracts used as lipid oxidation inhibitors in meat and meat products have a long history and there are several review papers on this topic (Karakaya, Bayrak, & Ulusoy, 2011; Shah, Bosco, & Mir, 2014; Kumar, Yadav, Ahmad, & Narsaiah, 2015; Ahmad, Gokulakrishnan, Giriprasad, & Yatoo, 2015). Methanolic extracts from the fruit of *Prunus mume* were found to reduce TBARS values of precooked chicken breast meat patties at 4 °C for 3 days (Jo et al., 2006). Pomegranate juice phenolics inhibited TBARS values in spent hen breast meat samples up to 12 days of refrigerated storage at 4 °C (Vaithiyanathan, Naveena, Muthukumar, Girish, & Kondaiah, 2011). A waste product from industrial tomato paste production was found to yield efficient protection against lipid oxidation in pressurized chicken meat (Alves, Bragagnolo, da Silva, Skibsted, & Orlien, 2012). Rose polyphenols protected lipids against oxidation in naturally dry fermented sausages (Zhang, Jiang, Rui, Li, Chen, & Dong, 2017). Our results are consistent with these previous findings and suggest the potential application of CMJ as an antioxidant for the production of meat products with improved quality traits. The inhibitory effect of CMJ on lipid oxidation may be attributed to its phenolics and other biochemical compounds that contribute to antioxidant activity.

### 3.2.2 Carbonyls

Protein oxidation is another major cause of meat quality deterioration in addition to lipid
oxidation and has drawn increasing attention in recent years. The extent of protein oxidation was assessed by measuring the formation of carbonyls. Carbonyl contents of all samples exhibited identical increasing trend during processing and storage of dried-minced pork slices (Fig. 1B), indicating the occurrence of protein oxidative reactions. This carbonyl accumulation is attributed to the direct (primary) oxidation of amino acid side chains or indirect (secondary) carbonylation (Rysman, Van Hecke, Van Poucke, De Smet, & Van Royen, 2016). Furthermore, carbonyl groups can be formed by scission of oxidative peptides, or in the presence of oxidizing sugars or lipids (Ganhão, Morcuende, & Estévez, 2010). The carbonyl content of the control sample increased faster and reached 4.01±0.22 nmol/mg protein after a two-step heat treatment. Though samples treated with CMJ exhibited carbonyl contents lower than 3.52 nmol/mg proteins, β-cyclodextrin did not provide any additional protective effect against protein oxidation during the heat processing stage. At the end of the storage, the carbonyl content of the control sample reached 5.56±0.14 nmol/mg protein while others were lower than 4.31 nmol/mg proteins. Jia et al. (2012) found that black currant extract significantly inhibited carbonyl formation in pork patties at 6 and 9 days of storage. Similar results were reported by Andres, Petron, Adamez, Lopez, and Timon (2017), who demonstrated good antioxidant properties for tomato and red grape extracts in lamb meat patties. Parallel to the variation of TBARS, β-cyclodextrin provided decreased carbonyl formation in the slices containing mulberry juice during the storage period.

The precise mechanisms involved in the antioxidant actions of plant phenolic on dried-minced pork slices system are not well understood. Several studies have demonstrated that phenols from mulberry act as efficient radical scavengers which can block the prooxidant action of reactive oxygen species (ROS) on proteins (Rice-evans, Miller, Bolwell, Bramley, & Pridham,
1995). Additionally, some phenolic compounds such as C3G, which is abundant in mulberry, display metal-chelating activities. This might be particularly relevant in heat treated meat products since phenols may hinder the prooxidant action of non-heme iron by chelation. Both antioxidant mechanisms can explain the results obtained since certain ROS such as hydroxyl radicals and iron are directly involved in the formation of protein carbonyls and TBARS from meat products (Estevez, Kylli, Puolanne, Kivikari, & Heinonen, 2008).

3.3 Phenolic compound stability in dried-minced pork slices during preparation and storage

3.3.1 Total phenol stability

Total phenolic content changes in dried-minced pork slices with different treatments during preparation and storage are shown in Fig. 2. The total phenol decreased throughout preparation and storage of pork slices. Total phenolic contents in dried-minced pork slices enriched with CMJ, with or without β-cyclodextrin addition dropped from 190.67±1.21 μg GAE/g to less than 160 mg GAE/g dried product before heating. This notable loss was caused by the oxidation effect and phenolic extractability change. On one hand, the mixing process brought in large amount of oxygen which triggered the oxidation reaction; on the other hand, the minced meat stuffing was rich in proteins, lipids and polysaccharides, which could interact with polyphenols and modify their extractability from samples (Mazzaracchio, Pifferi, Kindt, Munyaneza, & Barbiroli, 2004). Given that mulberry juices contain anthocyanins which are prone to degradation during thermal processing, decreasing of total phenolic content in dried-minced pork slices may partly be due to the loss of anthocyanins. With 1% CMJ added, the recovery of total phenolic content after two-step heat processing was 60.7±1.21% (after heat pump drying process) and 54.26±1.32% (after baking process), respectively. Though β-cyclodextrin lowered this loss, the effect still
existed during the heating process (Fig.2). Some authors have ascribed the phenolic reduction
during storage to lipids and protein oxidation reactions (Ribas-Agustí, Gratacós-Cubarsí, Sárraga,
Guàrdia, García-Regueiro, & Castellari, 2014). However, this was not the same in our study since
the total phenol contents did not undergo any significant reduction during storage ($P<0.05$). The
satisfactory stability of phenolic compounds in dried-minced pork slices was probably related to
vacuum packaging which increased protection against oxidation. These results are in concordance
with the work of Fernández-López et al. (2007), who found that hesperidin, a phenolic compound
from orange by-products, was very stable in dry fermented sausages over time. Furthermore,
though oxidation destroyed available phenolics, additional chemical substitutes that release
phenolics might make up for this initial loss during the storage (Moore, Luther, Cheng, & Yu,
2009).

### 3.3.2 Flavonoid stability

Total flavonoid contents in dried-minced pork slices during manufacturing and storage are
presented in Fig.3. The detrimental effect of mixing on flavonoid compounds might be responsible
for lower total flavonoid content for both groups before heat treatment than the initial values upon
addition (180.85±0.21 μg CE/g). Mixing may involve cellular structure damage, oxidative and
hydrolytic enzyme release that can degrade flavonoids and other antioxidant compounds
(Wojdyło, Figiel, & Oszmiański, 2009). Heat pump drying process, as a main dehydration step,
significantly reduced the flavonoids content in dried-minced pork slices. Based on the recovery of
catechin, a greater recovery of flavonoids was observed in slices made with β-cyclodextrin
(58.70±1.74 %) than the control (49.97±2.04%). Previous works also have indicated that heat
treatment of fruit juices and plant extracts results in a significant loss of flavonoids and changes in
isoflavone forms (Slavin, Lu, Kaplan, & Yu, 2013). Nonetheless, differences in flavonoid content during the second heat process were insignificant. Two previous studies have reported a similar retention of total isoflavones during the baking process of a soy and wheat bread. This stability was ascribed to tautomeric effect since they had substitutes from malonyl glucoside to acetylglucosides and β-glucosides during baking (Shao, Duncan, Yang, Marcone, Rajcan, & Tsao, 2009). After two-step heat processing, flavonoid contents in dried-minced pork slices were 84.01±0.54 μg/g dried product (without β-cyclodextrin) and 101.6±0.29 μg/g dried product (with β-cyclodextrin). During storage, total flavonoid content of dried-minced pork slices only with the addition of CMJ varied from 84.1±0.21 to 64.1±0.49 μg/g dried product, while samples containing both CMJ and β-cyclodextrin were more stable. Slices with β-cyclodextrin addition had higher final flavonoid contents (90.03±0.36 μg/g dried product) which was attributed to changes in flavonoid form and protective effects of β-cyclodextrin (Shao, Duncan, Yang, Marcone, Rajcan, & Tsao, 2009; Slavin, Lu, Kaplan, & Yu, 2013).

### 3.3.3 Anthocyanin stability

The effects of thermal processing on food anthocyanins stability have been reviewed (Patras, Brunton, O'Donnell, & Tiwari, 2010), but the available data on the topic were limited. C3G and C3R are the predominant anthocyanins in mulberry juice (Cheng, Liu, Chen, Zhang, & Zhang, 2016), and their stability in dried-minced pork slices during manufacturing and storage were studied. Variations of total and main monomeric anthocyanins are presented in Fig.4. Before baking, the difference of anthocyanin recovery between the two groups was insignificant (P>0.05), and about 77.87% anthocyanins were recovered in the products. This result is consistent with previous findings, which suggested that proteins, lipids and polysaccharides can interact with
polyphenols and finally modify their extractability (Bordenave, Hamaker, & Ferruzzi, 2014). The decrease in anthocyanin content in dried-minced pork slices with supplementation of CMJ was time-dependent. Both C3G and C3R degradation are noted during the whole study period, but the distinction in total anthocyanin recovery was remarkable ($P<0.05$). Total anthocyanin recovery of product with β-cyclodextrin (56.29±0.39%) was much higher than that without β-cyclodextrin (45.15±0.62%). In addition, drying under lower temperatures for longer time (temperature 55°C, humidity 60%, drying until the sample moisture content close to 18%) resulted in a high loss of anthocyanins. Baking process under higher temperatures for limited time (180 °C, 3 min) also degraded C3G and C3R in a high amount. Without β-cyclodextrin, the dried-minced pork slices lost 52.88±0.12% C3G and 46.43±0.23% C3R during the first heat treatment, increased to 60.45±0.31% and 58.01±0.16% after the second baking period and 71.40±0.32 and 68.32±0.16% losses during the storage. At the end of the storage, 4.32±0.31μg/g dried product C3G and 5.81±0.53μg/g dried product C3R were recovered. With β-cyclodextrin added, the anthocyanins recovery was remarkably enhanced. The recoveries for C3G and C3R from dried-minced pork slices prepared with β-cyclodextrin were respectively higher by 5.11% and 5.89% compared to the control (without β-cyclodextrin). This was perhaps because of the thermodynamic interactions between anthocyanins and β-cyclodextrin, which enhanced the stability of anthocyanins (Del Valle, 2004).

3.4 Color stability in dried-minced pork slices during preparation and storage

The color stability for dried-minced pork slices with and without CMJ is shown in Fig.5. Mulberry juice, which is rich in anthocyanins, enhanced the redness of the dried-minced pork slices. Before baking, slices with mulberry juice displayed a distinctive purple color because of
In all groups, the total loss reached 4-6 units throughout the manufacturing process (Fig. 5A).

The decline in redness was mainly due to oxidation of myoglobin, forming metmyoglobin, which is brown in color (Jia, Kong, Liu, Diao, & Xia, 2012), and anthocyanins degradation. Contrary to a*-value, L*-value showed a smooth increase in all groups during manufacturing. Slices with both mulberry juice and β-cyclodextrin showed the highest L*-value (Fig. 5B). Jia et al. (2012) and Andres et al. (2017) also reported a similar color deterioration, a steady decrease in a*-value and increase L*-value in raw pork patties and lamb patties, respectively, during storage. After heat processing, the color of the product became more natural than the brown color in the control and retention of the initial purple color, which might be more acceptable as reported by Jia et al. (2012). Although the color changes were obvious during manufacturing, they become stable during the storage with mulberry juice. This can be interpreted from the protective effect of phenolic compounds on dried-minced pork slices. As revealed, the discoloration in pork was due to the formation of metmyoglobin and non-enzymatic browning reactions between lipid oxidation products and amine groups. Therefore, antioxidant ingredients, such as anthocyanins, flavonoids, and other phenolic compounds, may form products that can protect the slices against discoloration. Our findings are in agreement with previous results, demonstrating that phenolic-rich extracts, for example, extracts from mustard leaf kimchi (Lee, Choi, Choi, Han, Kim, Shim, et al., 2010) and avocado peel and seeds (Turgut, Soyer, & Işıkçı, 2016), can effectively inhibit the color change in pork during storage. Ganhão, Morcuende, and Estévez (2010) found that blackberry, which is rich in anthocyanins, enhanced the color of cooked burger patties. However, discoloration in slices enriched with mulberry juice was remarkably reduced by β-cyclodextrin.
addition during heating process and storage, even though no obvious color difference was observed before baking ($P>0.05$). This further verified the protective effect of $\beta$-cyclodextrin on stability of pigments present in mulberry juice.

4. Conclusion

To develop balanced meat products is a challenge for food technologists and represents an appealing research line for producing novel foods. For the first time, concentrated mulberry juice rich in phenolic compounds was added to formulations of dried-minced pork slices for the preparation of functional meat products. In situ testing confirmed that concentrated mulberry juice is a highly effective antioxidant in dried-minced pork slice because it inhibited both lipid and protein oxidation and stabilized the color during preparation and storage. Heat processing is the primary factor causing loss of phenolic compounds, which could be reduced by the presence of $\beta$-cyclodextrin. In spite of the protective effect of $\beta$-cyclodextrin on phenolic compounds, reducing phenolic compounds loss is still a barrier that hampers the health promoting properties of the product. On the basis of the present results, it may be suggested that concentrated mulberry juice provides a potentially functional ingredient to be used (at level of 1.0%) for improving shelf-life quality of dried-minced pork slices. Such processed meat product would be highly desirable from a diet/health standpoint as they contain phenol compounds. These results establish a solid basis for further studies, such as the use of plant polyphenol, to develop processed meat products with higher nutritional value. We also suggest that concentrated mulberry juice be used in other meat products such as sausage as natural pigments and bio-preservatives to substitute synthetic pigments and preservatives. Furthermore, bioavailability of phenolic compounds and digestability of protein in this functional meat product also need to be investigated for the commercialization of
the new formula and technology in future.

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Figure lists:

Fig.1. Influences of CMJ on TBARS values (A) and carbonyl content (B) of dried minced pork slices during heat process and storage.

Fig.2. Phenolic content variation of dried minced pork slices during preparation and storage.

Fig.3. Flavonoid variation of dried minced pork slices during preparation and storage.

Fig.4. Anthocyanin variation of dried minced pork slices during preparation and storage.

Fig.5. Lightness (L*) and redness (a*) on dried minced pork slices during preparation and storage.
Fig. 1. Influences of CMJ on TBARS values (A) and carbonyl content (B) of dried minced pork slices during heat process and storage. Error bars refer to the standard deviations obtained from triplicate sample analysis. MJ: mulberry juice; β-CD: β-cyclodextrin. a-c, Values with different letters within the same sampling time are significantly different (P<0.05); A-E, Values with different letters within the same treatment are significantly different (P<0.05).
Fig. 2. Phenolic content variation of dried minced pork slices during preparation and storage. Error bars refer to the standard deviations obtained from triplicate sample analysis. a-b, Values with different letters within the same sampling time are significantly different ($P<0.05$); A-E, Values with different letters within the same treatment are significantly different ($P<0.05$).
Fig. 3. Flavonoid variation of dried minced pork slices during preparation and storage. Error bars refer to the standard deviations obtained from triplicate sample analysis. a-b, Values with different letters within the same sampling time are significantly different ($P<0.05$); A-E, Values with different letters within the same treatment are significantly different ($P<0.05$).
Fig. 4. Anthocyanins variation of dried minced pork slices during preparation and storage. Error bars refer to the standard deviations obtained from triplicate sample analysis. A-E, Values with different letters within the same treatment are significantly different ($P<0.05$).
Fig. 5. Lightness (L*) and redness (a*) on dried minced pork slices during preparation and storage.

Error bars refer to the standard deviations obtained from triplicate sample analysis. a-c, Values with different letters within the same sampling time are significantly different \((P<0.05)\); A-E, Values with different letters within the same treatment are significantly different \((P<0.05)\).
Highlights:

- CMJ dramatically minimized lipid and protein oxidation of dried-minced pork slices.
- CMJ is rich in phenolics and has good antioxidant activity.
- Antioxidant ingredients added to products were unstable during thermal treatment.
- β-cyclodextrin exhibited protective effect on phenolic compounds.
Table list:

Table 1 Antioxidant characteristics of CMJ.
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<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFC</td>
<td>mg CE/g d.m.</td>
<td>18.39±1.21</td>
</tr>
<tr>
<td>TPC</td>
<td>mg GAE/g d.m.</td>
<td>19.13±0.64</td>
</tr>
<tr>
<td>TAC</td>
<td>mg/g d.m.</td>
<td>4.91±0.18</td>
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<tr>
<td>RSA</td>
<td>mg/ml</td>
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<tr>
<td>MQA</td>
<td>mg/ml</td>
<td>0.31±0.07</td>
</tr>
<tr>
<td>RP</td>
<td>-</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD from three determinations (n=3). TPC: total phenolic content as gallic acid equivalent; TFC: total flavonoid content as catechin equivalent; TAC: total anthocyanin content as C3G equivalent. RSA: radical scavenging activity, expressed as IC$_{50}$, concentration (mg/ml) at 50% scavenging of DPPH radical; MQA: metal chelating activity, expressed as IC$_{50}$, concentration (mg/ml) at 50% metal chelating activity; RP: reducing power at 1mg/ml, expressed as A$_{700}$. d.m: dry matter.