EFFECTS OF DIFFERENT DRYING AND PROCESSING METHODS ON

VITAMIN C, COLOR, PHENOLICS, ANTIOXIDANT ACTIVITY, AND

MOGROSIDE V OF LUO HAN GUO (*SIRAITIA GROSVENORII*) DRINK

by

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And approved by

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ABSTRACT OF THE THESIS

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Luo Han Guo fruit (LHG), native to Guangxi province of China, is rich in mogrosides, polyphenols, vitamins, and many other nutrients. Drying and processing methods are known to impact the final quality of food product. In this study, traditional hot-air dried Luo Han Guo (HAD-LHG) and low temperature-vacuum dried Luo Han Guo (LTVD-LHG) fruit samples were used to make Luo Han Guo aqueous drink that was subjected to high hydrostatic pressure processing (HHPP) and thermal pasteurization. Unpasteurized LHG drinks were used as controls. Color, vitamin C, total phenolic content, mogroside V content, and cellular antioxidant activity in HepG2 cells were measured and analyzed for the LHG drinks.

Results showed that pH values of original LHG aqueous drink were highly affected by the drying methods of LHG fruit. HAD-LHG drink had pH values of 4.4 - 4.8, while pH values of LTVD-LHG were around 5.8 - 6.1. No significant differences of color

parameters (L*, a*, b*, C*, hue, browning index) of LHG drink were found after treated by HHPP or thermal pasteurization. Whereas color indices were greatly affected by drying methods of LHG. The color of HAD-LHG drink was dark brown, and the color of LTVD-LHG drink was light yellow. The browning index of HAD-LHG drink (199 ± 8) was much higher than that of LTVD-LHG drink (21 ± 5).

Drying methods of LHG fruit largely affected their vitamin C content, total phenolic content, and mogroside V content. LTVD-LHG had much higher vitamin C content and mogroside V content than HAD-LHG, but HAD-LHG drink exhibited significantly higher total phenolic content (499 ± 114 mg GAE/100 g dried LHG) than LTVD-LHG drink (136 ± 40 mg GAE/100 g dried LHG). Drink processing methods showed no significant effects on total phenolic content and mogroside V content of each LHG drink. The cellular antioxidant activity (CAA) values of HAD-LHG drink was not significantly affected by drink processing methods. But CAA values of LTVD-LHG drink would be decreased after thermal pasteurization when comparing to high pressure processing and unpasteurized control groups.

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1. INTRODUCTION

1.1 *Siraitia grosvenorii* and its fruit Luo Han Guo

Figure 1: Fresh Luo Han Guo on the vine

(source: www.sanherb.com)

Luo Han Guo (LHG) in Chinese refers to the fruit of *Siraitia grosvenorii* Swingle (**Fig. 1**)*,* formerly called *Momordica grosvenorii,* belonging to Cucurbitaceae family, whose scientific classification is shown in **Table 1**. LHG is also called Arhat fruit, Buddha fruit, Monk fruit, or Longevity fruit. The *S. grosvenorii* plant is a dioecious herbaceous perennial vine endemic in southern China and northern Thailand (Lim, 2012). In China, it is grown primarily on the slopes of Guangxi and Guangdong mountains (Tang, et al., 2007a), and to a lesser degree in Guizhou, Hunan and Jiangxi (Lim, 2012). Yongfu County, Longsheng County, and Lingui County in northern Guangxi are the places of the origin of *S. grosvenorii* (Zeng, et al., 2011). The plant has been cultivated for hundreds of years and is rarely found in the wild now. The sweet fruit was first mentioned in the records of the 13th century by monks who used it (Dharmananda, 2004). LHG has long been consumed by local people as a beverage material and in traditional medicine.

Table 1: Family of Luo Han Guo

Kingdom	*Plantae*
Order	*Cucurbitales*
Family	*Cucurbitaceae*
Tribe	*Joliffieae*
Genus	*Siraitia*
Subgenus	*S. grosvenorii*

1.1.1 Cultivation of *S. grosvenorii* (Luo Han Guo)

S. grosvenorii plants grow in a limited climatic region. They have to be planted on the slopes of cool, tropical or subtropical mountains with high relative humidity ($\geq 75\%$). The suitable conditions for *S. grosvenorii* growth are the altitude of 150 meters - 800 meters, temperature of 15 ⁰C - 30 ⁰C (59 ⁰F - 86 ⁰F), annual rainfall of 1500 mm - 2000 mm, and annual sunlight of 1100 h - 1600 h. *S. grosvenorii* plants are light-loving but strong light-avoiding. They belong to short day plants, preferring 6 h - 7 h sun light per day. Plants should be surrounded by mists, or lent shadows by mountain slope (Bai, et al., 2009). Soil needs to be fertile, warm, damp, and well-drained; neither sandy nor sticky soils are suitable for this plant (Lim, 2012).

The growth period of *S. grosvenorii* can be divided into young seedling, flowering, fruit set, and withering periods. In Guangxi Province, young seedling period of *S. grosvenorii* starts from March to April; flowering period occurs from May to July; and fruit occurs from July to September. Luo Han Guo fruit is harvested from September to November.

Most aboveground leaves and stems die away in winter, underground thick roots survive and produce new sprouts in the following spring (Tang, et al., 2007b).

Table 2: Major cultivars of Luo Han Guo

Cultivars of Luo Han Guo	Characteristics / Description
Chang-Tan Guo	Long or ovate ellipsoid with thin downs and 9-11 veins on the surface Chang-Tan Guo has the best quality, requiring higher ecological conditions.
La-Jiang Guo	Pear-shaped or ellipsoid fruit with very thick downs La-Jiang Guo having high quality, are suitable for mountainous areas
Dong-Gua Guo	Long cylindrical shape with rounded ends like winter melon It has white downs and six inconspicuous or conspicuous hexagonal prisms. It has high yield rate.
Hong-Mao Guo	Small, pear-shaped fruit with thick downs
Qing-Pi Guo	Large, flat ellipsoidal fruit with thick downs; most widely cultivated now. Hot air dried fruit remains a little green color.
Cha-Shan Guo	Small, subglobose fruit with thick downs

Luo Han Guo is 5 cm - 7 cm in diameter, broadly ellipsoid, oval or subglobose with a round, smooth, hard, and thin skin/shell covered by fine hair (downs). It contains a sweet, soft and edible pulp, and a large quantity of seeds (Wang, et al., 2003). The major cultivars of LHG are shown in **Table 2** (http://zt.gllhg.com/pinzhong). Depending on the

size and shape, LHG can be sorted into four groups as shown in **Table 3** (Wang, et al., 2003).

Table 3: Categories of Luo Han Guo by shape and size

Size of Luo Han Guo	Transverse Diameter (cm)	
	Long-shape (Ellipsoidal) fruit*	Round-shape (Subglobose) fruit
Extra Large fruit (XL)	> 5.7	> 6.4
Large fruit (L)	5.3 ~5.7	5.7 ~ 6.4
Medium fruit (M)	4.8 ~5.3	5.3 ~ 5.7
Small fruit (S)	4.5 ~ 4.8	4.5 ~ 5.3

*Long-shape fruit has the ratio of its long diameter to short diameter larger than 1.2.

1.1.2 Composition of Luo Han Guo

Luo Han Guo contains cucurbitane-type triterpenoids, phenolic compounds, saccharides, proteins, lipids, vitamins, and minerals (Li, et al., 2014). The cucurbitane-type triterpenoids, also called mogrosides, are the main and active components of Luo Han Guo. Mogrosides were first reported by C.H. Lee in an English report in 1975 (Lee, 1975). Takemoto isolated and analyzed the compounds and gave the structure of sweeteners in late 1970s (Takemoto, et al., 1978) to early 1980s. Ever since mogrosides IV, V, and VI were isolated by Takemoto, more than forty similar compounds have been identified in the fruit. Mogrosides share the same mogrol group with different glucose units (structures shown in **Fig. 2**) (Li, et al., 2014; Chen, et al., 2005). Most of them taste sweet, and no caloric or glycemic properties of these sweeteners are showed, for the

glycosidic bonds will not be broken by human digestive system (Suzuki, et al., 2005).

Therefore, mogrosides have the potential to be used as a substitute of sugar.

Basic structure of cucurbitanes
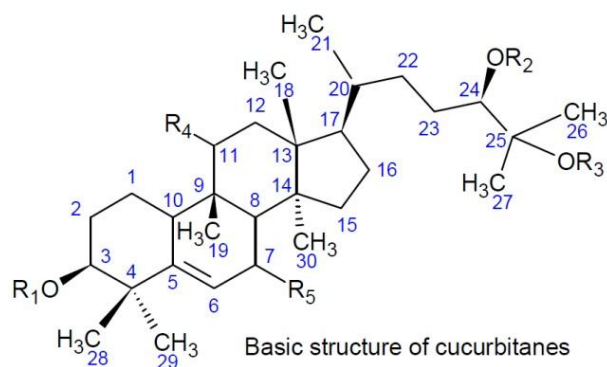
	Compound name	R_1	R_2	R_3	R_4	R_5
1	Mogrol	H	H	H	α-OH	H_2
2	Mogroside IA	H	—Glc	H	α-OH	H_2
3	Mogroside IE_1	—Glc	H	H	α-OH	H_2
4	Mogroside IIA_1	H	—Glc$\overset{6}{—}$Glc	H	α-OH	H_2
5	Mogroside IIA_2	—Glc$\overset{6}{—}$Glc	H	H	α-OH	H_2
6	Mogroside IIB	—Glc	H	—Glc	α-OH	H_2
7	Mogroside IIE	—Glc	—Glc	H	α-OH	H_2
8	Mogroside $IIIA_1$	H	—Glc$\overset{6}{\underset{2}{<}}$Glc / Glc	H	α-OH	H_2
9	Mogroside $IIIA_2$	—Glc$\overset{6}{—}$Glc	—Glc	H	α-OH	H_2
10	Mogroside IIIE	—Glc	—Glc$\overset{2}{—}$Glc	H	α-OH	H_2
11	Mogroside III	—Glc	—Glc$\overset{6}{—}$Glc	H	α-OH	H_2
12	Mogroside IV_A	—Glc$\overset{6}{—}$Glc	—Glc$\overset{6}{—}$Glc	H	α-OH	H_2
13	Mogroside IV_E	—Glc$\overset{6}{—}$Glc	—Glc$\overset{2}{—}$Glc	H	α-OH	H_2
14	Mogroside V	—Glc$\overset{6}{—}$Glc	—Glc$\overset{6}{\underset{2}{<}}$Glc / Glc	H	α-OH	H_2

15	Mogroside VI	—Glc$\overset{6}{\underset{2}{<}}$Glc Glc	—Glc$\overset{6}{\underset{2}{<}}$Glc Glc	H	α-OH	H$_2$
16	Siamenoside I	—Glc	—Glc$\overset{6}{\underset{2}{<}}$Glc Glc	H	α-OH	H$_2$
17	Neomogroside	—Glc$\overset{6}{—}$Glc$\overset{2}{—}$Glc	—Glc$\overset{6}{\underset{2}{<}}$Glc Glc	H	α-OH	H$_2$
18	Isomogroside V	—Glc$\overset{4}{—}$Glc	—Glc$\overset{6}{\underset{2}{<}}$Glc Glc		α-OH	H$_2$
19	Grosmomoside I	—Glc	—Glc$\overset{6}{\underset{2}{<}}$Glc Glc		α-OH	H$_2$
20	11-Oxomogrol	H	H	H	=O	H$_2$
21	11-Oxomogroside IA$_1$	H	—Glc	H	=O	H$_2$
22	11-Oxomogroside IE$_1$	—Glc	H	H	=O	H$_2$
23	11-Oxomogroside IIA$_1$	H	—Glc$\overset{6}{—}$Glc	H	=O	H$_2$
24	11-Oxomogroside IIIE	—Glc	—Glc	H	=O	H$_2$
25	11-Oxomogroside III	—Glc	—Glc$\overset{6}{—}$Glc	H	=O	H$_2$
26	11-Oxomogroside IV$_A$	—Glc$\overset{6}{—}$Glc	—Glc$\overset{6}{—}$Glc	H	=O	H$_2$
27	11-Oxomogroside V	—Glc$\overset{6}{—}$Glc	—Glc$\overset{6}{\underset{2}{<}}$Glc Glc	H	=O	H$_2$
28	7-Oxomogroside IIIE	—Glc	—Glc	H	α-OH	=O
29	7-Oxomogroside V	—Glc$\overset{6}{—}$Glc	—Glc$\overset{6}{\underset{2}{<}}$Glc Glc	H	α-OH	=O
30	11-Deoxymogroside III	—Glc	—Glc$\overset{6}{—}$Glc	H	H$_2$	H$_2$

31. 20-hydroxy-11-oxomogroside IA$_1$

32. 5α, 6α−epoxymogroside IE$_1$
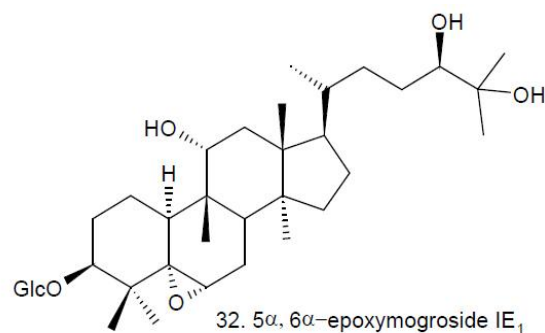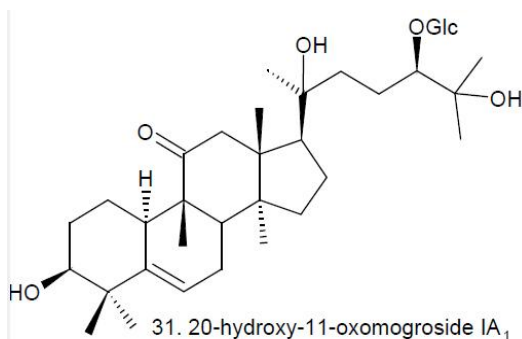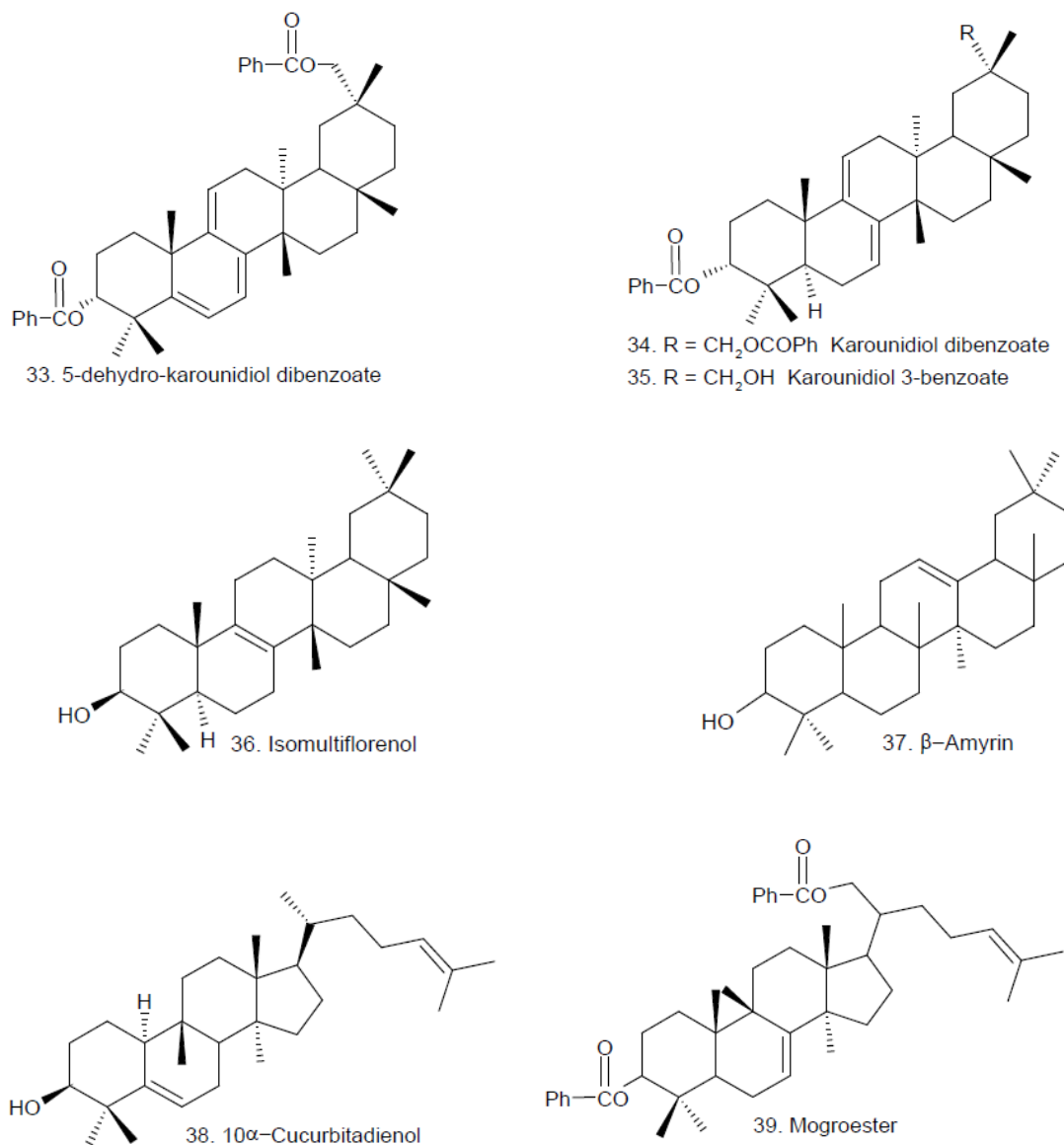
Figure 2: Structures of triterpenoid compounds isolated from *S. grosvenorii*

The compounds above are collectively called mogrosides, with one to six glucose units attached. Molecules with at least three sugar units are essential for the occurrence of sweet taste. The structure of mogrosides also affect their taste: Glycosides of the 11α-hydroxy series (R_4: α-OH) taste sweet, while glycosides of the 11β-hydroxy (R_4: β-OH) are tasteless; dehydro derivatives and 11-oxo compounds (more in unripe fruit) taste

bitter. Mogrosides IV, V, and siamenoside I are the major sweet principles and their intensities of sweetness are assessed at 233~392, 250~425, and 563 times sweeter than 5% sucrose respectively (Matsumoto, et al., 1990).

Phenolic compounds are also present in *S. grosvenorii*, including flavonoids, phenolic acids, anthraquinones, alkaloids, sterols, aliphatic acids, etc. The structures of flavonoids and other phenolic compounds found in *S. grosvenorii* fruits are shown in **Fig. 3** (Li, et al., 2014).
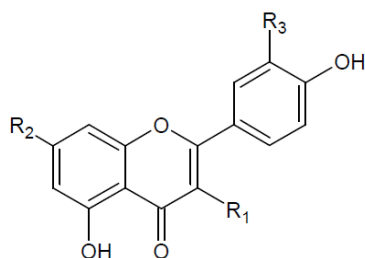
	Compound Name	R_1	R_2	R_3
1	Kaempferol	H	OH	H
2	Kaempferol 7-O-α-L-rhamnopyranoside	H	O-rha	H
3	[β-D-glucopyranosyl (1→2)]-α-L-rhamnopyranoside]	O-rha	O-rha (2→1) glc	H
4	Kaempferol 3, 7-di-α-L-rhamnopyranoside (kaempferitrin)	O-rha	O-rha	H
5	Quercetin-3-O-β-D-glucopyranoside-7-O-α-L-rhamnopyranoside	O-glc	O-rha	OH
6	7-Methoxy-kaempferol 3-O-α-L-rhamnopyranoside	O-rha	OCH$_3$	H
7	7-Methoxy-kaempferol 3-O-β-L-glucopyranoside	O-glc	OCH$_3$	H
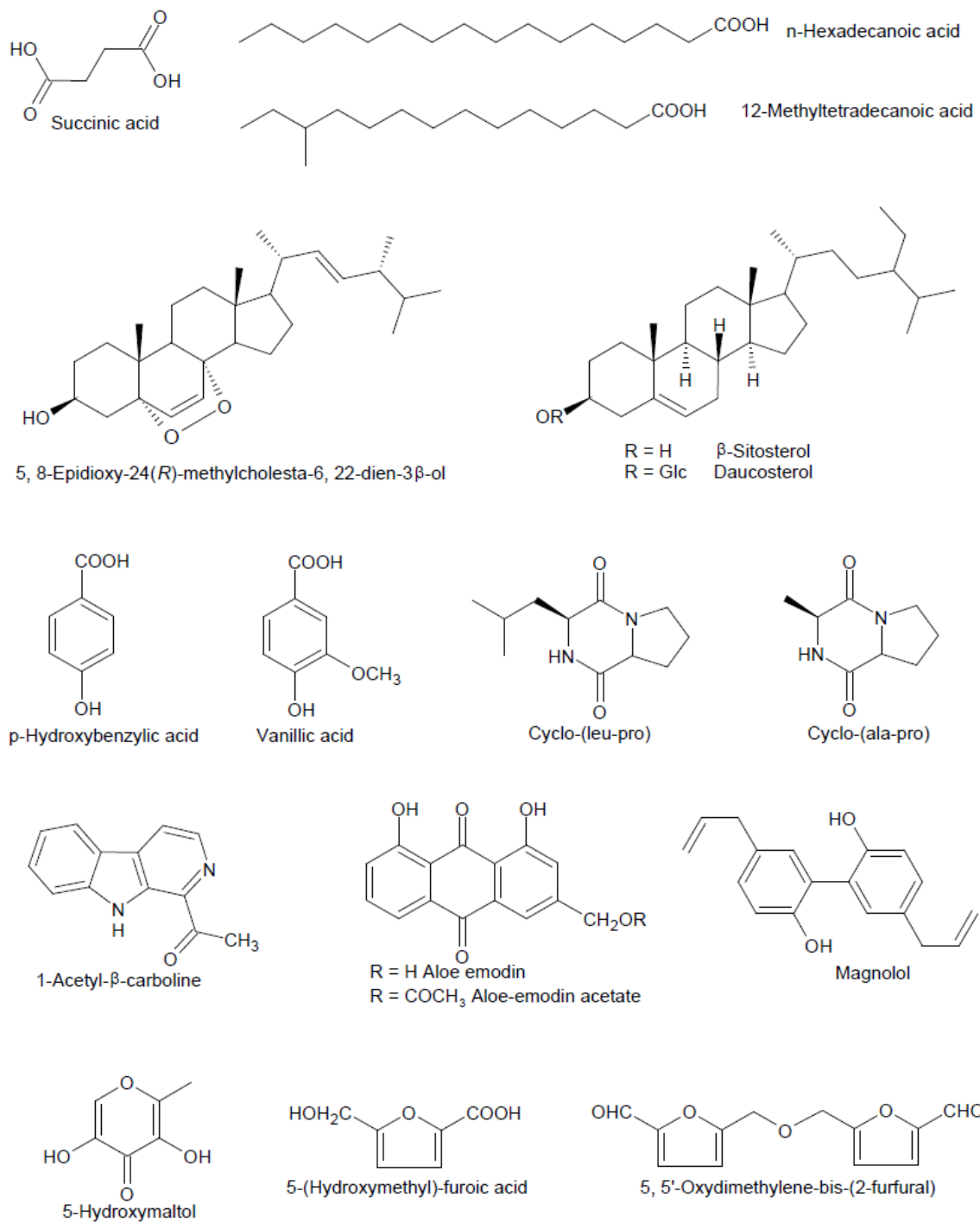
Figure 3: Structures of polyphenols in *S. grosvenorii*

Besides mogrosides and phenolic compounds, Luo Han Guo also contains saccharides, protein, vitamin and minerals. Monosaccharides and polysaccharides, such as glucose, arabinose, fructose, xylose and other sugars have been found from Luo Han Guo fruit, with the highest amount in the pulp and the lowest amount in the seeds. Crude protein content was between 8.67% to 13.35% in the dried fruit. Eighteen amino acids including eight essential amino acids have been obtained from the hydrolysis products of *S. grosvenorii* fruit. Fresh LHG is rich in Vitamin C. Seed oil of LHG contained a number and large quantity of fatty aldehydes, such as fagni aldehyde. LHG also contains sixteen minerals, such as K, Ca, P, Mg, Fe, Zn, Mn, Al, Cu, Pb, Cd, and Se (Li, et al., 2014).

1.1.3 Functions of Luo Han Guo

In southern China, Luo Han Guo is reputed to aid longevity and considered as a cooling drink used to clear away heat accumulated by metabolism or external heat. Luo Han Guo had been recorded in the books in Chinese: *Dictionary of Traditional Chinese medicine*, *National Herbal Compendium*, *Lingnan Herbs Recorded*, *Color Atlas of Chinese Herbal Medicine*, *Chinese Herbal Medicine: Materia Medica*, and *The Chinese Pharmacopoeia* as a herbal medicine used to eliminate lung heat, phlegm, and scrofula swelling (Hsu, et al., 1986), stop chronic cough, treat sore throat, hoarseness, chronic laryngitis, chronic bronchitis and other respiratory ailments. Luo Han Guo is also considered as a remedy for minor stomach and intestinal troubles (Lim, 2012). It promotes bowel movements, cures constipation and chronic enteritis, and relieves sunstroke (Lu H. C., 1986).

Modern research has demonstrated that LHG and its extracts, especially mogrosides, have a lot of physiological benefits. Konoshima & Takasaki (2002) and Takasaki, et al., (2003) reported that mogroside V and 11-oxomogroside V significantly inhibit the two-stage carcinogenesis test of mouse skin tumors induced by peroxynitrite and 7, 12-dimethylbenz anthrancene as initiators. *S. grosvenori* extract not only can be a sugar substitute, but also attenuate pathological conditions and prevent complications of type 1 (Qi, et al., 2006) and type 2 (Suzuki, et al., 2007) diabetes. *S. grosvenori* extracts display anti-hyperglycemic effects via improving insulin response of diabetic mice (Suzuki, et al., 2007) and inhibiting maltase in small intestinal epithelium (Suzuki, et al., 2005). Di, Huang, & Ho (2011) stated that mogrosides can inhibit inflammation induced by lipopolysaccharides (LPS) in RAW 264.7 cells. Zhang, Hu, & Lu (2011) suggested that *Siraitia grosvenori* polysaccharide (SGP) possesses immunomodulatory and antioxidant effects.

The anti-oxidant effects of LHG extracts have been extensively studied. Extracts from *S. grosvenorii* have antioxidant activity against free radicals and lipid peroxidation (Shi, et al., 1996), against LDL oxidation (Takeo, et al., 2002), against ROS generation and consequently hepatocarcinogenesis (Matsumoto et al. 2009). Chen and others (2007) reported that mogroside V and 11-oxo-mogroside V had significant inhibitory effects on reactive oxygen species (O^{2-}, H_2O_2 and *OH). Qi and others (2008) demonstrated that mogrosides are capable of scavenging free radicals, lower oxidative stress, serum glucose, and lipid levels in diabetic mice.

Numerous toxicity studies have been done, including acute, subacute (Marone, et al., 2008; Song, et al., 2006; Qi, et al., 2006), subchronic oral toxicity (Qin, et al., 2006; Jin, et al., 2007), cytotoxicity (Li, et al., 2007), and other animal or human studies about LHG or its extract. None of them reported any mortality or adverse clinical findings of LHG and its extract. Recently, *Siraitia grosvenorii* Swingle fruit (Luo Han Guo) extracts have been classified as generally recognized as safe (GRAS) by U.S. Food and Drug Administration (FDA, 2015).

1.1.4 Market of Luo Han Guo products

Luo Han Guo has been used as a food ingredient in Chinese cuisine to enhance flavor. It is also used in traditional Chinese medicine for centuries. Before 1965, Luo Han Guo was consumed in Guangxi, Guangdong provinces and nearby places, only to a limited extent with annual production less than 1 million fruit. The fruit became more and more popular due to its potential as a source for low-calorie sweetener, which was first studied by Lee (1975) and then Takemoto (1978). Luo Han Guo products have grown steadily since then. Nowadays, the annual production of fruits has been increased to 300-400 million fruit. Luo Han Guo concentrated extracts, particularly the non-caloric sweetener compounds, have been widely used as ingredients in drug and food industries. A lot of products made from Luo Han Guo have been developed and are available in the market in China, such as LHG drink and beverage, LHG tea, LHG herbal jelly, LHG candy, LHG grain products, LHG cough syrup and juice, LHG throat lozenges, and LHG effervescent tablet. **Figure 4** showed some of LHG products available in the market.

Figure 4: Commercial Luo Han Guo products

Most products are currently sold in China, companies from other countries also started developing products with LHG as an ingredient. **Table 4** list some commercial Luo Han Guo products and its manufacturers (BioVittoria Limited, 2009).

Table 4: Luo Han Guo products on the market

Product	Manufacturer	Country
Luo Han Guo (LHG) Beverage	Guilin Songda Food Ltd.	China
LHG Tea; LHG & Ginkgo Tea		
LHG Beverage; LHG-Ginseng Tea	Guilin Shun Chang Food Ltd.	China
LHG Tea; LHG-Wolfberry Tea		
Premium LHG-Glossy Ganoderma Tea	Guilin Guilong Food Factory	China
Premium LHG-American Ginseng Tea		
Premium LHG & Ginseng Tea		

LHG Drink	Yipeitong Ltd.	China
LHG Drink	OsloFoodie	Norway
LHG Beverage	BioValley	China
Zhizhonghe LHG Beverage	Zhizhonghe Company Ltd.	China
LHG Instant Beverage	Tea Plum GB	China
Instant Luo Han Kuo	Khao-La-Or Laboratories Ltd.	Thailand
LHG Tea	Qingfutang Company	China
Dayinxiang LHG Tea	Shantou Great Impression Co., Ltd.	China
LHG Sweet-Scented Osmanthus Tea	Guilin Grocery Food Ltd.	China
Specially-Made LHG Tea	China Guangxi Luo Han Guo	China
LHG Food	Yongfu Technology Bureau	China
Tianduo LHG Food	Tianduo Food Ltd.	China
LHG Paste	Huarentang Ltd.	China
LHG & Ginseng Grains	GB Luorensheng	China
Jin Shangzi Bai Cao (LHG Tablet)	Top Fragrance Enterprise Ltd.	China
Lim On Tong Pei Pa Koa (LHG Flavor)	Kingto Lim On Tong	China
LaKanTo Cooking Sugar (LHG Sugar)	Saraya Co., Ltd	China
LHG Tabletten	Energia Vital	Germany
Kwei Feng Kräuter Tee	Chinesische Lebensmittel	Germany
LHG Cough Syrup / Cough Beverage	Yong Fu Pharmaceutical Factory	China
Multi-Ingredient LHG Cough Beverage	Guangxi JinHaiTang Pharmaceutical Ltd.	China
Multi-Ingredient LHG Cough Beverage	Guangxi TianTianLe Pharmaceutical Ltd.	China
Multi-Ingredient LHG Cough Beverage	NanNing WeiWei Pharmaceutical Ltd.	China

1.2 Drying of Luo Han Guo fruit

Besides cultivar types and maturity stages of fruits, bioactive components in fruit-based products are heavily dependent on post-harvest processing, storage and manufacture processing. Although Luo Han Guo can be eaten in fresh form, they are usually dried before further use due to preservation concern and the fact that fresh LHG fruit is not a good source of pulp and juice, having unattractive flavors and a tendency to form off-flavors by fermentation (Dharmananda, 2004).

Drying or dehydration is defined as "The application to remove the majority of the water present in foods by evaporation or sublimation." (Fellows, 2000). The main purpose of drying is to achieve extended shelf life through reduction of water activity so that enzyme activity and microbial growth will be inhibited. The temperature used for drying of LHG is usually not sufficient to inactivate microorganisms or enzymes, which means any increase in moisture content of foods during storage will cause rapid spoilage. Therefore, blanching of LHG at 80 ºC - 100 ºC for 30 s - 10 min is usually carried out before drying. The flow chart in **Fig. 5** shows the post-harvest processing of Luo Han Guo fruit.
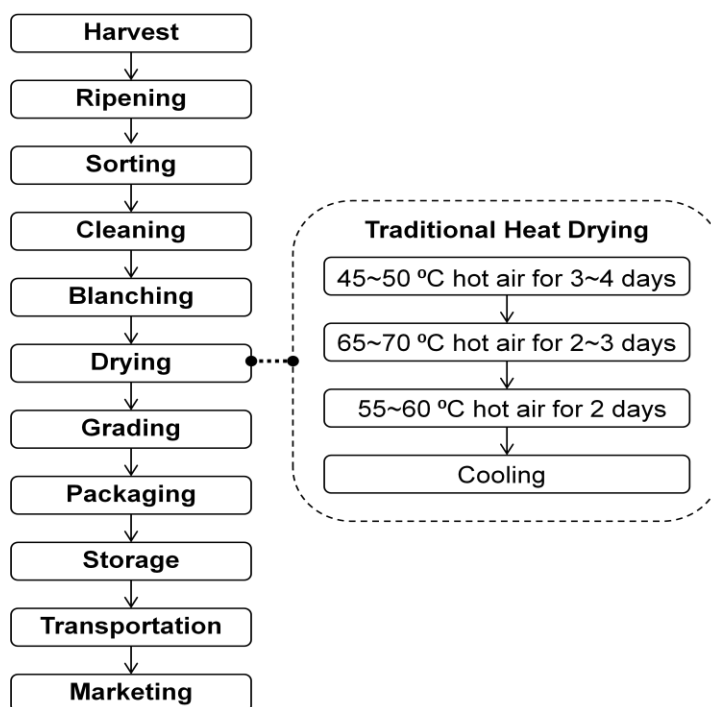
Figure 5: Post-harvest processing of Luo Han Guo fruit

1.2.1 Hot air drying

Drying plays a crucial role in the final quality of Luo Han Guo products. Heated air is used to remove moisture in the foods during traditional hot air drying. Three factors control the capacity of removing moisture from a food, which are: 1. air temperature; 2. air velocity; 3. air humidity (amount of water vapor in air). The inter-related properties of air-water vapor system can be expressed by a psychrometric chart (Fellows, 2000). Luo Han Guo fruit are usually dried at 45 ⁰C - 70 ⁰C air temperature for 6 - 8 days (Liu, et al., 2012), as shown in **Fig. 5**.

When LHG are placed in a drier, they go through several periods of drying. During the first period, the rate of water evaporation from LHG surface is the same as rate of water

moving from interior to fruit surface. The surface remains wet; and the critical factor affecting drying is air velocity. This constant-rate period of drying last for 3 days ~ 4 days at 45 ºC - 50 ºC. In the second period, the surface of LHG dries out; the overall drying rate is decided by the rate of water moving from interior to fruit surface. Water migration within LHG becomes the critical factor affecting the overall drying rate. This falling-rate period lasts 2 - 3 days, and within which most heat damage to fruit occurs. Air temperature is usually increased to 65 ºC - 70 ºC to balance the drying rate and reduce heat damage. In the last period when moisture content reaches desired amount, air temperature will be lowered to 55 ºC - 60 ºC for cooling and it lasts for 2 days.

Kiln dryers were used to dry out Luo Han Guo in ancient days. Standard sizes of kiln dryers for LHG were length of (2 - 2.5) m, width of (0.8 - 1) m, and height of 1.5 m (Wang, et al., 2005). Cabinet / tray dryers are more commonly used in modern days. Cabinet dryers are fitted with perforated trays or shallow mesh, each of which contains a layer of fruit. Hot air is uniformly blown at 0.5-5 m/s through a system of baffles and ducts. Cabinet dryers are used for small-scale production with low capital and maintenance costs (Fellows, 2000).

Drying brings about degradation of appearance and nutritional quality of Luo Han Guo. Depending on the quality, final dried fruit via traditional hot air drying can be categorized into three classes as shown in **Table 5** (Wang, et al., 2003).

Table 5: Quality classification of traditionally dried Luo Han Guo

Item	Quality Classification		
	Superior class	First class	Second class
Total Sugar	≥ 18.0 %	18.0 % ~ 16.5 %	16.5 % ~ 15.0 %
Water solubility	≥ 40.0 %	40.0 % ~ 37.5 %	37.5 % ~ 32.0 %
Moisture	≤ 15 %	≤ 15 %	≤ 15 %
Description	Excellent uniformity. • Very thin shiny skin with intact fine hairs. • Full of pulp and flesh, with thin fibers. • The core is yellow brown. • No scars. • No dents. • No scorched area. • No noising fruit* or bitter taste fruit.	Good uniformity. • Thin shiny skin with a lot of fine hairs. • Sufficient pulp and flesh, with thin fibers • The core is yellow brown. • Scars should not exceed 10% of total surface area. • No dents. • No scorched area. • No noising fruit* or bitter taste fruit.	Good uniformity. Some deforms are allowable: • Scars should not exceed 5% of total surface area. • Fruit with dents should not exceed 5% of whole bulk. Dents should not exceed two per fruit. • Scorched area should not be more than 10% per fruit. Weight of burned part should not exceed 10%. • Noising fruit* should not exceed 2%. Bitter taste fruit should not exceed 5%.

* Noising fruit: The interior of LHG shrivels separately from its skin upon drying, which make striking noise when being rocked. This is due to the non-maturity of the fruit.

1.2.2 Low temperature vacuum dryings

Processors strive in modifying drying methods to increase product quality, efficiency, and productivity, as well as reduce drying time and labor. Nowadays, new low temperature drying techniques are applied for drying Luo Han Guo, which include freeze drying (Sun, et al., 2011b), freeze-vacuum drying (Liu, et al., 2012), microwave drying (Sun, et al., 2011a), microwave-vacuum drying (Liu, et al., 2013a), microwave-vacuum infrared drying (Shen & Shen, 2012), and freezing followed by microwave-vacuum drying (Liang, 2013). Details of some drying procedures of LHG are showed in **Fig. 6**.

Freeze Drying

Freeze Drier
Control temperature as follows:
Step 1: Maintain -40 ~ -60 ℃ for 1-3 h
Step 2: Increase temp. to -25 ~ -15 ℃
    Rate: 1.2 ~ 7.5 ℃ / h
Step 3: Increase temp. to 20 ~ 25 ℃
    Rate: 3.5 ~ 10 ℃ / h
Step 4: Maintain 20 ~ 25 ℃ for 4-8 h

(source: patent CN 102125249 A)

Freeze-Vacuum Drying

Freezer
Control temperature as follows:
    -12 ~ -18 ℃ for 10-15 h
    -20 ~ -30 ℃ for 3 - 5 h

Freeze-Vacuum Drier
Cold trap temperature: -40 ~ -60 ℃
Vacuum degree: 25-70 Pa
Control heat plate temperature:
    5 - 7 ℃ for 3-5 h
    8-10 ℃ for 5-7 h
    20-25 ℃ for 3-5 h
    30-40 ℃ for 3-5 h
    45-55 ℃ for 3-5 h
    60-70 ℃ for 3-5 h
    75-85 ℃ for 3-5 h
    65-70 ℃ for 2-4 h
    50-60 ℃ for 3-5 h

(source: patent CN 102224838 B)

Microwave-Vacuum Drying

WBZ-80 Intelligent Microwave Vacuum Drier
Microwave frequency: 2450 MHz
Microwave output power: 20 kW
Vacuum degree < -0.08 MPa
Temperature: 45-50 ℃
Drying time: 90-150 min

(source: patent CN 103004956 A)

Figure 6: New drying technologies for Luo Han Guo fruit

Vacuum drying, as an advanced drying technology, has been explored and applied to fruits, vegetables and some other products. In a vacuum condition, the boiling point of water and the water vapor concentration from product surface will be sharply reduced. For example, the boiling temperature of water is 100 ℃ at the atmospheric pressure (101.325 kPa), while it reduced to 20 ℃ at the absolute pressure of 2.3 kPa (Richter Reis, 2014). Therefore, low temperature drying will be achieved and damage of product quality during heat drying will be avoided in vacuum drying. Besides, vacuum also eliminates oxygen so that oxidation degradation is greatly reduced (Feng, et al., 2012).

Freeze-vacuum drying and microwave-vacuum drying are the most known vacuum drying processes. Freeze-vacuum drying is based on sublimation of ice in product. When vacuum degree is lower than 0.006 atm, increasing temperature of frozen products will directly trigger ice sublimation rather than melting. Vacuum drying is more costly than atmospheric drying, and freeze-vacuum drying is the most expensive. But freeze-vacuum drying provides the product with the highest quality (Richter Reis, 2014).

Microwave-related vacuum drying is the fastest drying method now, providing dried products with medium quality. Compared to other drying methods, microwave-based vacuum drying dramatically reduces the process time and cost, increases energy efficiency and some qualities of dried products (Gunasekaran, 1999). Recently, microwave-related vacuum dryings have been commercially used in LHG, with drying temperature of 40 - 50 ℃ for 4 - 5 hr. Microwave is a unique volumetric electromagnetic heating that directly couples energy and moisture in LHG and consequently generate internal water vapor. Frequency of industrial microwave is 915 MHz, and that of home / restaurant microwave is 2540 MHz. Microwave drying alone has several limitations and drawbacks, such as short penetration depth of microwave and the inherent non-uniformity of microwave electromagnetic field, resulting in partial overheating, scorching and off-flavor development (Wang, et al., 2003). Combining microwave drying with various other dehydration methods can overcome the limitations of single drying methods. Examples include microwave-infrared vacuum drying, microwave-freeze vacuum drying, microwave-spouted bed drying, and microwave finish drying following other drying methods like hot air drying or osmotic dehydration (Zhang, et al., 2006).

1.3 Processing of Luo Han Guo drink

1.3.1 Thermal pasteurization

Thermal pasteurization is the most extensively used method in the food industry to increase digestibility of foods and achieve microbiological safety of the products (Rawson, et al., 2011). Unlike sterilization, which applies severe treatment to completely inactivate all microorganisms, pasteurization is a mild treatment designed to inactivate enzymes and to minimize possible health hazards from pathogenic microorganisms. Pasteurization reduces the damage to nutrients and sensory properties such as aroma, taste, texture and color. Temperature of thermal pasteurization ranges from 60 ^{o}C to 140 ^{o}C, depending on pH and water activity of the products and desired shelf life. Pasteurized foods will be shelf stable at room temperature if they are high-acid or acidified products (pH < 4.6), since the high acidity precludes the growth of *Clostridium botulinum*, a pathogen which may produce the deadly botulinum toxin (Bates, Morris, & Crandall, 2001). While for low acid foods (pH ≥ 4.6), shelf life of pasteurized foods under refrigeration would be extended to several days or weeks. According to the difference of pH, the heat treatment conditions for pasteurized high-acid products are shown in **Table 6** (Toledo, 2007).

Table 6: Conditions of thermal pasteurization of high acid products

pH of products	Processing Temperature		Processing time (s)
	(ºC)	(ºF)	
< 4.0	87.8	190	60
4.0	96.1	205	30
4.1	100	212	30
4.2	102.2	216	30
4.3 ~ 4.5	118.3	245	30

If starch or sugar is a component of a product, the time / temperature for the next higher pH should be used.

Since no published references regarding thermal pasteurization of LHG drink were found, we referred to the pasteurizing conditions of some other fruit-based products. When pasteurization process is based on an equivalent F value at 93.3 ºC (200 ºF), the processing time for tomato products are: 1 min at pH 4.1; 3 min at pH 4.2; 5 min at pH 4.3; using a z value of 8.9 ºC (16 ºF). The $F_{93.3ºC}$ for pineapple juice are: 5 min at pH = 4.0 ~ 4.3; 10 min at pH > 4.3; using a z value of 8.33 ºC (15 ºF) (Toledo, 2007).

1.3.2 High hydrostatic pressure processing (HHPP)

High hydrostatic pressure processing (HHPP) is a cold (or non-thermal) pasteurization technology to reduce the number of microorganisms in foods to an acceptable level. Liquid and solid foods, with or without packaging can be subjected to isostatic pressure between 40 and 1200 MPa via water as a pressure transmitting medium. Pressures

between 400 and 700 MPa are more commonly used. In HHPP, pressure is increased from atmospheric pressure to high pressure, held for certain time, and released rapidly. Less than 20 min of pressure holding time is practically used for the economic considerations. HHPP may be used in conjunction with monitored temperature from 4 ºC to 120 ºC to achieve higher food quality, or increase the rate of inactivation of microbes and denaturation of proteins. The isostatic character of HHPP overcomes problems of lack of uniformity in processing, such as conventional heating and microwave processing (Karwe, Maldonado, & Mahadevan, 2014).

1.3.2.1 Equipment and procedures of HHPP

10 liters vertical HHPP vessel

Figure 7: 10 liters vertical HHP equipment used in this study

High pressure units are available in vertical, horizontal, or tilting modes. The vertical orientation vessels are usually for small to medium capacity. For high capacity vessels (320-1000 liters), horizontal vessels are commonly used for the concern of safety, loading and unloading convenience, and stability. High pressure can be generated by direct or indirect compression. Direct compression uses a piston to reduce the volume, while indirect compression is realized by continuously pumping pressure medium into a volume-fixed vessel until desired pressure is reached (Martin, et al., 2002). The vertical

batch indirect compression unit, which was also used in this research (**Fig. 7**), mainly consists of:

1) A cylindrical steel pressure vessel with high tensile strength, and its end closures;

2) A means for restraining the end closures (e.g. yoke, threads, pin);

3) Indirect pressure pumps for pressure generation, including a low pressure pump, and an intensifier that delivers high pressure process liquid for compression;

4) Control system and temperature control device;

5) Loading and unloading equipments.
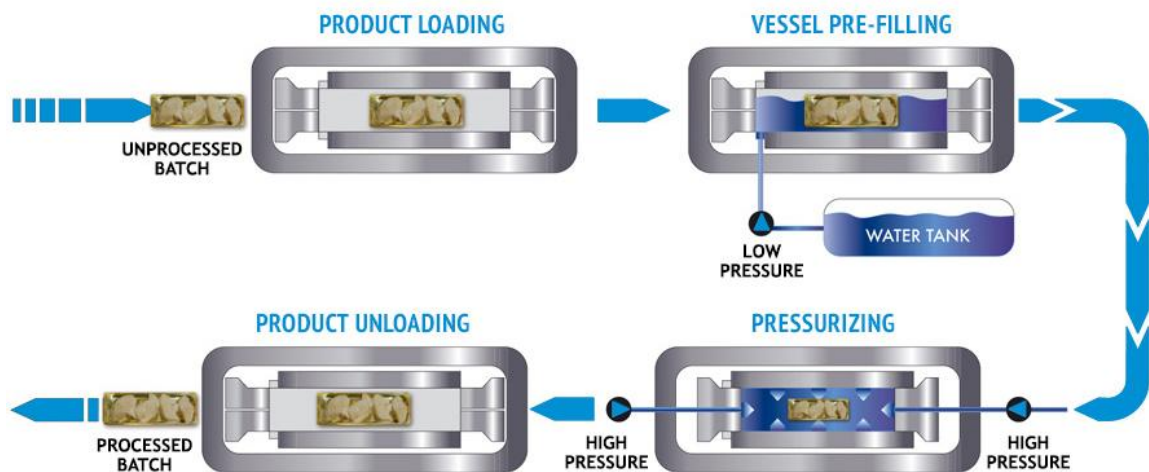
(source: www.hiperbaric.com)

Figure 8: Process steps in the operating high pressure processing

The steps of a batch-HHP process, shown in **Fig. 8**, are as follows:

1) Food products are loaded into the pressure vessel, then the vessel is sealed.

2) Pressure vessel is pre-filled with water, then the pressure relief valve is closed.

3) Water is pumped from a reservoir into the vessel via a pressure intensifier until desired pressure is reached; and the pressure is held for a desired time duration.

4) Pressure relief valve is opened to release pressure to atmospheric pressure.

5) Vessel is opened and products are unloaded.

The pressure and temperature curves during HHPP are shown in **Fig. 9**, consisting of compression /pressurization stage, pressure maintenance stage, and depressurization stage.
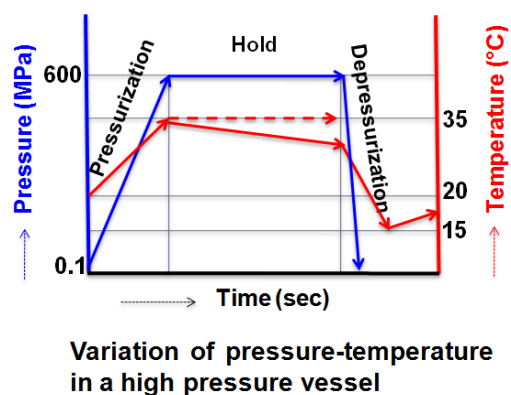
Figure 9: Typical pressure and temperature profile of HHPP

Although HHPP is considered as a non-thermal food processing technology, adiabatic heat is generated due to the work done during compression. The specific temperature rise during pressurization depends on the composition of the food. Typical values of compression adiabatic heating per 100 MPa pressure are: 3 ℃ for water, 6-9 ℃ for fatty foods and cooling oils, 30 ℃ for hexane and other synthetic chemicals. For most food materials, the adiabatic heating range is 3-6 ℃ (Rastogi, et al., 2007).

1.3.2.2 Effects of HHPP on food components

The main application of HHPP as non-thermal processing method, is ascribed to its capability of inhibiting and inactivating vegetative microorganisms, while having little effects on nutritional and sensory properties. Studies show that amino acids, vitamins, and any low molecular mass compounds are rarely affected by high pressure, whereas large microorganisms or large molecules like proteins, polysaccharides and nucleic acids can be altered by HHP treatment. This is because high pressure alone has little effect on covalent chemical bonds, but affects non-covalent chemical bonds, namely ionic bonds,

hydrogen bonds and hydrophobic interactions (Tauscher, 1995; 1998). Foods subjected to HHPP at room temperature will not undergo significant chemical changes, retaining original nutrients, color, texture or flavor, contributing to fresher, healthier, and higher quality products (Oey, et al., 2008). Horie and others (1991) reported that compared to the fresh strawberry jam, 95% vitamin C was retained in pressure-processed product. Root vegetables like potato and sweet potato, became softer, more pliable, sweeter and more transparent after HHPP, where starch molecules are partially degraded to increase sweetness and susceptibility to amylase activity (Fellows, 2000).

1.3.2.3 Application of HHPP in food industry

The first commercial high pressure processed products, fruit jams and juices, emerged in the market of Japan in 1990. Since then, a growing number of companies in Japan, Europe, USA and some other countries have started exploring and using this technology (Knorr, 1995). Currently, HHP processed products available on the market include fruit juices & jams, tomato salsa, guacamole, smoothies, fruit & vegetable puree, gold-band oysters, ready-to-eat meals, stewed packed ham, marinated chicken, meat, fish and seafood, and dairy products. Examples of HHP treated commercial products are shown in **Table 7** (Sun, 2005; Houska, et al., 2006).

Table 7: Commercially available HHP treated food products.

Product	Manufacturer	Country
Jams, fruit sauces, yogurt, jelly	Meida - Ya	Japan
Mandarin juice	Wakayama Food Industries	Japan
Tropical fruits	Nishin Oil Mills	Japan
Beef	Fuji Ciku Mutterham	Japan
Ham	Hormel foods	USA
Processed poultry products	Purdue farms	USA
Oysters	Motivatit seafoods; Joey Oysters;	USA
Hummus	Hannah International	USA
Guacamole, dips, ready meals, fruit juices	Avomex Inc., Keller, TX	USA
Juices, smoothies, teas	Suja Juice Co.	USA
Fruit and vegetable juices	Odwalla	USA
Juice	Hain Clestial Group Co.	USA
Vegetable beverages, juices, smoothies	Forager Project	USA
Orange juice	Ultifruit	France
Fruit juices	Pamryl	France
Fruit purees	Ata SpA	Italy
Sliced ham and tapas	Espuña	Spain
Apple juice	Frubaca	Portugal
Fruit juices and smoothies	Orchard House	UK
Juices, nectars, smoothie products	Grupo Jumex	Mexico
Red meat products	MLA Donor Company	Australia

1.3.3 Microbial studies of drink processing

Thermal pasteurization had been thoroughly and systematically studied. Thermal destruction of microorganisms commonly follows a first order logarithmic rate for death, which is called logarithmic order of death. That is, in a given time interval, the same percentage of contaminating microorganisms die regardless of their initial numbers when food is heated to certain high temperature. Two main parameters, D value (min) and z value (ºC), are determined from a death rate curve. The D value (or decimal reduction time) represents heating time needed to destroy 90% of the existing microbial population. The z value is defined as the temperature interval (ºC) that results in 10-fold change of the D value (Awuah, et al., 2007).

However, for high pressure treatment, more research is needed to establish the complete model of microbial death curve. The microbial mortality under thermal pasteurization and high pressure processing (Alpas, et al., 1999) would be influenced by some common factors, such as process pressure, time, and temperature; types and species of microorganisms; pH and composition of foods (Smelt, 1998).

1.3.3.1 Process pressure, time, and temperature

For thermal pasteurization, commercial sterility is used in food industry. The estimated kinetic sterilizing value (or Process Lethality F_0) at the coldest spot can be calculated by:

$F_0 = \int_0^t 10^{(T-T_0)/z} dt$. Where t, z, T and T_0 represent process time, z value, temperature at any given time, and reference temperature, respectively (Awuah, et al., 2007).

For high pressure pasteurization, moderately high pressures (300 MPa - 600 MPa) at room temperature would inactivate or kill vegetative cells of bacteria, yeasts, molds and other microorganisms present in foods, which help extending the shelf life of foods (Hoover, et al., 1989).

For pressure vessels and intensifiers of current commercial HHP food treatment, practical operating pressure is 580 MPa (85,000 psi). Pressure of 580 MPa with a holding time of 3 min at room temperature is used for shelf stable (commercially sterile) products that have pH < 4.0 and Aw ≈1. This pressure-time combinations have been proven to inactivate 10^6 cfu per gram of key food pathogens such as *E. coli* O157:H7, *Listeria* spp.*, Salmonella* spp.*, or Staphylococcus* spp. Acid foods with pH between 4.0 and 4.5 can achieve commercially sterility by using a pressure of 580 MPa and a holding time of 15 min. For low acid foods (pH ≥ 4.6), no satisfactory guidelines of holding time for 580 MPa have emerged (US FDA, 2014).

1.3.3.2 Types and species of microorganisms

Microorganisms show a wide range of sensitivity to heat or pressure. Microbial genotype and phenotype, stage of microbial growth, temperature when microorganisms form spores and some other factors will affect their heat or pressure resistance. Three types of microorganisms are present in foods, namely bacteria, yeast, and mold.

For bacteria, cells in the stationary or dormant phase are generally more heat- and pressure-resistant than cells in exponential growth phase (Pagán & Mackey, 2000). The

more developed the life form, the more sensitive it is. (US FDA, 2014). Spores of bacteria are the most resistant life forms known (Larson, Hartzell, & Diehl, 1918), some of which are capable of surviving high heat (100 ºC) for hours and surviving pressures as high as 1200 MPa. Combination of HHPP and heat, or with other treatments is required for effective elimination of bacterial spores in low-acid foods. Spore of *Clostridium botulinum* is the most lethal and most resistant microorganism, which is also the major indicator microorganism for food processing. (Jay, Loessner, & Golden, 2005)

Yeasts are spoilage microorganisms, but not a food pathogen. First-order inactivation curves of *Saccharomyces cerevisiae* ascospores in orange, apple, and a model juice system was studied by Zook et al. (1999), using pressures from 300 MPa to 500 MPa. HHPP inactivation of yeasts and vegetative bacteria in fruits is very effective because of their inherent low pH. Toxic mold growth is a safety concern in foods.

1.3.3.3 pH of foods and food composition

An important food property is the pH of food. Most microbes become more susceptible to pressure or heat in acidic environment. Acids enhance microbial inactivation during pressure or heat processing, as well as inhibit repair of sub-lethally injured cells and outgrowth of surviving cells after processing. (Garcia-Graells, et al., 1998; Linton, et al., 1999). *C. botulinum* will not grow to produce toxin in acid foods or acidified foods with pH ≤ 4.6 and water activity ≥ 0.85.

In a food matrix system, proteins, fats, sucrose and some other polysaccharides like colloids have protective effects on microorganism from heat processing (Fellows 2000). The presence of glucose, sucrose, sodium chloride or calcium acetate protect microbes against pressure damaging effect at acid or alkaline conditions. Microorganisms are protected in peanut butter under high pressure (Grasso, et al., 2010; D'Souza, et al., 2012)

1.4 Effects of processing on properties of Luo Han Guo

1.4.1 Color

Heat from both hot air drying of LHG and thermal pasteurization of LHG drink will affect the final product quality for consumption. Color alteration in thermally processed fruit beverages will be caused by enzyme inactivation, Maillard reaction, caramelization, and degradation of natural pigments. Enzymatic browning will be inhibited since enzyme will be inactivated by heat. Meanwhile, non-enzymatic browning will be generated and accelerated by high temperature through Maillard reaction and caramelization. Many natural food pigments are unstable to heat. In thermally processed vegetables and fruits, loss of green color occurs due to the conversion of chlorophyll to pheophytin and pyropheophytin (Damodaran, et al., 2008). The green LHG fruit will turn brown after being dried in the traditional hot air drier. Colorful anthocyanin pigments are unstable, the degrading level of which relies on the type of anthocyanin, temperature, pH, light and oxygen level (Boyles & Wrolstad, 1993). Carotenoids are relatively stable during heating, though heat sterilization induces cis/trans isomerization reactions, resulting in less intensely colored structure (Lee & Coates, 2003).

1.4.2 Vitamins

Table 8: Summary of vitamin stability

Nutrient	Neutral	Acid	Alkaline	Air or Oxygen	Light	Heat	Maximum cooking loss (%)
Vitamin A	S	U	S	U	U	U	40
Ascorbic acid	U	S	U	U	U	U	100
Biotin	S	S	S	S	S	U	60
Carotenes	S	U	S	U	U	U	30
Choline	S	S	S	U	S	S	5
Vitamin B12	S	S	S	U	U	S	10
Vitamin D	S	S	U	U	U	U	40
Folate	U	U	U	U	U	U	100
Vitamin K	S	U	U	S	U	S	5
Niacin	S	S	S	S	S	S	75
Pantothenic acid	S	U	U	S	S	U	50
Vitamin B6	S	S	S	S	U	U	40
Riboflavin	S	S	U	S	U	U	75
Thiamin	U	S	U	U	S	U	80
Tocopherols	S	S	S	U	U	U	55

S —— stable (no important destruction); U —— unstable (significant destruction).

Caution: these conclusions are oversimplifications and may not accurately represent stability under all circumstances.

Vitamins are among the most heat labile nutrients in fruits. Thermally induced losses of vitamins depend upon the chemical nature of vitamins and their chemical environment, including pH, light, relative humidity, concentration of dissolved oxygen, presence or absence of transition metals or other reactive compounds. **Table 8** lists the summary of vitamin stability under diverse conditions (Damodaran, et al., 2008).

Water soluble ascorbic acid (or vitamin C) is easily degraded by heat and thus oftentimes used as a marker of quality changes in fruits and vegetables processing. Ascorbic acid in most fruits and vegetables is highly available to humans (Gregory, 1993). It has reducing, antioxidative, and therapeutic properties. Ascorbic acid plays a vital role in metabolic processes like osteogenesis, iron absorption, collagen biosynthesis, and also in activating immune response, healing wound, detoxifying the organism, preventing the clotting of blood vessels. It will easily undergo up to 100% degradation during food processing. LHG is rich in vitamin C, with concentrations of (339-461) mg/100 g in fresh fruit, but it reduces to (24.6-38.7) mg/100 g in traditionally hot air dried fruit. Low temperature vacuum dried LHG has been claimed to have 760 mg/100 g dried fruit (Peng, et al., 2014). Freeze vacuum dried LHG has been claimed to have 1350 mg /100 g dried fruit (Liu, et al., 2013).

Degradation of vitamin C follows either an aerobic or anaerobic pathway. In the aerobic pathway, L-ascorbic acid converts to L-dehydroascorbic acid (DHAA), then hydrolyze to 2,3-diketogulonic acid (DKGA) and further degrade to form a wide array of other nutritionally inactive products. Although DHAA exhibits approximately the same vitamin

activity as ascorbic acid, hydrolysis of DHAA occurs very readily, and the concentration of DHAA in foods is substantially lower than ascorbic acid. Oxidation to DHAA represents an essential and rate-limiting step of the oxidative degradation of vitamin C. The oxidative rate is strongly affected by oxygen concentration, pH, and water activity, and also can be catalyzed by metal ions, heat and light. DHAA is more stable at pH 2.5-5.5 (Damodaran, et al., 2008).

Anaerobic degradation of ascorbic acid is most significant in canned products, but relatively insignificant in most other foods. Anaerobic degradation exhibits a maximum rate at pH 3 - 4. In most cases, rate constants for anaerobic degradation of ascorbic acid will be 2-3 orders of magnitude less than those for the oxidative reaction. The presence of certain sugars (ketoses) can increase the rate of anaerobic degradation.

Ascorbic acid effectively inhibits enzymatic browning primarily by reducing orthoquinone products. However, it has been demonstrated that ascorbic acid degradation is associated with discoloration reactions and will contribute to browning (Kacem, et al., 1987). An intermediate product, 3,4-dihydroxy-5-methyl-2(5H)-furanone, of dehydration following decarboxylation during anaerobic degradation of ascorbic acid, has a brownish color. In addition,. DHAA and dicarbonyls can participate in Strecker degradation with amino acids, which can generate reddish or yellowish products.

1.4.3 Phenolic compounds / polyphenols / phenolics

Phenolic compounds (i.e. polyphenols or phenolics), important constituents of human diet, consist of one or more aromatic rings bearing one or more hydroxyl groups. Polyphenols are products of the secondary metabolism of plants and express their antioxidant capacity by scavenging free radicals and chelating metal. Molecular weight of phenolic compounds ranges from less than 100 Da (phenol) for simple molecules to greater than 30,000 Da for highly polymerized ones. More than 8,000 phenolic structure variants are currently known, which can be classified into up to 10 different classes by their basic chemical structure according to Bravo, et al. (1998) and Huang, et al. (2010), as shown in **Table 9**.

Polyphenols like quercetin, kaempferol, myricetin are heat sensitive compounds. However, studies have showed that thermal processing improves the total phenolic content and antioxidant activity. In the studies of grain sprouts and seedlings, most autoclaved samples showed an increase in total phenolic contents varying from 7% to 50% (Randhir, Kwon, & Shetty, 2008). Thermally processed sweet corn (115 ºC, 25 min) (Dewanto, et al., 2002a) and tomatoes (88 ºC, 15 min) (Dewanto, et al., 2002b) showed 44% and 34% higher antioxidant activity, respectively. The phenomenon could be explained by thermal extraction of more free available phytochemicals, and the protective effect of the antioxidant properties from some Maillard reaction products as well (Nursten, 2005).

Table 9: Classification of polyphenols

Class	Subdivision	Examples
Simple phenols		Phenol, cresol, thymol, catechol, resorcinol, orcinol, pyrogallol, phloroglucinol
Phenolic acid	Hydroxybenzoic acids	p-Hydroxybenzoic acid, Gallic acid, vanillic acid, syringic acid, vanillin
	Phenylacetic acids	
	Hydroxycinnamic acids	Ferulic acid, caffeic acid, p-coumaric acid, chlorogenic acid, sinapic acid
	Phenolic acid analogs	
Coumarins	Hydroxylcoumarins	Aesculin, esculetin, scopoletin, escopoletin
	Furocoumarins & isofurocoumarin	Psoralen & isopsoralen
	Pyranocoumarins	Xanthyletin, xanthoxyletin, seselin, khellactone, praeuptorin A
	Bicoumarins & dihydroisocoumarins	Bergenin
Quinones	Benzoquinones	Embelin, embelinol, embeliaribyl ester, embeliol
	Phenanthraquinones	Tanshinone I, IIA, and IIB
	Naphthoquinones	Shikonin, alkannan, acetylshikonin
	Anthraquinones	Rhein, emodin, chrysophanol, aloe-emodin, physcion, purpurin, pseudopurpurin, alizarin, munjistin, emodin-glucoside, emodin-maloyl-glucoside
Curcuminoids		Curcumin, demethoxycurcumin, bisdemethoxycurcumin

Stilbenes		Resveratrol, oxyresveratrol, trans-resveratrol, piceatannol, pterostilbene
Flavonoids	Flavones	Luteolin, apigenin, baicalein, chrysin; apigetrin, vitexin, baicalin
	Flavonols	Quercetin, kaempferol, myricetin, morin, galangin; rutin, quercitrin, astragalin
	Flavanones	Naringenin, hesperetin, eriodictyol; naringin, hesperidin, liquiritin
	Flavanonols	Taxifolin
	Flavanols	Catechin, epicatechin, epigallocatechin, epicatechin gallate (ECG), epigallocatechin gallate (EGCG)
	Anthocyanins	Cyanidin, delphinidin, malvidin, peonidin, pelargonidin
	Chalcones	Butein, phloretin, sappanchalcone, carthamin
	Isoflavonoids	Daidzein, genistein, glycitein, formononetin
	Neoflavonoids	
	Biflavonoids	
Tannin	Hydrolysable tannins	Gallo-tannins, ellagi-tannins
	Condensed tannins	Proanthocyanidins
Lignans	Lignans	Cubebin, hinokinin, yatein, isoyatein
	Lignins	
	Lignanolides	Arctigenin, arctiin, matairesinol secoisolariciresinol
	Cyclolignanolides	Chinensin
	Bisepoxylignans	Forsythigenol, forsythin

	Neolignans	Magnolol
Others		Schizandrins, schizatherins, wulignan

1.5 Oxidation and antioxidation

Oxidation is a chemical reaction, involving increase in the oxidation state or electron loss of molecules, which occurs under atmospheric oxygen or when reactive oxygen species (ROS) are present. ROS consist of both oxygenated free radical species (superoxide radical and the hydroxyl radical) and non-radical nature species like ozone and hydrogen peroxide. In organisms, oxidative stress will cause damage to cell structure and cell function by reactive oxygen molecules and chronic excessive inflammation. Oxidation can be initiated by free radicals-mediated chain reactions, which proceeds continuously in the presence of suitable substrates until a blocking defense mechanism appears.

Antioxidant compounds can terminate chain reaction by attacking free radicals and inhibit other types of oxidations, and therefore they inhibit or delay oxidation. Antioxidants are used for stabilization of foodstuffs, pharmaceuticals, cosmetics, polymeric products, and petrochemicals. (Pisoschi & Negulescu, 2011).

Recently, attention towards oxidative stress, chronic diseases, antioxidants and their relationships is growing. Epidemiological studies show that fruits, vegetables, nuts and grains protect against development of diseases caused by oxidative stress, such as hypertension, diabetes, obesity, coronary heart disease, cataract and cancer. There are

myriads of antioxidants in plants, such as carotenoids, phenolics, flavonoids, stilbenes, lignans, coumarins, and proanthocyanidins. LHG extract shows antioxidant activity, and has the capability of reducing morbidity and mortality rate.

1.5.1 Antioxidant compounds

Endogenous antioxidants include antioxidant enzymes like superoxide dismutase, catalase, peroxiredoxins, glutathione peroxidase, and non-enzymatic chemicals like uric acid, carnosine, bilirubin, albumin, metallothioneins. Due to the inefficiency of our endogenous defense system against ROS, the needs for exogenous / dietary antioxidants rise. Examples of exogenous antioxidants derived from natural source are vitamins, flavonoids, anthocyanins, some mineral compounds. Exogenous synthetic compounds are butylhydroxyanisole, butylhydrooxytoluene, gallates, etc.

Antioxidants can be classified into water-soluble (hydrophilic) and lipid-soluble (lipophilic) divisions. Hydrophilic antioxidants like vitamin C and flavonoids, react with oxygen species in the blood plasma and cell cytosol; whereas lipophilic antioxidants like β-carotene, vitamin D, vitamin E, vitamin K_3, protect cell membranes from lipid peroxidation (Pisoschi & Negulescu, 2011).

1.5.2 Antioxidant activity and its measuring assays

Due to the great variety of both antioxidant compounds and reactive oxygen species, there are no straightforward or standard methods for measuring antioxidant activity. The oxygen radical absorbance capacity (ORAC) method used to be the industry standard for antioxidant capacity of whole foods, juices and food additives. But United States

Department of Agriculture (USDA) withdrew ORAC database in 2012, stating that *in vitro* ORAC values have no relevance to *in vivo* or human effects (USDA, 2012).

Diverse analytical methods for measuring antioxidant activity are summarized in **Table 10**. The DPPH, ABTS, FRAP, PFRAP, CUPRAC, ORAC, HORAC, TRAP, fluorimetry methods belong to spectrometric techniques, relying on the reaction of a radical, radical cation or complex with an antioxidant compound that able to donate a hydrogen atom. Spectrometric methods are affected by many factors like temperature, time of analysis, character of a compounds, concentration of antioxidants and prooxidants (Pisoschi & Negulescu, 2011). Electrochemical technologies are rapid, simple and sensitive methods, which including cyclic voltammetry, differential pulse voltammetry, square wave voltammetry, amperometric method, and biamperometric methods. Electrochemical methods measure antioxidant capacity via the potential difference between working electrodes in both stationary and dynamic (or flow, or electomigration) system. Chromatographic methods were often used in antioxidant detection and separation.

Table 10: Summary of antioxidant activity assay (Pisoschi & Negulescu, 2011)

Assays	Principle of the method	End-product determination
Spectrometry		
DPPH	Antioxidant reaction with an organic radical	Colorimetry
ABTS	Antioxidant reaction with an organic cation radical	Colorimetry
FRAP	Antioxidant reaction with a Fe (III) complex	Colorimetry
PFRAP	Potassium ferricyanide reduction by antioxidants and subsequent reaction of potassium ferrocyanide with Fe3+	Colorimetry
CUPRAC	Cu (II) reduction to Cu (I) by antioxidants	Colorimetry
ORAC	Antioxidant reaction with peroxyl radicals, induced by AAPH (2,2'-azobis-2-amidino-propane)	Loss of fluorescence of fluorescein
HORAC	Antioxidant capacity to quench OH radicals generate by a Co (II) based Fenton-like system	Loss of fluorescence of fluorescein
TRAP	Antioxidant capacity to scavenge luminol-derived radicals, generated from AAPH decomposition	Chemiluminescence quenching
Fluorimetry	Emission of light by a substance that has absorbed light or other electromagnetic radiation of a different wavelength	Recording of fluorescence excitation / emission spectra
Chromatography		
Gas chromato-graphy	Separation of the compounds in a mixture is based on the repartition between a liquid stationary phase and a gas mobile phase	Flame ionization or thermal conductivity detection

High performance liquid chromatography	Separation of the compounds in a mixture is based on the repartition between a solid stationary phase and a liquid mobile phase with different polarities, at high flow rate and pressure of the mobile phase	UV-Vis (e.g. diode array) detection, fluorescence, mass spectrometry or electrochemical detection
Electrochemical Techniques		
Cyclic voltammetry	The potential of a working electrode is linearly varied from an initial value to a final value and back, and the respective current intensity is recorded	Measurement of the intensity of the cathodic / anodic peak
Amperometry	The potential of the working electrode is set at a fixed value with respect to reference electrode	Measurement of the intensity of the current generated by the oxidation / reduction of an electroactive analyte
Biamperometry	The reaction of the analyte (antioxidant) with the oxidized form of a reversible indicating redox couple	Measurement of current flowing between two identical working electrodes, at a small potential difference and immersed in a solution containing the analysed sample and a reversible redox couple

1.5.3 Cellular antioxidant activity assays (CAA)

Cellular antioxidant assay (CAA), developed by Wolfe and Liu (2007), is a biologically relevant assay to measure antioxidant activity. Numerous studies have suggested that the original compounds may not exhibit antioxidant activity in vitro but their metabolites do in vivo, and vice versa (Scalbert & Williamson, 2000). Compared to pure "test tube" chemical methods, cell-based CAA consider the bioavailability, uptake, metabolism, and other physiological conditions of antioxidants. Although assays based on animal or human studies reflect the most accurate antioxidant properties in vivo, they are costly, time consuming, and less suitable for initial screening of food antioxidant activity. CAA as a bridge between in vitro and in vivo methods is less expensive, relatively fast, takes into account absorption, distribution, metabolism, excretion (ADME), and interactions of food components (Shahidi & Ho, 2007).

The principle of CAA method, as shown in **Fig. 10**, is that the DCFH-DA probe uptaken by human hepatocarcinoma HepG2 cells are deacetylated to DCFH, and then oxidized to fluorescent DCF when peroxyl radical generator ABAP is added. The fluorescent intensity of DCF, proportional to the level of oxidation, is measured. Non-polar DCFH-DA entered into cells through cell membrane, will get deacetylated to polar DCFH by cellular esterases and trapped within the cells. If any antioxidant compounds are in the cell, the generation of DCF fluorescence will be inhibited since antioxidants will quench reactive oxygen species from ABAP decomposition, and thus less ROS will oxidize DCFH. Antioxidant effects can be exerted by either breaking peroxyl radical chain reaction at cell membrane, or by reacting with ROS in the cell.
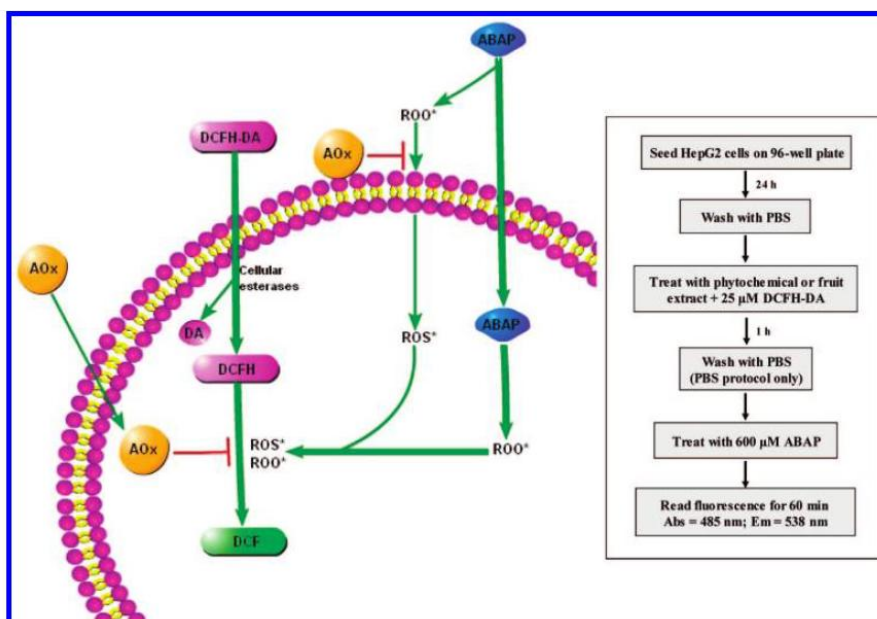
Figure 10: Method and proposed principle of CAA assay (Wolfe and Liu, 2007)

In the CAA method, appropriate selection of each reagent, component and procedure is necessary. HepG2 cells are better suited to address metabolism issues and provide consistent results with low coefficient of variation when compared to Caco-2, RAW 264.7, and other cell lines (Wolfe, et al., 2008). DCFH-DA is a versatile tool to measure oxidative stress in cells, owing to the capability to be oxidized by various arrays of oxidases, ROS, and RNS (reactive nitrogen species). Besides, the uptake of DCFH-DA is rapid, and its final concentration in the cells is relatively stable (Royall & Ischiropoulos, 1993). ABAP is an oxidant source to initiate peroxyl radicals. Although ABAP itself is not physiologically a radical, its decomposed products are a major type of ROS in vivo (Wang & Joseph, 1999). Wolfe and Liu (2007) recommended utilizing quercetin as a standard in CAA for the following reasons: 1) quercetin has high CAA activity and comparatively stable; 2) pure quercetin is economically available; 3) it is ubiquitously present in fruits, vegetables, and other plants.

2. HYPOTHESIS, OBJECTIVES AND RATIONALE

2.1 Hypothesis

- Aqueous drink made from low temperature vacuum dried LHG (LTVD-LHG) would have brighter color, higher total phenolic content, and higher antioxidant activity than that from traditional hot air dried LHG (HAD-LHG).

- High hydrostatic pressure (HHP) processed aqueous LHG drink would have brighter color, higher total phenolic content, and higher antioxidant activity than thermal pasteurized and unprocessed LHG drink.

2.2 Rationale

LHG is a fruit rich in bioactive compounds. But customers more often consume processed LHG drink rather than fresh fruit. The impact of processing technologies on the final quality of LHG is important but still unclear. Two stages of processing, namely drying methods of fruit and processing types of LHG drink, are the main factors that will affect the final properties of LHG drink. LHG drying processors strive to use new low temperature drying technologies, claiming that it preserves more nutrients than traditionally dried ones. Beverage processors strive to try new non-thermal processing to avoid heat damage on bioactive compounds. However, there is little scientific research supporting their claims or efforts. Therefore, we wanted to study the effects of drying and drink processing methods on the quality and properties of LHG drink, in order to provide some scientific basis for LHG processors.

2.3 Objectives

This research focused on comparing the effects of drying and drink processing methods on the color, vitamin C, total phenolic content, cellular antioxidant activity, and mogroside V content of Luo Han Guo drink. The objectives were:

1) To evaluate Vitamin C content of traditional hot air dried and low temperature vacuum dried Luo Han Guo.

2) To compare color, total phenolic content, cellular antioxidant activity, and mogroside V content of aqueous LHG drink made from traditional hot air dried and low temperature-vacuum dried fruits.

3) To compare color, total phenolic content, cellular antioxidant activity, and mogroside V of LHG drink after thermal pasteurization and non-thermal HHPP.

3. DESIGN OF EXPERIMENTS

Preliminary microbial studies were first conducted to decide the HHPP conditions to achieve at least 5 log reduction of target microorganisms required by FDA (US FDA, 2004).

Since both hot air dried LHG (HAD-LHG) and low temperature-vacuum dried LHG (LTVD-LHG) were directly purchased from venders in China, differences between just two batches of LHG samples would not be sufficient to demonstrate that the difference was due to different drying methods rather than batch variations. Therefore, two factors randomized complete block design (RBD) was chosen. In the design, factor 1 (x_1) was sample variables: HAD-LHG was in block 1 (row 1), while LTVD-LHG was in block 2 (row 2). Each block has three batches of LHG supplied by different venders. If the batch differences within each block is significantly smaller than differences between the two blocks, then we can determine with sufficient confidence that the drying methods affect the final quality of LHG products. **Table 11** shows the randomized block design (RBD) used in this research. A, B, C represented three different batches of HAD-LHG from three different suppliers. X, Y, Z represented another three different batches of LTVD-LHG from the other three different suppliers.

Factor 2 (x_2) was treatment variables of LHG drink: unprocessed control, thermal pasteurized, and HHP processed groups. Three responses containing color, total phenolic content (TPC), cellular antioxidant activity (CAA), and mogroside V content were measured. Results were obtained through triplicate experiments.

Table 11: Two-factor randomized block design table

Drying methods	Drink processing methods		
	Control (Unprocessed)	Thermal Pasteurization	HHPP
Hot air dried LHG	*A, B, C*	*A, B, C*	*A, B, C*
Low temperature-vacuum dried LHG	*X, Y, Z*	*X, Y, Z*	*X, Y, Z*

4. MATERIALS AND METHODS

4.1 List of used chemicals and biological substances

The chemicals and biological substances listed were used in this research:

a. Sample preparation:

- L(-)-Malic acid, 99% (ACROS ORGANICS AC15059100, Geel, Belgium)

b. Vitamin C measurement:

- Acetic acid, Glacial (Fisher Scientific A38-500, Fair Lawn, NJ)

- Ascorbic acid (SUPELCO® 47863, Bellefonte, PA)

- Boric acid (EMD Chemicals BX0865-11, Darmstadt, Germany)

- Hydrochloric acid (Fisher Scientific A144s-500, Fair Lawn, NJ)

- Metaphosphoric acid (ACROS ORGANICS 450251000, Geel, Belgium)

- Norit® Carbon, Decolorizing black powder (Fisher Scientific C170-500,)

- o-phenylenediamine dihydrochloride, 99+% (ACROS organics 218480250, Geel, Belgium)

- Sodium hydroxide (Fisher Scientific S318-1, Fair Lawn, NJ)

- Sodium acetate trihydrate (Fisher Science Education S93352, Rochester, NY)

- Sulfuric acid (Fisher Scientific A510-P500, Fair Lawn, NJ)

- Thymol blue, sodium salt, indicator (ACROS organics 81012-93-3, Geel, Belgium)

c. Microbial Studies:

- Agar, 80-100 mesh (Fisher Scientific BP2641-1, Fair Lawn, NJ)

- Dextrose (Fisher Scientific D16-3, Fair Lawn, NJ)

- Difco™ Peptone Water (BD 218071, Sparks, MD)

- *Saccharomyces cerevisiae* strain BY 4741 (Dr. George Carman's Lab, Rutgers University)

- Yeast extract (Growcells MCMA-0601, Irvine, CA)

d. Folin-Ciocalteu assay:

- Folin-Ciocalteu reagent (Sigma-Aldrich F9252-500ML, St. Louis, MO)

- Gallic acid (Sigma-Aldrich G7384-100G, St. Louis, MO)

- Polyvinylpyrrolidone, cross-linked (ACROS organics 22748500, Geel, Belgium)

- Sodium carbonate (Na_2CO_3) (Sigma-Aldrich S7795-1KG, St. Louis, MO)

e. Cytotoxicity & Cellular Antioxidant Activity (CAA) assay:

- 2,2'-azobis (2-amidinopropane) dihydrochloride (ABAP) (Sigma-Aldrich 440914, St. Louis, MO)

- 2',7'-dichlorofluorescin diacetate (DCFH-DA) (Sigma-Aldrich D6883-50MG, St. Louis, MO)

- CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI)

- Dimethyl Sulfoxide (DMSO) (Sigma-Aldrich 41639-100ML, St. Louis, MO)

- Fetal Bovine Serum (Invitrogen 26140, Carlsbad, CA)

- Hanks' Balanced Salt Solution (HBSS) (Invitrogen 14025, Carlsbad, CA)

- HEPES (Sigma-Aldrich, St. Louis, MO)

- HepG2 cells (ATCC HB-8065, Manassas, VA)

- L-glutamine (Sigma-Aldrich, St. Louis, MO)

- Minimum Essential Medium, Eagle with Earle's Balanced Salt Solution (EMEM) (ATCC 30-2003, Manassas, VA)

- Penicillin-streptomycin (Invitrogen 15070, Carlsbad, CA)

- Phosphate buffer saline (PBS) (Sigma-Aldrich, St. Louis, MO)

- Quercetin (Merck KGaA QU995, Darmstadt, Germany)

- Trypsin-EDTA (0.25%), phenol red (Invitrogen, Carlsbad, CA)

- Williams' Medium E (Invitrogen 12551-glutamine, Carlsbad, CA)

f. High Performance Liquid Chromatography (HPLC):

- Acetonitrile (HPLC grade, 99.9%) (Thermo Fisher scientific 61001-0040, Waltham,

   MA)

- Mogroside V (Purity ≥ 98%) (Shanghai Tauto Biotech, Shanghai, China)

- Water (HPLC grade) (Fisher Scientific W5SK-4, Fair Lawn, NJ)

- Phosphoric acid (HPLC grade) (Fisher Scientific, Fair Lawn, NJ)

4.2 Preliminary microbial equivalence studies

U.S. FDA states "The HACCP regulation requires you to use treatments capable of
consistently achieving at least a 5-log reduction (using ten as the base number) in the
level of the pertinent microorganism in your juice." (US FDA, 2004). Therefore, the
range of HHPP conditions to achieve desired safety level for the LHG drink need to be
determined. Based on the pH of LHG drink (pH = 4.20 - 4.25), yeast will be a target
microorganism. *Saccharomyces cerevisiae* was chosen for the microbial safety study
(Alemán, et al., 1996).

Luo Han Guo drink to be tested for log reduction of yeast, was sterilized at 121 ºC / 15
psi steam pressure for 15 min. This will eliminate the existing microorganisms in the

drink before the test. The juice was then inoculated with a BY4741 strain of *Saccharomyces cerevisiae*, which was obtained from Dr. Carman's Lab at Rutgers University. Luo Han Guo drink has enough nutrients itself to support the growth of the yeasts. The drink was then incubated for 1 - 2 days at 30 ℃ in a shaking incubator to ensure uniform dispersion of yeast. The cultured drink was then packed and subjected to thermal and high hydrostatic pressure processing. For HHP processing, the preliminary experiments were conducted from least treatment condition 300 MPa 2 min to severe treatment condition 600 MPa 10 min (**Fig. 11**).
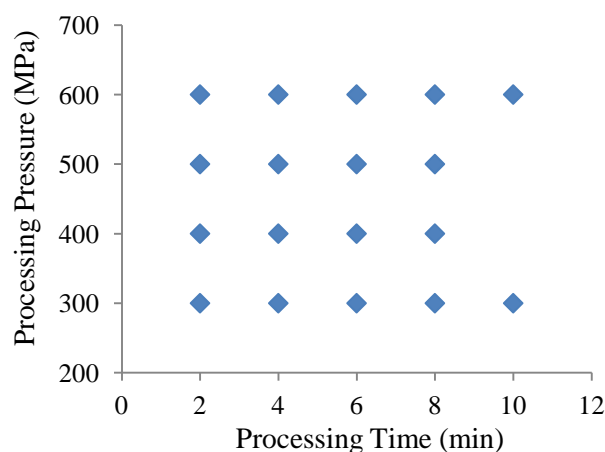
Figure 11: HHPP conditions for S. cerevisiae

YPD agar and peptone water was prepared, autoclaved at 121 ℃/ 15 psi stream pressure for 15 min. The YPD agar was prepared by dissolving 10.0 g Yeast Extract, 20.0 g Peptone, 20.0 g Dextrose, 15.0 g Agar in DI water to make 1 liter medium. Autoclaved YPD agar aliquot of 20 mL was immediately transferred to each petri-dish (Fisher Scientific, Pittsburg, PA) to make solidified YPD agar when cooling down. The peptone water was made by dissolving 1.5 g of peptone powder into 1 L deionized water.

Unprocessed cultured drink as control group was diluted $\times 10^{-3}$, $\times 10^{-4}$, and $\times 10^{-5}$ times, and processed cultured drink was diluted $\times 10^{0}$, $\times 10^{-1}$, $\times 10^{-2}$ times with peptone water to reach yeast concentration of approximately 300-3000 CFU/ml. Then, 100 μL diluted drink was dropped onto the pre-made YPD agar plate and spread uniformly. The entire dilution and plating process was done in a sterile environment. After that, the plates were incubated at 30ºC for 3 days. The plates were enumerated for the number of colonies. Only when the number of colonies was within the range of 30-300 /plate, the corresponding dilution was chosen to determine the final concentration of yeast in Luo Han Guo drink. Concentration of yeast (Y) can be expressed as

$$Y\frac{CFU}{mL} = \frac{Number\ of\ CFU\ counted}{Volume\ plated\ (mL) \times total\ dilution\ used}.$$

4.3 Luo Han Guo Samples

The Luo Han Guo (LHG) variety used in this research was Qing-Pi cultivar, large fruit (long diameter = 6.11 ± 0.23 cm, transverse or short diameter = 5.66 ± 0.15 cm, ratio of long and short diameter = 1.08 ± 0.04). Three batches of traditional hot-air dried LHG fruits were obtained from farmers' market in Guilin City, China; Naning Ningfu Trading Co., China; and Royal King® Herba Natural Products, Inc. Three batches of low temperature-vacuum dried LHG fruits were bought from Guilin Qinli® Zhen-Jin Luo Han Guo Co. Ltd, China; ASGO® Guilin Ronghe Food Manufactory; and Long-Ji® Longji Luo Han Guo Processing Manufactory, China. **Figure 12** showed one HAD-LHG (**a**), one LTVD-LHG fruit (**b**), and their powder. All of them were blended by Waring Laboratory Blender (Dynamics Corporation of America, New Hartford, CT) into powder, sieved through No. 30 USA Standard Testing Sieve (Opening: 600 micron. Fisher

Scientific, Pittsburgh, PA).   Every 500 mg LHG powder aliquot was put into a 6.2 cm $\times$ 6 cm SpecialTea[TM] heat-sealable paper teabag (Orlando, FL), and would be heat-sealed by a foot operated AIE-402CH heat sealer (American Int'nl Electric Inc, City of Industry, CA).

Figure 12: (a) HAD-LHG & its powder and (b) LTVD-LHG & its powder

4.3.1 Ascorbic acid (AA) measurement of Luo Han Guo fruit

Total vitamin C of LHG fruit was measured according to 45.1.15 AOAC Official Method 967.22 Microfluorometric Method (1968). Briefly, 0.1 mg/mL ascorbic acid (AA) standard solution was made by dissolving AA in metaphosphoric acid - acetic acid solution (HPO_3 : CH_3COOH : H_2O = 3 : 8 : 89 w/v/v). LHG solution was made by dissolving and filtering LHG powder in metaphosphoric acid - acetic acid - sulfuric acid solution (HPO_3 : CH_3COOH : H_2SO_4 : H_2O = 3 : 8 : 0.7 : 88.3 w/v/v/v). The H_2SO_4 was utilized to bring down the pH of final solution, and its percentage or amount was not fixed, but depended on the initial pH of variable samples. After that, 2% (v/w) of hydrochloric acid-washed Noric[®] Carbon powder was added to AA and LHG solution, well mixed, and filtered.

Test solution was made by fully mixing 5% AA or LHG filtrate, 5% sodium acetate solution ($CH_3COONa : H_2O = 50 : 50$), and 90% distilled water. Blank solution was made by fully mixing 5% AA or LHG filtrate, 5% boric acid-sodium acetate solution ($H_3BO_3 : CH_3COONa : H_2O = 3 : 48.5 : 48.5$ w/v/v), and 90% distilled water. Then 2 ml of test or blank solution, 5 ml of 0.2 mg/ml o-phenylenediamine·2HCl was mixed, and stood 35 min at room temperature avoiding light. The fluorescent readings of final solution were measured by HORIBA Jobin Yvon FluoroMax-3 Spectrofluorimeter (Horiba, Ltd., Kyoto, Japan) (**Fig. 13**), with excitation filter of wavelength 350 nm, and range of emission filter of wavelength 390 nm ~ 490 nm (Fluorescence maximum at ca 430 nm).

Figure 13: HORIBA Jobin Yvon FluoroMax-3 Spectrofluorimeter

The principle of microfluorometric method is that ascorbic acid is oxidized to DHAA in the presence of Norit Carbon. DHAA reacts with o-phenylenediamine to produce fluorophor, whose intensity is proportional to DHAA concentration. For blank solution, H_3BO_3-DHAA complex was formed prior to the addition of o-phenylenediamine, leaving the extraneous fluorescence that will be subtracted during calculation.

4.3.2 Luo Han Guo aqueous drink and pH measurement

Luo Han Guo drink of 20 mg / mL was made by adding a teabag of 500 mg LHG powder

(**Fig. 14a**) and 25 mL Great Value® distilled water (Wal-Mart Stores, Inc, AR) at room

temperature, with powder to water ratio 1:50 (w/v) into a 12.5 cm × 10 cm MRE pouch

made from heat sealable film (Printpack Inc., Williamsburg, VA).

(b)

(a)
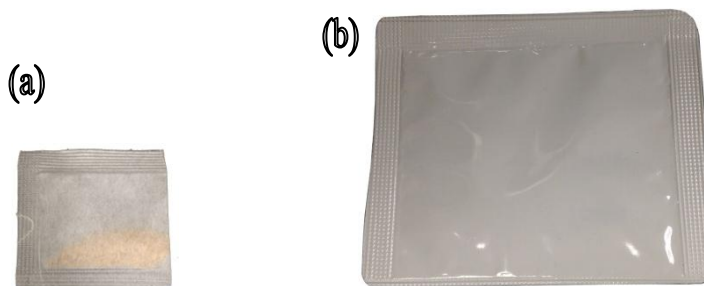
Figure 14: (a) LHG powder in a teabag and (b) LHG drink in the pouch

The pH of LHG aqueous drink was measure by Thermo Scientific Orion^{TM} Star A111 pH

Benchtop Meter (Waltham, MA). All samples of LHG drink were adjusted to pH = 4.20

~ 4.25 by 20% L-malic acid solution (L-malic acid : water = 20 : 80 w/w). The pouches of

LHG drink (**Fig. 14b**) would be heat-sealed by AIE-402CH heat sealer (American Int'nl

Electric Inc, City of Industry, CA) and ready for drink processing.

4.4 Thermal pasteurization and HHPP of Luo Han Guo drink

Pouches of LHG drink were left in MGW Lauda K6KS water-bath (Lauda-Königshofen,

Germany) for thermal pasteurization. A pouch had a C-4 flexible Ecklund-Harrison

thermocouple (Ecklund-Harrison Technologies Inc., Fort Myers, FL) attached to it (**Fig.**

15). The thermocouple was then connected to a data acquisition system, which consisted

of a high speed USB carrier NI USB9162 (National Instruments, Austin, TX) connected

to a computer. Software Labview® 2010 (National Instruments, Austin, TX) cooperated with MATLAB® was used to record real time, temperature data and calculate F-values. Based on the pH of Luo Han Guo drink (acidified to pH = 4.20 ~ 4.25), the reference pasteurization conditions were at 200ºF (93.3ºC) for 10 min with z-value of 16ºF (8.8ºC) (Toledo, 2007).
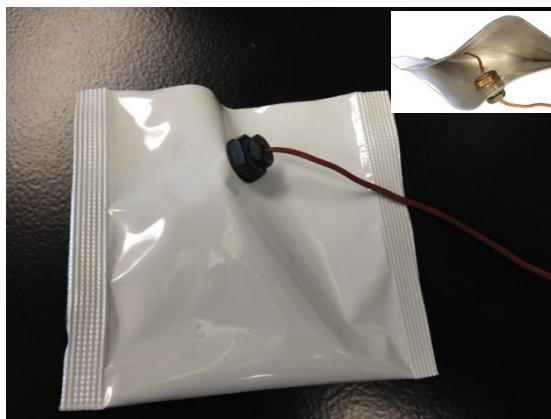
Figure 15: A pouch with C-4 flexible Ecklund-Harrison thermocouple

HHPP were carried out by using Rutgers 10 liter pilot plant HHPP facility, which was manufactured and assembled by Elmhurst, Inc. (Albany, NY). Based on current commercial HHP equipments, pH of sample (pH = 4.20 ~ 4.25), and concern of microbial safety, pressure of 580 MPa (85,000 psi) with a holding time of 15 min (US FDA, 2014) were chosen to process LHG drink.

Control groups were pouches of both HAD-LHG drink and LTVD-LHG drink without any processing, which would only be shaken on Fisher ScientificTM Nutating Mixer at room temperature for 1 h.

4.5 Color measurement of Luo Han Guo drink

Color of Luo Han Guo drink was measured by a Konica Minolta CR-410 chroma meter (Osaka, Japan) (**Fig. 16a**) based on Commission Internationale de l'éclairage L*, a* and b* (CIELAB) uniform color space (**Fig. 16b**). Standard illuminant D_{65} and 2° observer angle were used. Illuminants D series were recommended by CIE in 1966 to represent daylight that was more completely and accurately than illuminants B and C. The subscript "65" are the first two digits of their correlated color temperature 6504 K. (Sahin & Sumnu, 2006). Calibration was done with a white standard Y=93.38, x=0.3172 and y=0.3331. Each 10 mL of Luo Han Guo drink sample was placed in a circular clear glass dish with inner diameter 57 mm and outer diameter 61 mm that perfectly fits the top of light port of the colorimeter. The CIELAB coordinates (L*, a*, b*) values were directly measured. L* values represent the lightness, in which 0 means absolute black and 100 means absolute white; a* represents greenness (- value) and redness (+ value), whereas b* represents blueness (- value) and yellowness (+ value) (Konica Minolta Sensing, Inc., 2007).
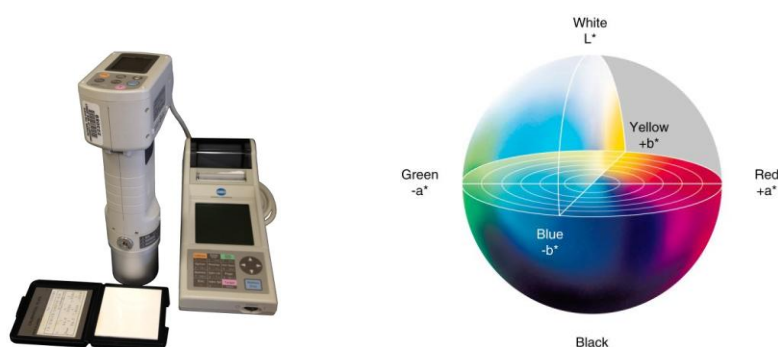
Figure 16: (a) Konica Minolta CR-410 chroma meter and (b) CIELAB color space

Chroma (C*) indicating the color intensity or saturation, is defined by the disparity of a particular color and grey color with the same lightness. Hue angle (h°), indicating what color class samples belong to, is expressed in forms of degree, starting from red at 0°, to yellow at 90°, to green at 180°, and to blue at 270° (Pathare, et al., 2013). Numerical total color difference ΔE* indicates the overall color difference from the standard plate without dimension (Konica Minolta Sensing, Inc., 2007). The browning index (BI) is an important parameter when enzymatic or non-enzymatic browning takes place (Castañón, et al., 1999; Palou, et al., 1999). Chroma, hue angle, total color difference, browning index can be calculated using the following equations:

(1) Chroma: $C^* = \sqrt{(a^*)^2 + (b^*)^2}$

(2) Hue angle: $h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right)$

(3) Total colour difference: $\Delta E^* = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2}$

(4) Browning Index: $BI = 100 \times \left(\frac{X - 0.31}{0.17}\right)$, where $X = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*}$

The meaning of different level of ΔE* value was shown in **Table 12**, in accordance with CIE 94 method.

Table 12: Meaning of ΔE* value by CIE 94 method

ΔE* value	Meaning
0 - 1	A normally invisible difference
1 - 2	Very small difference, only obvious to a trained eye
2 - 3.5	Medium difference, also obvious to an untrained eye
3.5 - 5	An obvious difference
> 6	A very obvious difference

4.6 Total phenolics: Folin-Ciocalteu assay

The clear and intense yellow Folin-Ciocalteu Reagent (FCR) was first developed by Folin & Ciocalteu (1927) to quantitatively determine tyrosine (having phenolic OH-group) and tryptophane in proteins. Folin-Ciocalteu reagent were mainly prepared by sodium tungstate ($Na_2WO_4 \cdot 2H_2O$) and sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$), forming analogous yellow compounds without any green color. The reagent will turn to blue color when it is reduced by involved polyphenols. The blue intensity is proportional to phenolic contents; therefore, Folin-Ciocalteu reagent was used as a colorimetric method to measure total phenolic contents. Singleton & Rossi (1965) improved this method by establishing specific procedures and conditions for obtaining more reliable data, which were: (1) gallic acid as reference standard for polyphenols; (2) plot of reaction time and temperature for initial development of maximum color and onset of color fading based on different alkaline concentrations; (3) ratio of sample, FC reagent, and sodium carbohydrate; (4) monitoring optical density at 765 nm.

The Folin-Ciocalteu method used in this project was based on the method described by Singleton & Rossi (1965) and Sensoy, et al., (2006) with some modifications. The procedures were as follows: (1) The standard gallic acid samples with concentrations range of 0-400 (0, 25, 50, 100, 150, 200, 300, 400) μg / ml were prepared to develop the reference curve. (2) LHG drink samples were prepared with concentration of 5, 10 and 20 mg dried fruit powder per milliliter deionized water. (3) 200 μL of gallic acid or LHG drink sample, 3 mL DI water, and 200 μL Folin-Ciocalteu reagent were added into a test tube; fully mixed by votex. (4) After 1 min and before 8 min, 750 μL of 200 g / L

sodium carbonate (Na_2CO_3) solution were added into the test tube. (5) After 90 min at room temperature (23.5 °C ± 0.5 °C), 200 μL / well of each sample was pipetted into a Corning® 96 well clear polystyrene plate (Catalog No. 3370). The absorbance of the samples in the plate was measured at 725 nm using a BioTek Synergy™ HT spectrophotometer (Winooski, VT) (**Fig. 17**). For each sample, absorbance reading value was corrected by subtracting the blank reading, measured via replacing standard or sample by deionized water. The absorbance readings from samples were calculated by means of interpolation into gallic acid standard curve. The final results were expressed as mg GAE (Gallic Acid Equivalents) per 100 g of dried Luo Han Guo fruits ± standard deviation (SD) for triplicates.

Figure 17: BioTek Synergy™ HT spectrophotometer

In Folin-Ciocalteu assay, phenolic value will be overestimated in the presence of interfering compounds such as ascorbic acid (reducing compounds), aromatic amines (indoles, purines, guanine, xanthine, uric acid, etc.) (Singleton, et al., 1999), bi-sulfite, ferrous ion (Singleton & Rossi, 1965), and sugars (glucose, fructose, and maltose) (Magalhães, et al., 2010). Ascorbic acid has higher interfering effects than sulfite,

maltose and fructose. PVPP was used to avoid interference, since it only binds flavonoids and other polyphenols, leaving interfering compounds unadsorbed (Bridi, et al., 2014).

Based on the research of McMurrough, et al. (1995), Bridi, et al. (2014), and preliminary studies, 10% (w/v) insoluble adsorbent PVPP was added into aqueous LHG drink, mixed for 30 s by vortex, then shaken for 15 min by Fisher Scientific^{TM} Nutating Mixer. Supernatant was taken after being centrifuged for 8 min at 10000 g. The above PVPP-binding procedures were repeated for 3 cycles. Final supernatant was measured by Folin-Ciocalteu Assay to get the post-PVPP results. Pre-PVPP results were obtained by carrying out Folin-Ciocalteu Assay with LHG drink sample without any PVPP treatment. Most phenolics would be adsorbed to PVPP, leaving the inference compounds in the liquid part. The accurate polyphenolic contents were obtained by subtracting Folin-Ciocalteu results of post-PVPP samples from that of pre-PVPP samples.

4.7 Cellular antioxidant activity (CAA) assay

The cellular antioxidant activity assay used in this study was as per Wolfe & Liu (2007) with minor modifications. The details are as follows:

4.7.1 Preparation of chemicals, media and samples

A 1 mM stock solution of quercetin, as a standard of CAA, was freshly made in DMSO prior to use. Stock solution of 5 mM DCFH-DA in methanol and 200 mM ABAP in water was prepared and stored at -20 ℃. Treatment medium consisted of Williams'

Medium E (WME) supplemented with 2 mM L-glutamine and 10 mM Hepes. Processed LHG drink was filtered through vacuum filter with the filtrate stored at -60 ºC as samples.

4.7.2 Cell culture and cytotoxicity

HepG2 cells were grown in growth medium that consisted of EMEM supplemented with 10% FBS, and 50 μg/mL penicillin-streptomycin. HepG2 cells were maintained at 37 °C and 5% CO_2. Cells used in the study were between passages 5 and 10.

Cytotoxicity assay was carried out using CellTiter 96® AQ$_{ueous}$ One Solution Cell Proliferation reagent (Promega, Madison, WI), which contains tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS] and an electron coupling reagent phenazine ethosulfate (PES). MTS will be bioreduced by cells into a colored formazan product. This conversion is achieved by NADPH or NADH produced by metabolically active cells. This is a simple colorimetric assay that the quantity of formazan formed, via measuring absorbance at 490 nm, is directly proportional to the number of living cells in culture. Briefly, HepG2 cells were seeded at 6×10^4 / well into a 96 well-plate in 100 μL of growth medium, and incubated for 24 h at 37 ºC and 5% CO_2. The growth medium was removed and the cells were washed with 100 μL PBS. 100 μL of treatment medium, containing LHG drink, were then applied to the cells followed by incubation at 37 ºC and 5% CO_2 for 1 h. After incubation, 20 μL / well of CellTiter 96® AQueous One Solution Reagent was added into the 96-well plate without removing the treatment medium. The plate was incubated for 90 min at 37 ºC and 5% CO_2, and the absorbance was measured at

490 nm. The concentrations of LHG drink were considered cytotoxic when the absorbance reduction percentage exceeded 10% of corresponding wells compared to the control wells (only treatment medium and CellTiter 96® Reagent) (Progema, 2012).

4.7.3 Cellular antioxidant activity assay

HepG2 cells, counted by hemacytometer, were seeded into a 96 well-plate at a density of 6×10^4 /well in 100 µl of growth medium. The cells were only seeded into the inner wells of a 96 well-plate. The outer wells were filled with PBS to create a thermal mass, and to reduce temperature fluctuations and signal variations. The plates were incubated for 24 h at 37 °C. After that, the growth medium was removed and the cells were washed with 100 μL PBS. Triplicate wells were treated for 1 h with 100 µl / well of quercetin standards and Luo Han Guo samples made in treatment medium, containing 25 µM DCFH-DA. The cells were then washed with 100 μL PBS. Following that, 100 μL of HBSS containing 600 μM ABAP was applied to each well. The plate was immediately placed in a pre-heated (37 ℃) BioTek Synergy™ HT spectrophotometer (Winooski, Vermont), controlled by KC4™ Data Analysis Software for kinetic fluorescence measurement. The excitation filter of wavelength/slit width = 485 nm/20 nm and emission filter of wavelength/slit width = 545 nm/40 nm was used, with a sensitivity setting of 50. The fluorescence reading was taken every 5 min for 1 h. Triplicate wells of control and blank were also included. Control wells contained treatment medium with DCFH-DA and HBSS with ABAP. Blank wells contained treatment medium with DCFH-DA and HBSS without ABAP, the source of oxidants.

4.7.4 Quantification of CAA results

Initial reading was subtracted from all fluorescence readings of each curve. The net curve area is equal to curve area of samples and controls minus blank curve area. The curve area (A) was calculated using a trapezoidal method as follow:

$$A = \sum_{i=1}^{12} \frac{(f_n + f_{n+1}) \cdot \tau}{2}$$

where f_n is the fluorescence value at n^{th} reading number; and τ is the interval time period of readings in minutes, which is 5 minutes here. There are totally 13 fluorescence readings. The CAA unit was calculated using the formula as following:

$$CAA\ unit = 1 - \frac{net\ Area\ of\ Sample}{net\ Area\ of\ Control}$$

As CAA units of different concentrations of quercetin and Luo Han Guo samples obtained, the dose-response curve (or CAA unit vs. concentration plot) were generated. The dose-response curve was then converted to median effect plot of log (f_a/f_u) versus log (concentration). f_a is the area fraction affected (CAA unit); and f_u is the area fraction unaffected (1 - CAA unit). EC_{50} value is the concentration at which $f_a/f_u = 1$, $\log(f_a/f_u) = 0$ or CAA unit = 50 as calculated from the linear regression of the median effective plot.

EC_{50} values were converted to CAA unit of μmoles of quercetin equivalents (QE)/ 100 g dried Luo Han Guo fruit, derived by normalizing the EC_{50} of Luo Han Guo samples to the EC_{50} of quercetin as a reference. EC_{50} were stated as mean $\pm$ standard deviation (SD).

4.8 Mogroside V: High performance liquid chromatography (HPLC)

UltiMate 3000 HPLC (Thermo Scientific, USA) was used to measure mogroside V in LHG drink. Mogroside V standard was purchased from Shanghai TAUTO® Biotech Co., Ltd., China. The HPLC procedure was referred to Lu, et al., (2012). Briefly, the analysis was carried out on a ZORBAX SB-C18 column (4.6 mm $\times$ 250 mm, 5 μm; Agilent, USA). The mobile phase was 0.05% phosphoric acid in water (M) - acetonitrile (N) using a gradient program of 3%-13.5% (N) in 0-8 min, 13.5%-35% (N) in 8-35 min, 35% (N) in 35-45 min. The flow rate was set at 0.8 ml/min, and the wavelength of detection is 203 nm. LHG aqueous drink was filtered through 0.45 μm filters prior to HPLC analysis and the inject volume is 30 μL. Results were expressed as mg of mogroside V/100 g initial dried LHG.

4.8 Statistical analysis

All results were presented as mean $\pm$ SD based on triplicate performance. Statistical analysis was performed using SAS version 9.4 software (SAS Institute, Cary, NC). Analysis of variance and separation of means were performed using PROC GLM (General Linear Model). When effects were significant, multiple comparisons among means were carried out by using Tukey's HSD test. Data were considered significant when p value < 0.05.

5. RESULTS AND DISCUSSION

In this study, both hot air dried Luo Han Guo (HAD-LHG) and low temperature vacuum dried Luo Han Guo (LTVD-LHG) samples were procured from different venders to account for batch variation and establish any sample difference between drying methods with confidence. HAD-LHG samples were obtained from farmers' market in Guilin City, China; Naning Ningfu Trading Co., China; and Royal King® Herba Natural Products, Inc., and were symbolized by letters *A, B, C* respectively. LTVD-LHG samples were obtained from Guilin Zhenjin Luo Han Guo Co., China; ASGO® Guilin Ronghe Food Manufactory, China; and Longsheng county Long-Ji® Luo Han Guo Processing Manufactory, China, and were symbolized by letters *X, Y, Z* respectively. The analytical results were presented and discussed in this section.

5.1 Preliminary microbial equivalence studies

The pH values of LHG drink had been modified to 4.2 by L-malic acid in this study, hence the growth of spore-forming food pathogen would be inhibited in the acidic environment. Mold, yeast, and lactic bacteria became the major concerns. We utilized *Saccharomyces cerevisiae* yeast, a common spoilage microorganism in food, to examine if the conditions of HHPP treatment meet the FDA requirements. Any processing method was required to have the ability to achieve at least 5 log reduction of target microorganisms (US FDA, 2004). Pressures of 300 MPa - 600 MPa with pressure holding time varying from 2 min - 10 min were used to treat LHG drink cultured by *S. cerevisiae*. The log reduction numbers were shown in **Table 13**.

Results showed that more than 5 log reduction of *S. cerevisiae* was obtained by all HHPP conditions used in this study. Conditions of pressure equal to or higher than 400 MPa, with a pressure holding time equal to or longer than 4 min would achieve more than 6 log reduction of yeast. The results demonstrated that high pressure conditions of 580 MPa 15 min we used in the following experiments were sufficient to achieve acceptable microbiological quality of aqueous LHG drink.

Table 13: Log reduction of *Saccharomyces cerevisiae* cultured in LHG drink under

HHP treatment (n=2)

Log reduction of		Pressure			
S. cereviasiae		300 MPa	400 MPa	500 MPa	600 MPa
	2 min	6.0	5.2	5.4	> 6.1
Pressure	4 min	6.0	> 6.1	> 6.1	> 6.1
holding	6 min	> 6.1	> 6.1	> 6.1	> 6.1
time	8 min	> 6.1	> 6.1	> 6.1	> 6.1
	10 min	> 6.1			> 6.1

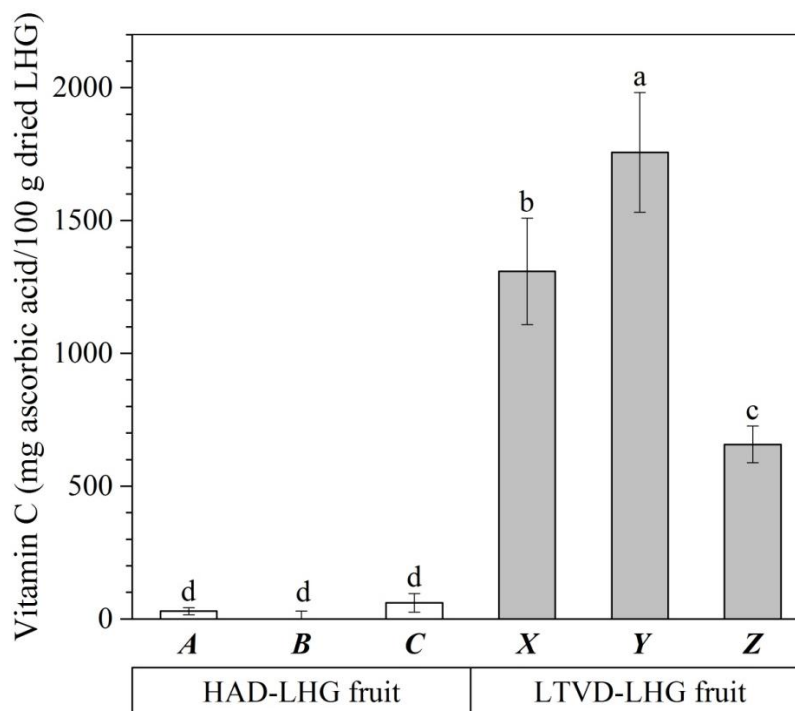## 5.2 Vitamin C content of Luo Han Guo fruit

Figure 18: Vitamin C content of dried LHG fruit

Vitamin C content of the 6 batches of LHG fruit were shown in **Fig. 18**. It was noticed that vitamin C contents of all LTVD-LHG fruit were greatly higher than that of HAD-LHG fruit. HAD-LHG had lower than 100 mg ascorbic acid per 100 g dried LHG, whereas LTVD-LHG had more than 600 mg ascorbic acid per 100 g dried LHG. Sample *Y* showed very high vitamin C content, reached as high as 2000 mg ascorbic acid per 100 g dried LHG. Fresh LHG is rich in vitamin C, with concentrations of (339-461) mg/100 g in fresh fruit (Li, et al., 2014). The vitamin C content of kiwifruit, which was widely recognized as high vitamin C fruit, varied from (26 ± 3) mg /100 g fresh weight to (206 ± 20) mg/100 g fresh weight of different cultivars (Nishiyama, et al., 2004). When converting these data to dry basis without any possible loss, the vitamin C of kiwifruit

varied from (156 ± 20) mg /100 g dry basis to (1475 ± 142) mg /100 g dry basis. Therefore, LHG fruit had even higher vitamin C content than kiwifruit, and low temperature vacuum drying better preserved vitamin C than hot air drying of LHG. This could be explained by the fact that heat-sensitive compounds such as vitamin C would be damaged during heat drying.

Another noticed thing was that although *X, Y, Z* samples were dried by similar method, the batch to batch variation was very high among LTVD-LHG samples. *Y* samples showed much higher level of vitamin C content than *X* and *Z* samples. The differences may due to different vitamin C content of fresh LHG fruit, disparity of drying equipment, or variable process conditions used by different LTVD-LHG suppliers.
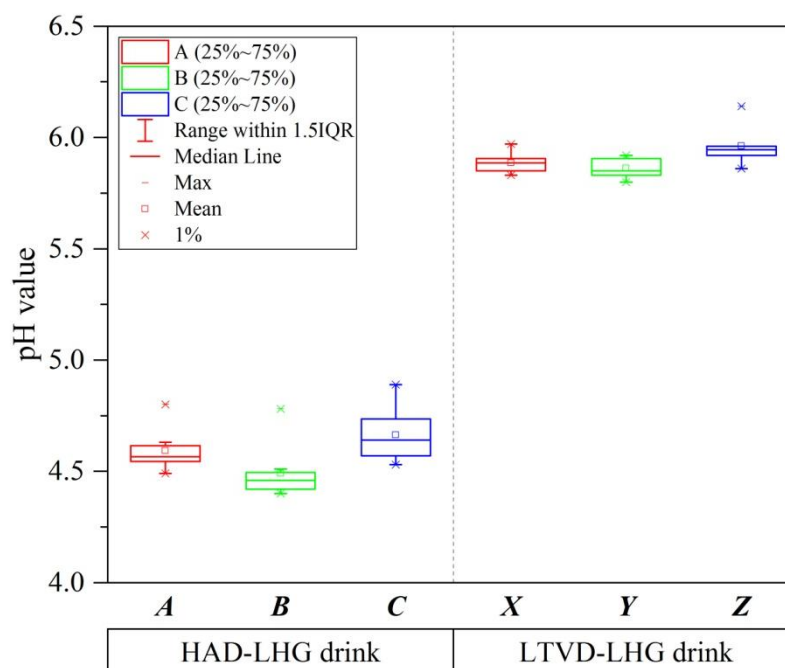
5.3 pH of Luo Han Guo drink

Figure 19: The original pH of HAD-LHG drink and LTVD-LHG drink.

As shown in **Fig. 19**, the pH values of HAD-LHG were between 4.4 ~ 4.9, while the pH values of LTVD-LHG were significantly higher, that were between 5.6 ~ 6.1. This indicated that drying methods of LHG fruit affected the final pH of LHG drink. Hot air drying resulted in more acidic compounds in LHG as compared to low temperature vacuum drying.

5.4 Color of Luo Han Guo drink

Table 14 showed the pictures of 10 mL LHG drink in the same circular glass dish with inner diameter of 57 mm and outer diameter of 61 mm. For each batch of LHG drink, the color changes were not significant among control groups, thermal pasteurized, or high pressure processed drink. However, the images illustrated that the color between HAD-LHG drink and LTVD-LHG drink samples was obviously different by naked eyes. The color of aqueous drink from HAD-LHG was dark brown, while that from LTVD-LHG was light yellow and was much brighter than HAD-LHG drink. Color difference of LHG drink within the same fruit drying method could also be visually noticed.

The browning phenomenon of HAD-LHG drink could result from caramelization and Maillard reaction during 5~7 days of traditional heat drying of LHG. While low temperature-vacuum drying below 50 ℃ would rarely trigger these reactions. Therefore, LTVD-LHG drink would retain more original color of fresh LHG.

Table 14: Pictures of 10 mL LHG drink in a glass disc (Φ_{in} = 57 mm)

	Control	HHPP	Thermal pasteurization
HAD-LHG drink *A*			
HAD-LHG drink *B*			
HAD-LHG drink *C*			
LTVD-LHG drink *X*			
LTVD-LHG drink *Y*			
LTVD-LHG drink *Z*			

Color characteristics L*, a*, b* for LHG drink were summarized in **Table 15**. Those parameters were generally not affected by either thermal pasteurization or high pressure pasteurization, but affected by sample variations based on two-way ANOVA statistical method ($p < 0.05$) followed by Tukey significant difference test. The effect of fruit drying methods on the color of LHG drink was significantly different. Lightness (L*) values of HAD-LHG drink (L*: 43 ~ 56) were much lower than that of LTVD-LHG drink (L*: 90 ~ 94). HAD-LHG drink had positive a* values (a*: 26 ~ 32) indicating red color, while LTVD-LHG drink had small negative a* (a*: -2.5 ~ -0.5)indicating light green color. HAD-LHG drink had significantly higher positive b* values (34 ~ 51) than LTVD-LHG drink (b*: 13 ~ 26), which means the yellow indices of HAD-LHG drink were much higher than that of LTVD-LHG drink.

Table 15: Color parameters L*, a*, b* of different processed LHG drink

Samples		Color parameters of 10 mL LHG drink in a glass dish		
		L*	a*	b*
Distilled water		96.82 ± 0.14 a	0.33 ± 0.02 e	2.59 ± 0.01 g
HAD-LHG drink (**A**)	Control	53.04 ± 0.16 de	27.54 ± 0.22 b	48.22 ± 0.43 a
	HHPP	53.65 ± 0.11 de	27.29 ± 0.25 b	48.81 ± 0.08 a
	Thermal	54.75 ± 0.49 de	26.44 ± 0.35 bc	50.17 ± 0.69 a
HAD-LHG drink (**B**)	Control	45.78 ± 1.00 f	30.66 ± 0.24 a	38.04 ± 1.47 b
	HHPP	45.28 ± 0.50 f	31.44 ± 0.21 a	37.40 ± 0.81 b
	Thermal	44.31 ± 0.67 f	30.45 ± 0.75 a	35.49 ± 1.25 b
HAD-LHG drink (**C**)	Control	52.91 ± 1.59 e	26.07 ± 0.97 cd	46.99 ± 1.76 a
	HHPP	52.77 ± 0.45 e	26.65 ± 0.14 bc	47.12 ± 0.61 a
	Thermal	55.37 ± 0.60 d	25.06 ± 0.22 d	49.89 ± 0.76 a
LTVD-LHG drink (**X**)	Control	90.89 ± 0.92 c	-1.39 ± 0.10 f	18.71 ± 0.82 de
	HHPP	93.04 ± 0.23 bc	-1.53 ± 0.03 f	16.69 ± 0.10 e
	Thermal	92.05 ± 0.09 bc	-1.59 ± 0.04 f	18.16 ± 0.16 de
LTVD-LHG drink (**Y**)	Control	91.88 ± 1.02 bc	-1.19 ± 0.30 f	23.43 ± 2.28 c
	HHPP	92.86 ± 0.97 bc	-1.50 ± 0.90 f	21.17 ± 1.32 cd
	Thermal	91.39 ± 0.55 bc	-1.31 ± 0.20 f	24.15 ± 1.32 c
LTVD-LHG drink (**Z**)	Control	92.25 ± 1.22 bc	-1.02 ± 0.15 f	16.05 ± 1.41 ef
	HHPP	93.44 ± 0.54 b	-1.15 ± 0.34 f	14.32 ± 1.12 f
	Thermal	92.26 ± 0.91 bc	-1.19 ± 0.15 f	16.10 ± 1.40 ef

Different lowercase letters within a column indicated significant differences ($p < 0.05$)

Although both drying methods and sample variables significantly affected color parameters L*, a*, b*, chroma, hue, and browning index of LHG drink, F ratios of drying methods were obviously higher than F ratios of sample variables (statistical analysis were shown in Appendix II). Therefore, drying methods were proved to highly affect the final color of LHG drink.

Drink processing methods showed insignificant effects on L*, hue, browning index or total color difference. It had significant effects on a*, b*, and chroma. But F ratios of pasteurization methods on a*, b* were clearly smaller than F ratios of sample variables. Taken together, drying methods but not drink processing methods were the critical factor to influence final color parameters of LHG drink.

Table 16: Chroma (C*), hue angle (h), and browning index (BI) of LHG drink

Samples		Color parameters of LHG drink			
		C*	h (°)	BI	ΔE*
Distilled water		2.61 ± 0.01 g	82.61 ± 0.27 b	2.90 ± 0.03 k	-
HAD-LHG drink (*A*)	Control	55.53 ± 0.31 a	60.27 ± 0.38 c	207.30 ± 1.67 ab	-
	HHPP	55.93 ± 0.06 a	60.79 ± 0.26 c	206.93 ± 0.42 abc	0.90 ± 0.19 a
	Thermal	56.71 ± 0.45 a	62.21 ± 0.63 c	207.62 ± 0.97 a	2.82 ± 0.90 a
HAD-LHG drink (*B*)	Control	48.86 ± 1.18 b	51.11 ± 1.06 d	191.01 ± 3.35 e	-
	HHPP	48.86 ± 0.51 b	49.94 ± 0.78 d	190.95 ± 2.09 e	1.25 ± 0.72 a
	Thermal	46.77 ± 1.31 b	49.37 ± 0.79 d	183.41 ± 4.67 f	3.01 ± 1.45 a
HAD-LHG drink (*C*)	Control	53.76 ± 1.08 a	60.96 ± 1.79 c	198.65 ± 0.92 d	-
	HHPP	54.14 ± 0.47 a	60.51 ± 0.45 c	200.97 ± 0.95 bcd	0.85 ± 0.25 a
	Thermal	55.83 ± 0.62 a	63.32 ± 0.51 c	200.54 ± 1.21 cd	3.94 ± 0.98 a
LTVD-LHG drink (*X*)	Control	18.76 ± 0.81 de	94.38 ± 0.53 a	21.35 ± 1.35 hij	-
	HHPP	16.76 ± 0.10 ef	95.24 ± 0.14 a	18.05 ± 0.19 ij	2.95 ± 0.22 a
	Thermal	18.22 ± 0.17 de	95.00 ± 0.07 a	20.13 ± 0.20 ij	1.30 ± 0.13 a
LTVD-LHG drink (*Y*)	Control	23.47 ± 2.26 c	92.97 ± 1.05 a	27.79 ± 3.76 gh	-
	HHPP	21.23 ± 1.26 cd	94.15 ± 2.58 a	24.05 ± 2.84 ghi	2.68 ± 1.41 a
	Thermal	24.18 ± 1.31 c	93.13 ± 0.64 a	28.84 ± 2.23 g	1.38 ± 0.62 a
LTVD-LHG drink (*Z*)	Control	16.09 ± 1.41 ef	93.64 ± 0.64 a	17.86 ± 2.05 ij	-
	HHPP	14.37 ± 1.10 f	94.67 ± 1.71 a	15.33 ± 1.73 j	2.13 ± 1.23 a
	Thermal	16.14 ± 1.39 ef	94.24 ± 0.64 a	17.78 ± 2.00 ij	1.24 ± 0.73 a

Different lowercase letters within a column indicated significant differences (p < 0.05)

As shown in **Fig. 20**, the browning index ($BI = 100 \times \left(\frac{X-0.31}{0.17}\right)$, where $X = \frac{a^*+1.75L^*}{5.645L^*+a^*-3.012b^*}$) of HAD-LHG drink was much higher than that of LTVD-LHG drink. Six batches of LHG samples were significantly different from each other. Batch to batch variation was much higher than drink processing method, which had insignificant ($p > 0.05$) effects on BI of LHG drink.
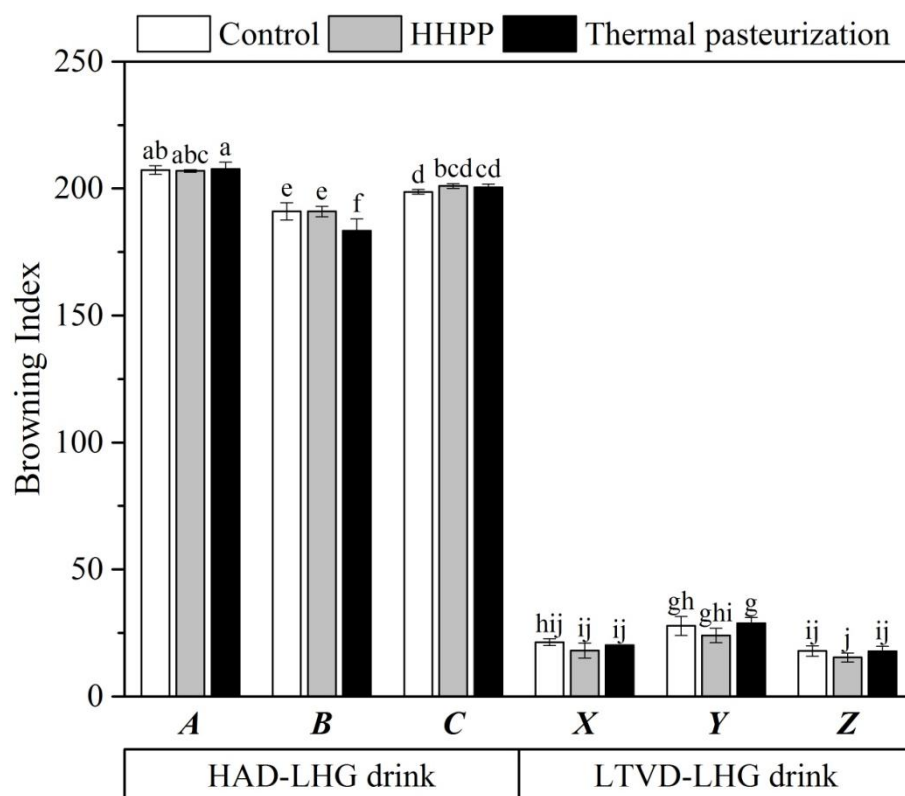
Figure 20: Browning indices of LHG drink (mean ± SD, n = 3)

5.5 Total phenolics of Luo Han Guo drink

Total phenolic content (TCP) of LHG drink was determined by means of colorimetric Folin-Ciocalteu method, with gallic acid as reference standard and 725 nm as its absorbance wavelength. Values for TPC of LHG drink were expressed as mg Gallic Acid Equivalent (GAE) / 100 g dried fruit. **Figure 21** shows the standard curve for gallic acid used to calculate the results in this experiment. Data of standard curve were the means of four duplicates ± standard deviation.
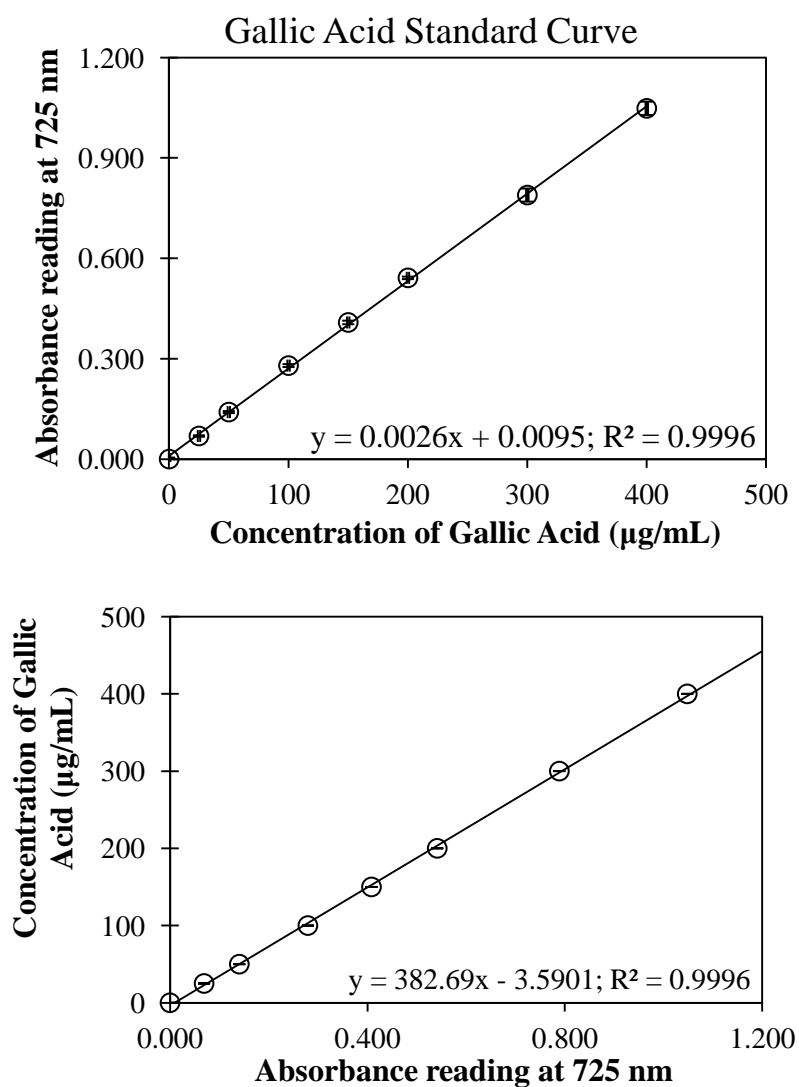
Figure 21: Standard curve of gallic acid in Folin-Ciocalteu assay.

Table 17: Total phenolic content of different processed LHG drink

Samples		Total phenolic results (mg GAE / 100 g dried fruit)		
		pre-PVPP result	post-PVPP result	Corrected TP result
HAD-LHG drink (*A*)	Control	1767 ± 88 d	1267 ± 81 e	500 ± 99 a
	HHPP	2071 ± 147 ab	1508 ± 51 a	563 ± 131 a
	Thermal	2089 ± 125 a	1438 ± 36 bc	591 ± 104 a
HAD-LHG drink (*B*)	Control	1932 ± 44 c	1437 ± 41 bc	496 ± 26 a
	HHPP	1932 ± 47 c	1398 ± 53 c	534 ± 31 a
	Thermal	2024 ± 69 b	1467 ± 39 ab	558 ± 48 a
HAD-LHG drink (*C*)	Control	1686 ± 40 e	1285 ± 30 e	401 ± 56 b
	HHPP	1726 ± 44 de	1340 ± 53 d	386 ± 34 b
	Thermal	1737 ± 50 de	1336 ± 35 d	401 ± 34 b
LTVD-LHG drink (*X*)	Control	655 ± 18 ij	510 ± 15 ij	145 ± 29 cd
	HHPP	630 ± 39 j	509 ± 19 ij	120 ± 30 cd
	Thermal	708 ± 41 i	546 ± 12 i	161 ± 39 c
LTVD-LHG drink (*Y*)	Control	878 ± 23 h	771 ± 21 h	107 ± 27 cd
	HHPP	952 ± 17 g	864 ± 21 g	88 ± 28 d
	Thermal	1059 ± 31 f	941 ± 15 f	118 ± 39 cd
LTVD-LHG drink (*Z*)	Control	634 ± 27 j	490 ± 16 j	144 ± 25 cd
	HHPP	659 ± 38 ij	498 ± 17 ij	161 ± 26 c
	Thermal	708 ± 35 i	532 ± 18 ij	176 ± 23 c

Different lowercase letters within a column indicated significant differences ($p < 0.05$)

Polyvinylpolypyrrolidone (PVPP), a highly crosslinked version of polyvinylpyrrolidone (PVP), is an insoluble polymeric adsorbent of phenolics. PVPP is often utilized to adsorb low aqueous phenolic compounds from beverages in order to reduce haze, precipitates, and off-flavors, as well as to improve the stability of products in wine, beer, and juices industry (Folch-Cano, Olea-Azar, & Speisky, 2013).

PVPP-phenolics binding is also one of the most commonly used methods to offset the overestimation of phenolic content resulting from reactions between Folin-Ciocalteu reagent and non-phenolic compounds such as ascorbic acid, sugars, and proteins. Durán-Lara, et al., (2015) indicate that adsorption equilibrium between phenolics and PVPP would be reached before 15 min. The adsorptivity between PVPP and phenolic compounds can be explained by adsorption percentage (AP). For the first cycle of adding PVPP, adsorption percentages of polyphenols are as follows: AP (all flavonoids) $\geq$ 90 %; AP (resveratrol) $\geq$ 90 %; AP (hydroxycinnamic acids) $\geq$ 70 %; AP (hydroxybenzoic acid) = 53 %. Complete removal of all polyphenols is impossible. However, most phenolic compounds can achieve AP $\geq$ 90 % after three consecutive cycles of PVPP addition (Bridi, et al., 2014). PVPP-Polyphenol complexes are driven by three forces: hydrophobic interaction between phenolic and pyrrolidinone rings, H bonds between hydroxyl functions and CO-N linkages, and van der Waals bonds with contact surfaces. (Laborde, et al., 2006).
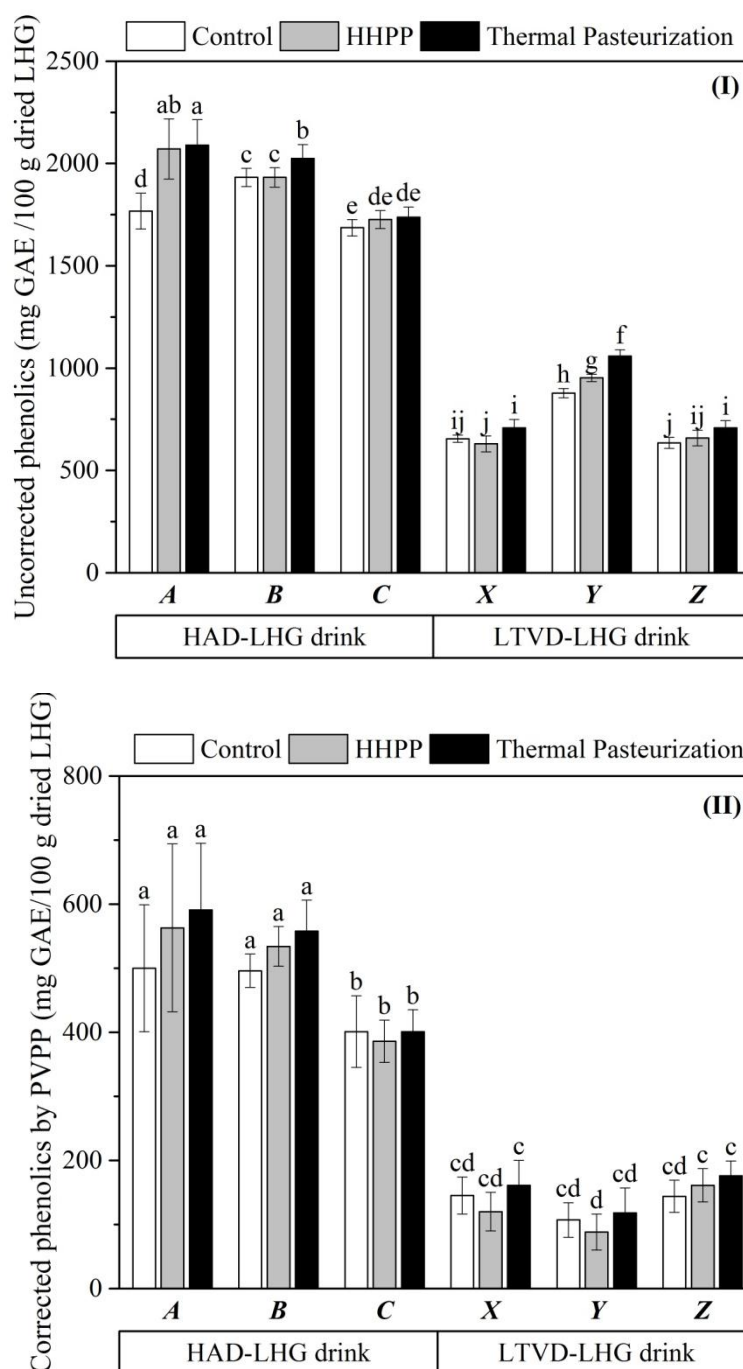
Figure 22: Uncorrected total phenolic content (I) and corrected total phenolic content by PVPP-assisted Folin-Ciocalteu assay (II) of control, HHP processed, and thermal pasteurized LHG drink made from HAD-LHG and LTVD-LHG. Statistically significant differences (p < 0.05) were indicated by lowercase letter.

Values for total phenolic content (TPC) of LHG drink were expressed as mg Gallic Acid Equivalent (GAE) /100 g dried fruit. As shown in **Table 17** and **Fig. 22**, uncorrected results of total phenolic content without PVPP-binding was significantly higher than that of corrected TPC by PVPP-binding. For HAD-LHG drink, uncorrected TPC values of (1600 - 2300) mg GAE/100 g dried LHG, were 3-5 times higher than corrected TPC values of (350 - 800) mg GAE/100 g dried LHG. For LTVD-LHG drink, uncorrected TPC values of (550 - 1100) mg GAE/100 g dried LHG, were 4-10 times higher than corrected TPC values (60 - 200) mg GAE/100 g dried LHG. This demonstrated that there was a large portion of interfering compounds of Folin-Ciocalteu assay present in LHG drink, resulting in a significant overestimation of total phenolics. Ascorbic acid was one of the interfering compounds (Bridi, et al., 2014). This could be illustrated by the comparatively high uncorrected TPC and comparatively low corrected TPC of *Y* LHG drink, which had highest ascorbic acid content among the 6 batches of LHG (**Fig. 18**).

For uncorrected TPC results, thermal pasteurization significantly increased most total phenolic content of LHG drink comparing to the control groups. However, neither thermal pasteurization nor HHPP showed any significant effects on the corrected TPC values. This may be explained as the interfering compounds of Folin-Ciocalteu assay would be increased by thermal pasteurization of LHG drink, while phenolic compounds would rarely be affected. In summary, HHPP or thermal pasteurization had little effects on total phenolic content of LHG drink, but fake increment of total phenolic content under thermal pasteurization might be reported if no PVPP were used for Folin-Ciocalteu method.

Drying methods of LHG fruit affected both corrected and uncorrected TPC of LHG drink. HAD-LHG drink showed greatly higher total phenolics than LTVD-LHG drink. This meant that hot air drying increased the total phenolic content in LHG. The result was in agreement with Que, et al., (2008) and López, et al., (2010), who reported an increase TPC of dried pumpkin flours and blueberries when drying temperature was increased. Our results might be explained by that some chemical reactions were triggered to form more phenolic substances during hot air drying.

5.6 Cellular antioxidant activity of Luo Han Guo drink
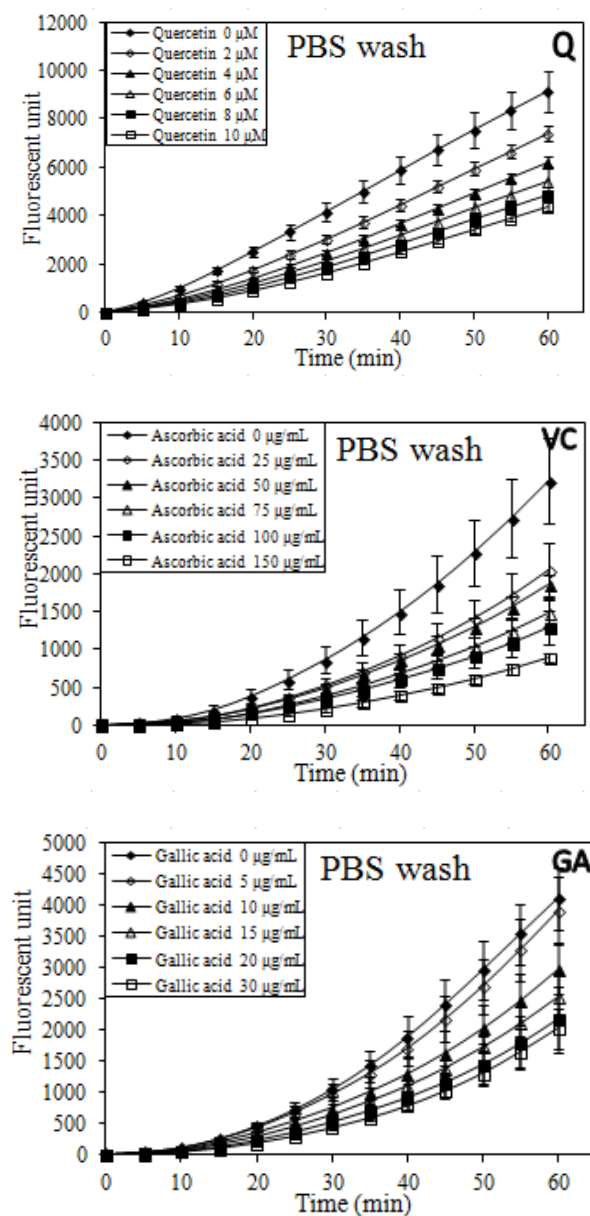
Figure 23: Peroxyl radical-induced oxidation of DCFH to DCF in HepG2 cells and the inhibition of oxidation by quercetin (Q), ascorbic acid (VC), and gallic acid (GA) over time in CAA assay. Each graph was generated from single experiment (mean ± SD, n=3).
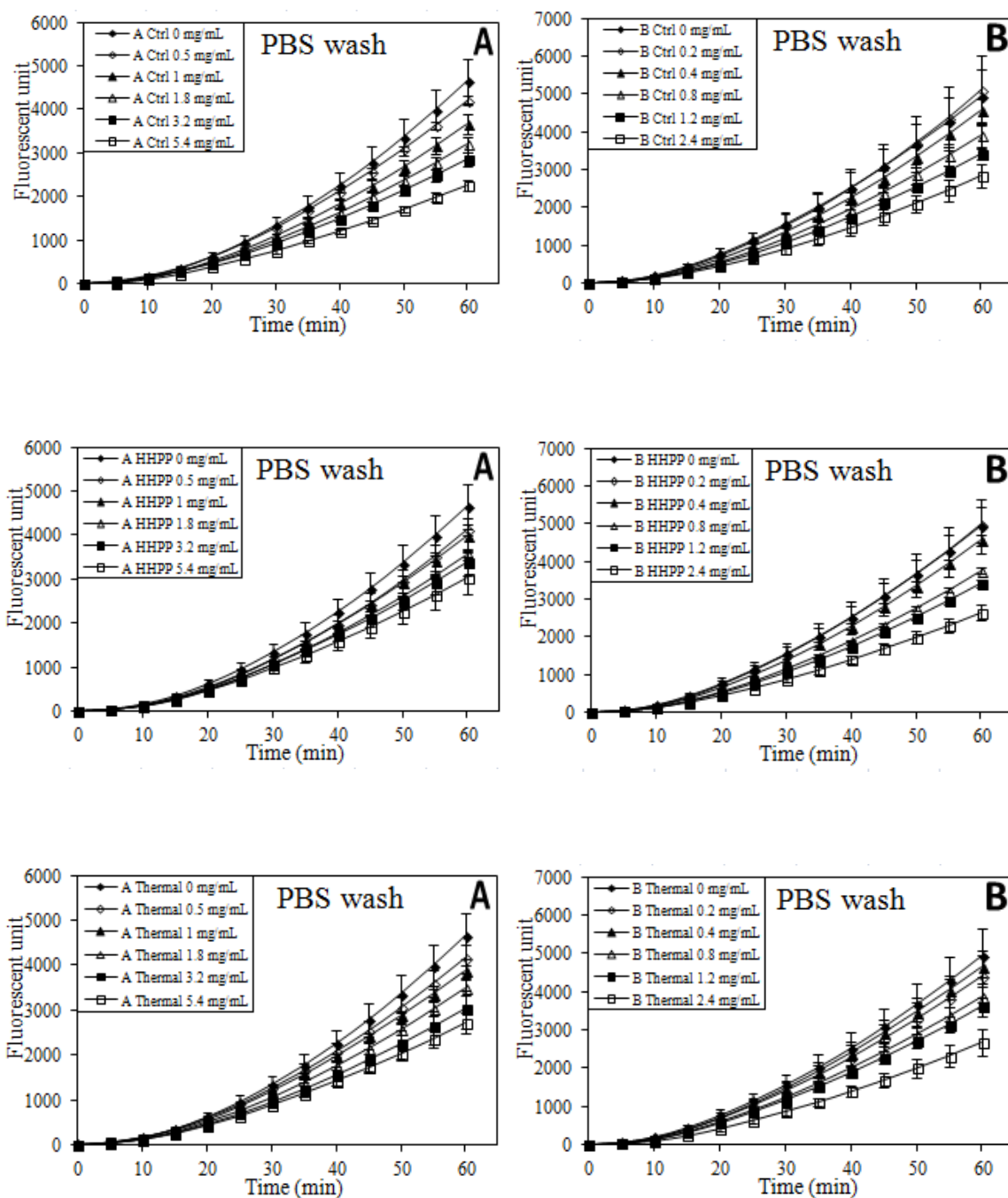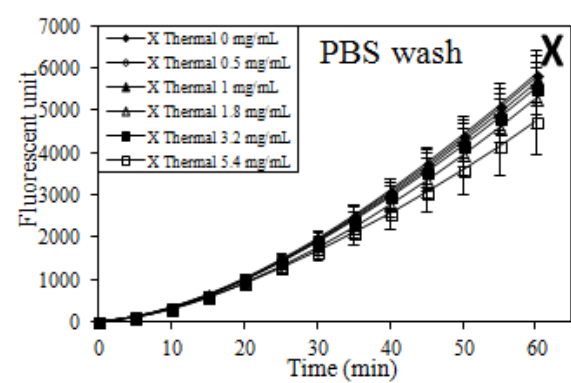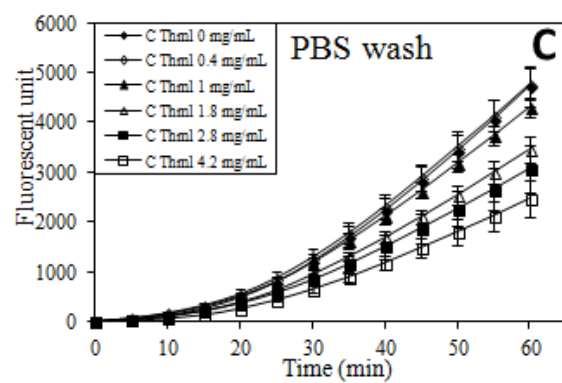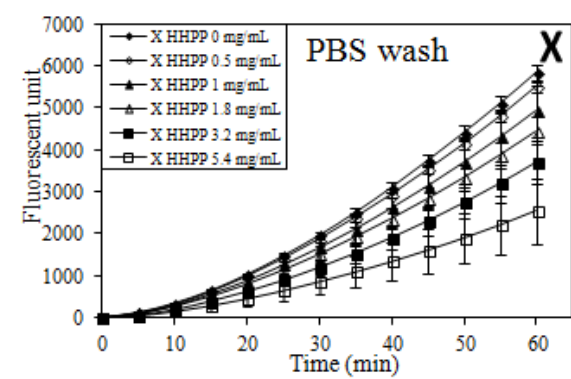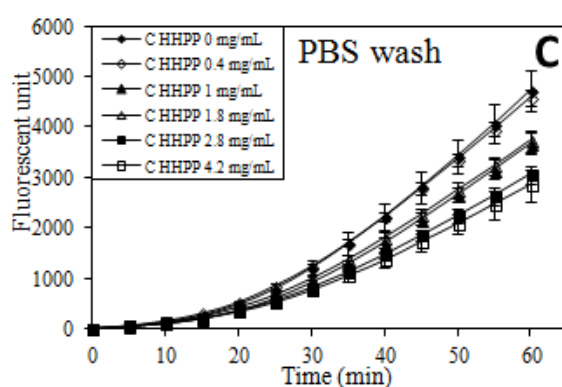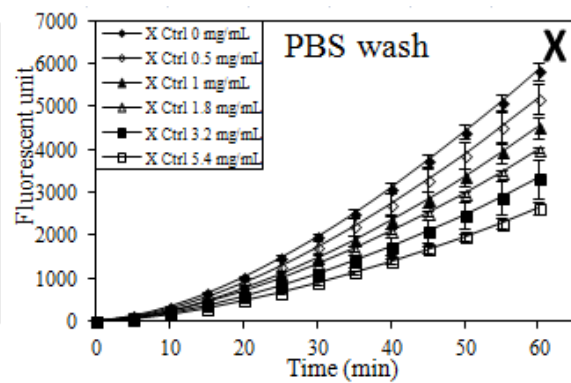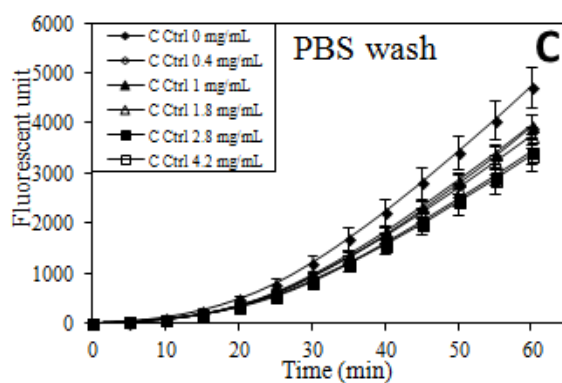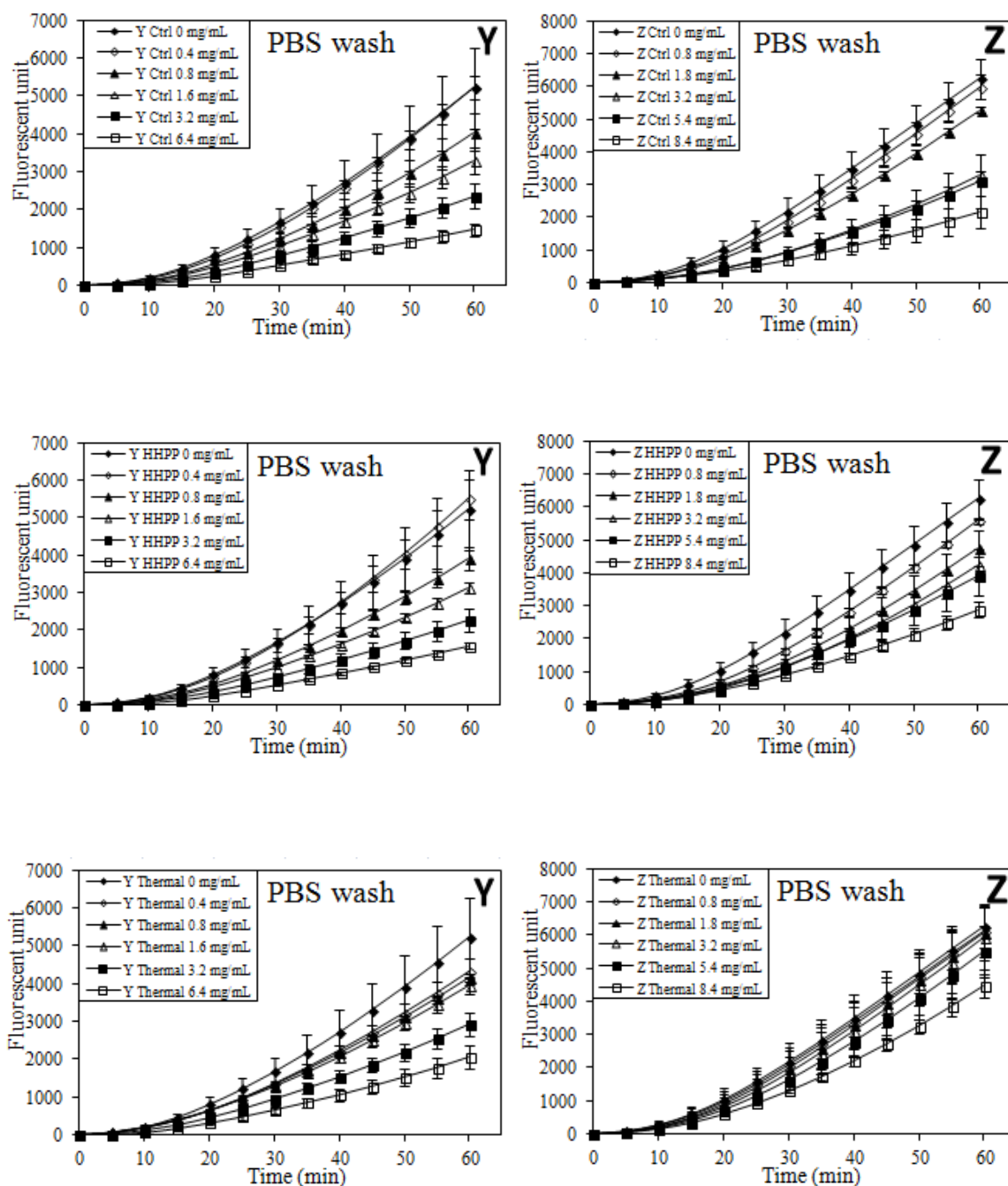
Figure 24: Peroxyl radical-induced oxidation of DCFH to DCF in HepG2 cells and the inhibition of oxidation by differently processed HAD-LHG drink (Samples *A*, *B*, *C*) and LTVD-LHG drink (Samples *X*, *Y*, *Z*) over time in CAA assay. Each graph was generated from single experiment (mean ± SD, n=3).
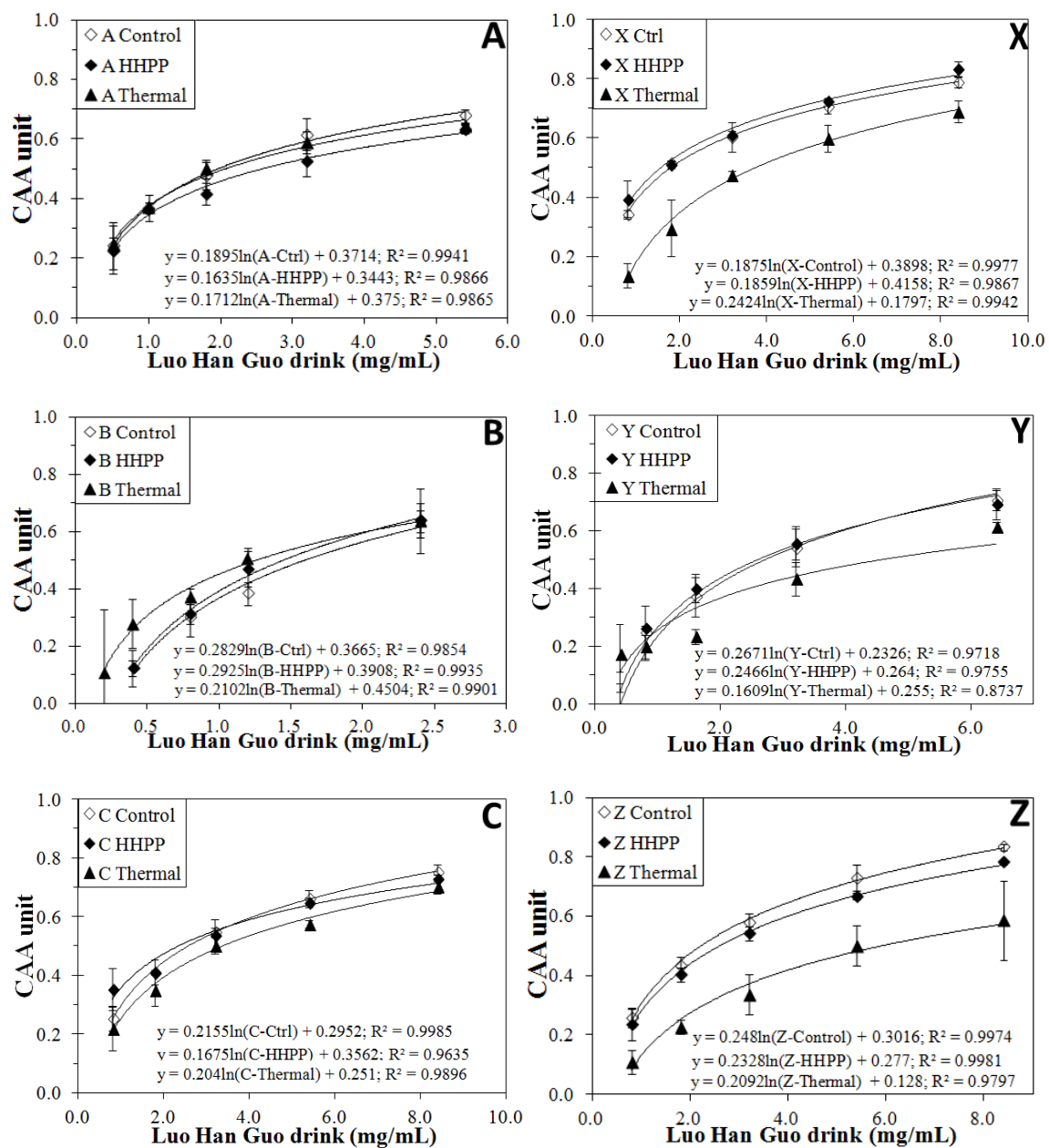
Figure 25: Dose-response curves for inhibition of peroxyl radical-induced oxidation of DCFH by unprocessed control, HHP processed, and thermal pasteurized HAD-LHG drink (Samples *A, B, C*) and LTVD-LHG drink (Samples *X, Y, Z*) in CAA assay. Each curve shown in graphs was from a single experiment (mean ± SD, n=3).
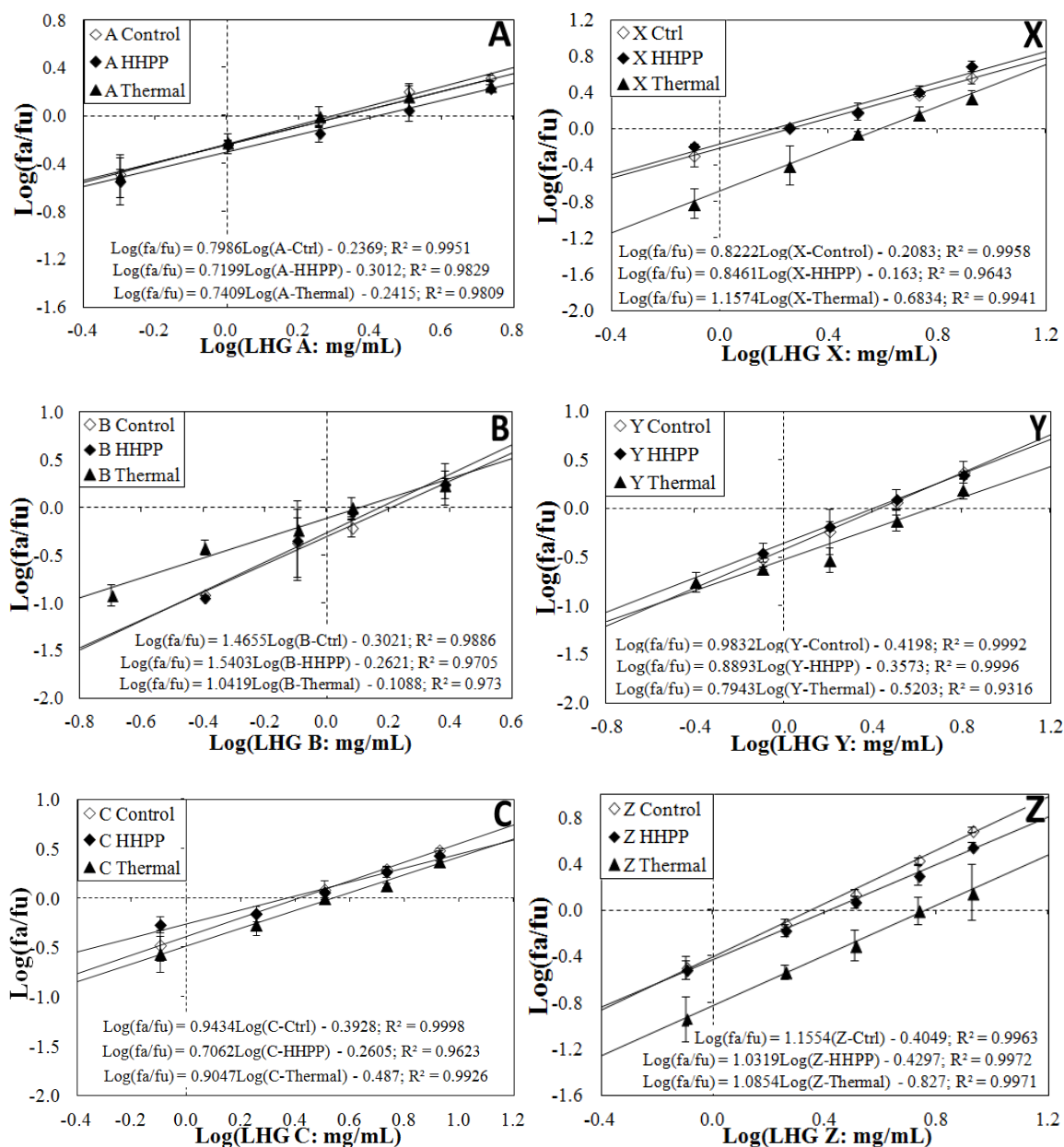
Figure 26: Median effect plots for inhibition of peroxyl radical-induced oxidation of DCFH by unprocessed control, HHP processed, and thermal pasteurized HAD-LHG drink (Samples *A, B, C*) and LTVD-LHG drink (Samples *X, Y, Z*) in CAA assay. Each curve shown in graphs was from a single experiment (mean ± SD, n=3).

Table 18: Cellular antioxidant activities of standard and LHG drinks expressed as

EC_{50} and CAA values (mean ± SD, n = 3)

Samples		EC_{50} (mg/mL)	CAA (μmol QE/100 g dried LHG)	Cytotoxicity: CC_{50} (mg/mL)
Quercetin standard		$7.23 \pm 0.12\,\mu$M	-	$> 10\,\mu$M
HAD-LHG drink (*A*)	Control	1.84 ± 0.15 abc	394 ± 33 abc	> 20
	HHPP	2.03 ± 0.55 abc	372 ± 90 abc	> 20
	Thermal	2.05 ± 0.52 abc	369 ± 103 abc	> 20
HAD-LHG drink (*B*)	Control	2.55 ± 1.17 abc	323 ± 131 abc	> 20
	HHPP	2.50 ± 0.99 abc	330 ± 157 abc	> 20
	Thermal	2.27 ± 1.16 abc	378 ± 183 abc	> 20
HAD-LHG drink (*C*)	Control	3.09 ± 1.21 abc	256 ± 86 abc	> 20
	HHPP	2.71 ± 0.92 abc	285 ± 84 abc	> 20
	Thermal	4.02 ± 1.47 abc	195 ± 62 abc	> 20
LTVD-LHG drink (*X*)	Control	1.54 ± 0.23 ab	478 ± 70 ab	> 20
	HHPP	1.90 ± 0.36 abc	390 ± 73 abc	> 20
	Thermal	4.78 ± 1.43 bc	159 ± 41 bc	> 20
LTVD-LHG drink (*Y*)	Control	1.68 ± 0.90 a	502 ± 205 a	> 20
	HHPP	2.22 ± 0.63 abc	349 ± 118 abc	> 20
	Thermal	6.64 ± 2.24 c	118 ± 40 c	> 20
LTVD-LHG drink (*Z*)	Control	2.52 ± 1.00 abc	317 ± 114 abc	> 20
	HHPP	2.95 ± 1.27 abc	276 ± 109 abc	> 20
	Thermal	10.93 ± 6.14 c	81 ± 42 c	> 20

Different lowercase letters within a column indicated significant differences (p < 0.05)

The CAA values in **Table 18** were expressed as *μ*mol quercetin equivalent (QE) per 100 g dried LHG. The average CAA values of LHG were around 300 *μ*mol QE/100 g dried LHG. Assuming all dried LHG contained 15% moisture according to **Table 5**, the CAA value could be converted to around 45 *μ*mol QE/100 g fresh LHG, which is similar to CAA values of strawberry (Wolfe, et al., 2008).

 For HAD-LHG drink, all high pressure processed, thermal pasteurized, and unprocessed ones had similar CAA values without any significantly difference. But for LTVD-LHG drink, samples after thermal pasteurization showed lower CAA level than levels of HHP processed and unprocessed control LTVD-LHG drink. This indicated that the antioxidant compounds in HAD-LHG would be more stable under heat treatment, while the antioxidant compounds in LTVD-LHG would be degraded after thermal processing.

Both pure gallic acid and ascorbic acid had high cellular antioxidant activity in accordance with the research of Wolfe and Liu (2007). Hence, total phenolics and vitamin C in LHG drink would both contribute to the CAA values. Total phenolics were comparatively heat stable from the summary of Rawson, *et al*. (2011), and vitamin C was widely accepted as a heat sensitive compound. The results of **Section 5.2** and **Section 5.5** showed that HAD-LHG had higher total phenolic content and lower vitamin C content than LTVD-LHG. Therefore, it is highly possible that the CAA values of HAD-LHG drink were mainly contributed by total phenolics, which were relatively

stable after thermal pasteurization, while the CAA values of LTVD-LHG drink were mainly contributed by vitamin C, which was unstable during thermal pasteurization.

5.7 Mogroside V content of Luo Han Guo drink

Mogroside V is a main component as well as a non-calorie sweetener in LHG products. It was determined by HPLC method. **Figure 27** illustrated the HPLC chromatogram of mogroside V standard solution, whose retention time appeared at 27.30 ± 0.05 min (peak *30*). The retention time for L-malic acid was 4.55 ± 0.02 min (peak *4*), which was added to modify pH and not innately present in LHG products.
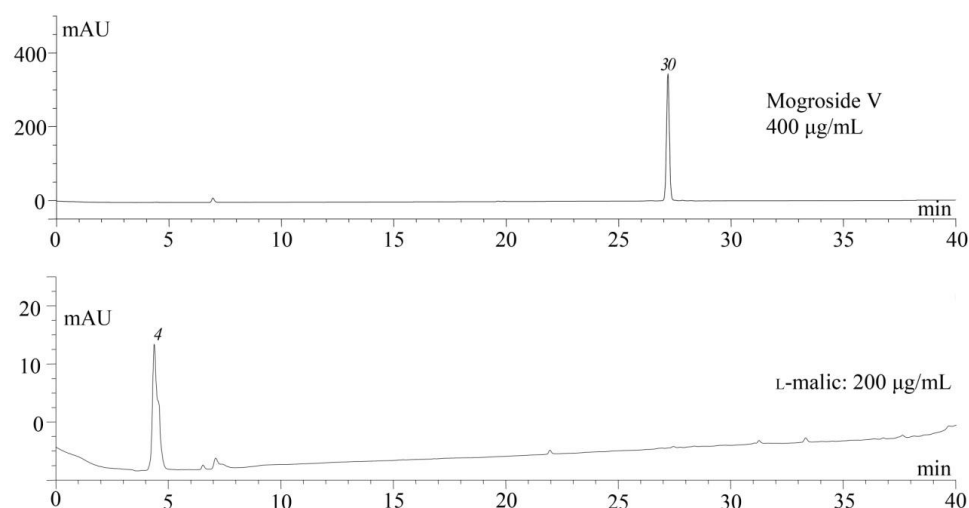
Figure 27: HPLC chromatograms of mogroside V standard and L-malic acid.

Figure 28 illustrated the HPLC chromatograms of 15 mg/mL unprocessed, HHP treated, and thermal pasteurized HAD-LHG and LTVD-LHG drink. The retention time of the *1-33* characteristic peaks was 2.91, 3.31, 3.68, 4.55, 5.36, 5.86, 6.11, 6.71, 7.03, 8.71, 9.31, 9.52, 9.79, 10.69, 10.92, 11.87, 12.6, 12.95, 13.50, 14.23, 14.85, 16.08, 16.68, 20.75, 21.88, 22.96, 23.93, 25.34, 26.34, 27.30, 29.77, 30.95, 33.28 min respectively. In

accordance with our results and the research of Lu and others (2012), peaks of *29, 31, 32, 33* in HPLC chromatograms were probably compounds of 11-oxomogroside V, mogroside IVA, mogroside III, and mogroside IIE.

All chromatograms of LTVD-LHG drink showed obviously more and higher peaks than HAD-LHG drink between retention time of 6 - 15 min (peaks *7-19*). When comparing to each other, HAD-LHG drink had some consistent specific peaks like peaks *5, 6, 22, 23*; and LTVD-LHG drink also displayed some consistent specific peaks like peaks *7-16, 25, 27*. The HPLC chromatograms of unprocessed, HHP treated, and thermally pasteurized HAD-LHG drink were similar to each other. However, unprocessed, HHP treated, and thermally pasteurized LTVD-LHG drink showed noticeable differences. HHP treated LTVD-LHG drink had unique peak *17*, *19*, and *28* that were not presented in unprocessed or thermally pasteurized LTVD-LHG drink. Thermally pasteurized LTVD-LHG drink displayed plenty of small peaks that were not presented in chromatograms of either control or HHP treated LTVD-LHG drink.

From the results gained, it was reasonably suggested that LTVD-LHG drink had more compounds, and higher content of compounds than HAD-LHG drink. Hot air drying could destroy most of the original compounds and also trigger the formation of several new compounds in LHG. Drink processing methods had more effects on LTVD-LHG drink rather than HAD-LHG drink. HHP processing increased the amount of one or two compounds, and thermal pasteurization might also form small amounts of new compounds in LTVD-LHG drink.
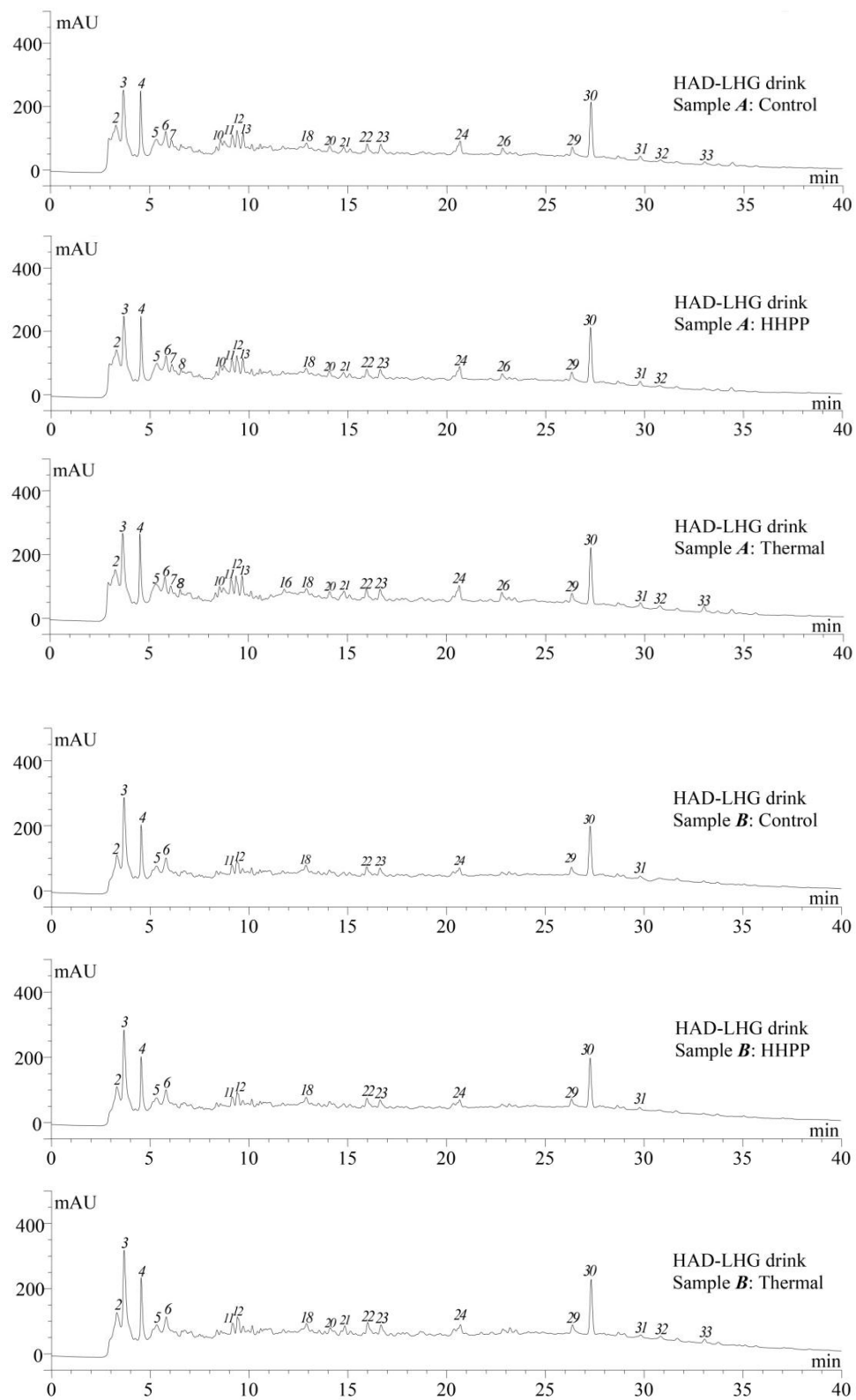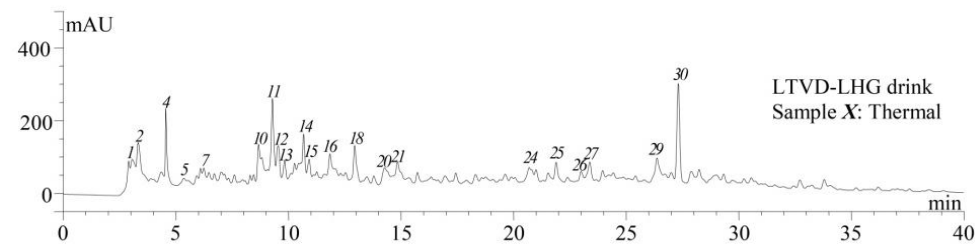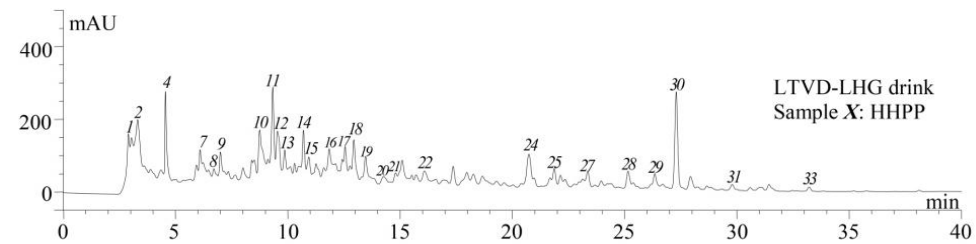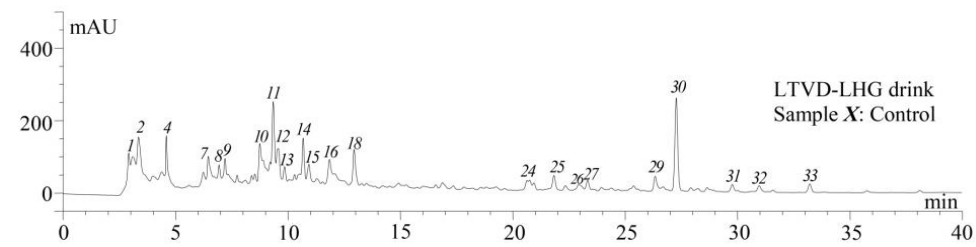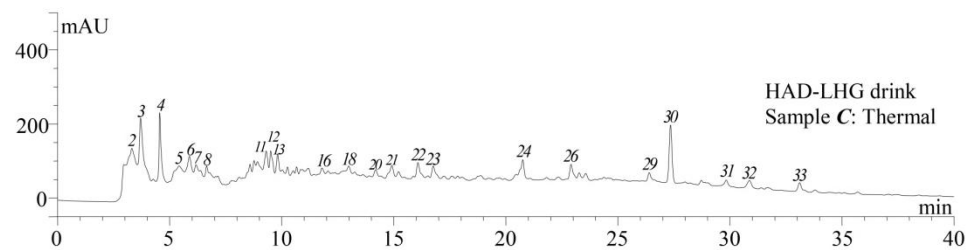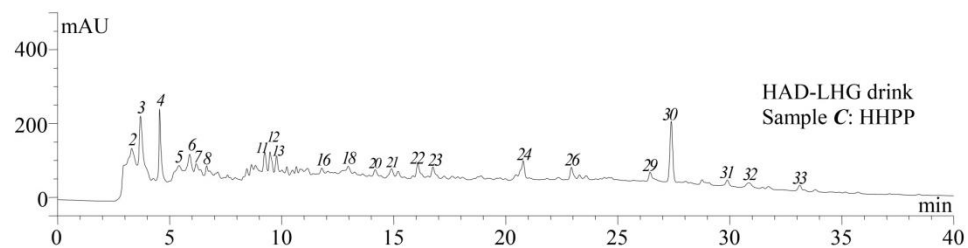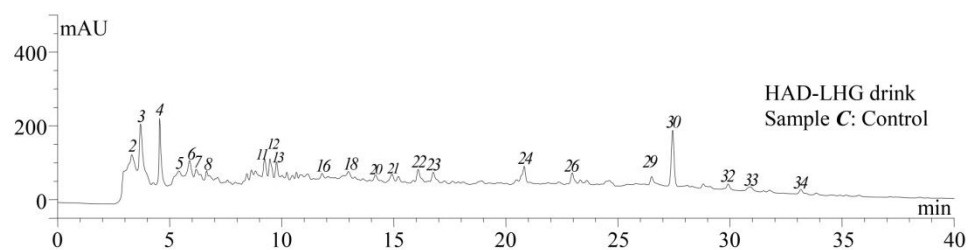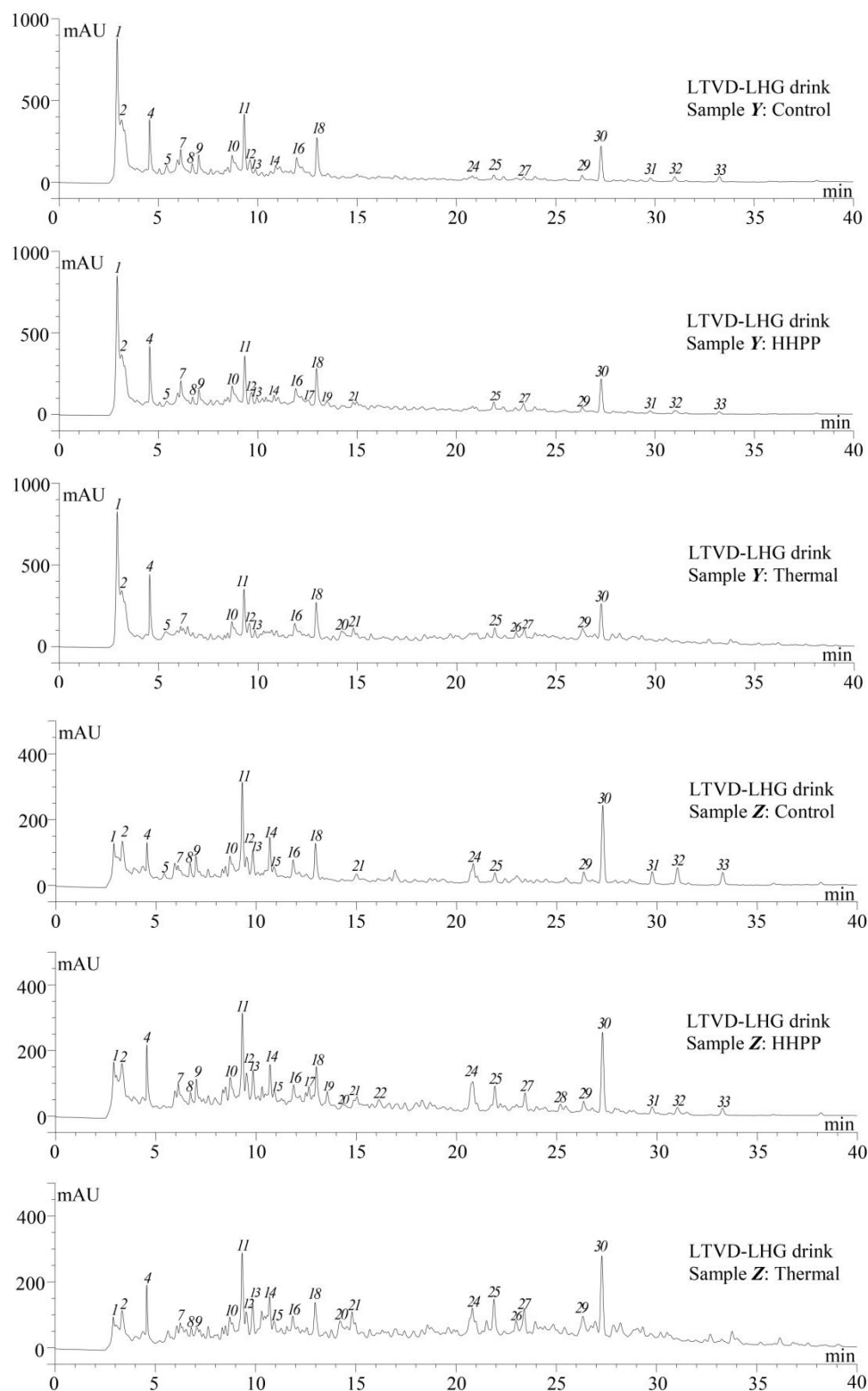
Figure 28: HPLC chromatograms of differently processed HAD-LHG drink and LTVD-LHG drink.
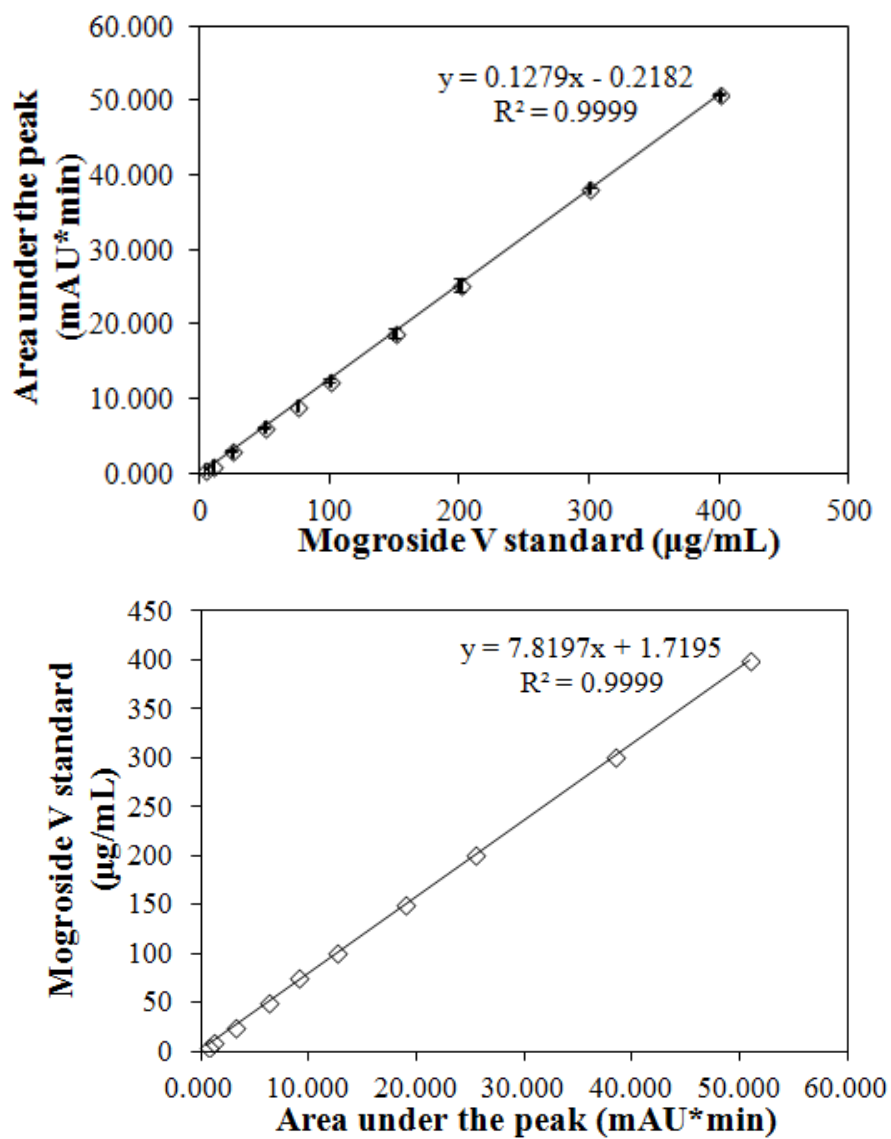
Figure 29: Mogroside V standard curve measured by HPLC

Mogroside V standard curve was made by plotting peak area versus concentration of mogroside V standard solution (5 - 400 μg/mL), as shown in **Fig. 29**. The content of mogroside V in LHG drink was calculated by interpolating area of peak *30* in HPLC chromatograms into the above standard curve. The results were summarized in **Table 18**, with data presented as mean of triplicates ± standard deviation.

Table 19: Mogroside V content in LHG drink via HPLC method (Mean ± SD, n=3)

Drying method	Sample letter	Mogroside V content (mg MGV/g dried LHG)		
		Control	HHPP	Thermal
	A	13.26 ± 0.05 [d]	13.15 ± 0.07 [d]	13.63 ± 0.26 [d]
HAD-LHG	**B**	11.78 ± 0.13 [e]	11.78 ± 0.20 [e]	13.00 ± 0.26 [e]
	C	11.65 ± 0.13 [e]	12.66 ± 0.07 [e]	12.17 ± 0.26 [e]
	X	20.03 ± 0.34 [a]	20.30 ± 0.14 [a]	19.85 ± 0.18 [a]
LTVD-LHG	**Y**	16.74 ± 0.38 [c]	15.94 ± 0.63 [c]	16.44 ± 1.13 [c]
	Z	18.49 ± 0.59 [b]	18.90 ± 0.37 [b]	18.17 ± 1.20 [b]

Different lowercase letters indicated significant differences ($p < 0.05$).

The statistical results showed that drink processing methods (HHP and thermal pasteurization) had no significant influence on mogroside V content of LHG drink when compared to each control drink. On the other hand, LTVD-LHG drink *X, Y, Z* exhibited an overall higher amount of mogroside V content (1500-2100 mg MGV/100 g dried LHG) than that of HAD-LHG drink *A, B, C* (1100-1400 mg MGV/100 g dried LHG). When accounting for sample variation, LHG drying methods still exhibited significant effects on the content of mogroside V in LHG drink. This could be explained that the degradation of mogroside V in LHG fruit would cease or be slowed down during low temperature drying rather than heat drying. In sum, HHPP or thermal pasteurization had little impact on mogroside V content of LHG drink. But low temperature drying of LHG resulted in more mogroside V content of LHG drink than hot air drying.

6. CONCLUSIONS

The conclusions of this study are summarized as below:

➢ The vitamin C contents of dried Luo Han Guo (LHG) fruit were highly affected by their drying methods. The magnitude of total vitamin C content of low temperature vacuum dried LHG (LTVD-LHG) was significantly greater than that of hot air dried LHG (HAD-LHG) measured by AOAC microfluorometric method. The average vitamin C of HAD-LHG was (36 ± 28) mg/100 g dried LHG. However, individual variation of vitamin C among LTVD-LHG from different venders was great as well, varying from (657 ± 69) mg/100 g dried LHG of sample *Z* to (1757 ± 225) mg/100 g dried LHG of sample *Y*.

➢ The pH of original LHG aqueous drink were highly affected by the drying methods of LHG fruit. HAD-LHG had pH values of 4.4 - 4.8, while pH values of LTVD-LHG was around 5.8 - 6.1.

➢ Drying methods significantly affected the color indices (L*, a*, b*, C*, hue, browning index) of LHG drink. The color of HAD-LHG drink is dark brown, and the color of LTVD-LHG drink is light yellow. The browning index of HAD-LHG (199 ± 8) is much higher than that of LTVD-LHG (21 ± 5). Drink processing methods (HHPP, thermal pasteurization, and unpasteurized control group) showed insignificant impact on color parameters .

➢ There was a great interfering compounds of polyphenols in LHG drink when directly using Folin-Ciocalteu reagent. The average total phenolic values with interfering compounds of HAD-LHG and LTVD-LHG were (1885 ± 169) mg GAE/100 g dried LHG and (765 ±153) mg GAE/100 g dried LHG respectively. However, the average corrected total phenolic values of HAD-LHG and LTVD-LHG by PVPP-binding Folin-Ciocalteu method were (499 ± 144) mg GAE/100 g dried LHG and (136 ± 40) mg GAE/100 g dried LHG respectively. The phenolic content of LHG aqueous drink was significantly affected by drying methods of the fruit, but insignificantly affected by drink processing methods.

➢ CAA values of HAD-LHG drink were not significantly affected by drink processing methods. But CAA values of LTVD-LHG drink would be decreased after thermal pasteurization when compared to the control and HHP processed drinks.

➢ LHG drying method significantly affected the HPLC results. LTVD-LHG drink displayed more compounds and higher content of the compounds than HAD-LHG drink in HPLC chromatograms.. LTVD-LHG drink showed significantly higher mogroside V content than HAD-LHG drink. Drink processing methods had little effects on HPLC chromatograms of HAD-LHG drink, but exhibited noticeable differences among differently processed LTVD-LHG drink. Drink processing methods showed insignificant effects on the mogroside V content of each LHG drink.

7. FUTURE WORK

Based on the results obtained, further investigations to complement this study were suggested below:

➤ Use some chemical assays like ORAC, DPPH, ABTS to measure the in vitro antioxidant activity of LHG drink.

➤ Headspace flavor profiles of LHG drink measured by GC-MS.

➤ Shelf life study

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APPENDIX I: Abbreviation

Abbreviation used in this research

ABAP   2,2'-azobis(2-amidinopropane) dihydrochloride

ABTS   2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid

ANOVA   analysis of variance

AOAC   association of official agricultural chemists

AP   adsorption percentage

BI   browning index

CAA   cellular antioxidant activity

CIELAB   Commission Internationale de l'éclairage L*, a*, and b*

CUPRAC   cupric reducing antioxidant capacity

CV   coefficient of variation

DCF   dichlorofluorescein

DCFH   dichlorofluorescin

DCFH-DA   dichlorofluorescin diacetate

DHA   docosahexaenoicacid

DHAA   L-dehydroascorbic acid

DKGA   2,3-diketogulonic acid

DPPH   2,2-diphenyl-1-picrylhydrazyl

DMSO   dimethyl sulfoxide

EC_{50} median effective concentration

ECG   epicatechin gallate

EGCG   epigallocatechin gallate

EMEM	minimum essential medium, eagle with earle's balanced salt solution
FBS	fetal bovine serum
FC	Folin-Ciocalteu
FCR	Folin-Ciocalteu reagent
FDA	Food and Drug Administration
FRAP	ferric reducing antioxidant parameter
GAE	gallic acid equivalents
GRAS	generally recognized as safe
HACCP	hazard analysis and critical control points
HAD-LHG	hot air dried Luo Han Guo
HBSS	Hank's balanced salt solution
HHP	high hydrostatic pressure
HHPP	high hydrostatic pressure processing
HORAC	hydroxyl radical averting capacity
HPLC	high performance liquid chromatography
LDL	low density lipoprotein
LHG	Luo Han Guo
LPS	lipopolysaccharides
MTS	3-(4,5-dimethyl-2-thiazolyl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt
LTVD-LHG	Low temperature-vacuum dried Luo Han Guo
ORAC	oxygen radical absorbance capacity
PBS	phosphate buffer saline

PFRAP	potassium ferricyanide reducing power
PMS	phenazine methosulfate
PVP	polyvinylpyrrolidone
PVPP	polyvinylpolypyrrolidone
QE	quercetin equivalents
RBD	randomized block design
RNS	reactive nitrogen species
ROS	reactive oxygen species
SD	standard deviation
SGP	*Siraitia grosvenori* polysaccharide
TPC	total phenolic content
TRAP	total radical-trapping antioxidant parameter
USDA	United States Department of Agriculture
WME	Williams' Medium E
YPD	yeast extract peptone dextrose

APPENDIX II: Statistical analysis

Table 20: Summary of ANOVA and Type I Sum of Squares analysis of color

parameters.

| | ANOVA | | Type I Sum of Squares analysis | | | | | |
| | | | Drying | | Pasteurization | | Sample variables | |
	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
L*	2308.950	< 0.0001	20379.326	< 0.0001	2.244	0.118	99.047	< 0.0001
a*	5917.419	< 0.0001	52691.495	< 0.0001	7.331	0.002	136.599	< 0.0001
b*	564.978	< 0.0001	4541.857	< 0.0001	4.696	0.014	133.341	< 0.0001
C*	1106.086	< 0.0001	9595.987	< 0.0001	3.650	0.034	87.838	< 0.0001
h (º)	1514.542	< 0.0001	13072.191	< 0.0001	1.399	0.257	138.449	< 0.0001
BI	6109.834	< 0.0001	54716.394	< 0.0001	1.019	0.369	67.394	< 0.0001
ΔE	0.327	0.948						

Table 21: Two-way ANOVA and Tukey HSD analysis of total phenolic content

Analysis of variance

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	29	173477314.289	5981976.355	132.273	< 0.0001
Error	402	18180224.653	45224.439		
Corrected Total	431	191657538.942			

Computed against model Y=Mean(Y)

Type I Sum of Squares analysis

Source	DF	Sum of squares	Mean squares	F	Pr > F
Sample-Processing	17	63200760.984	3717691.823	82.205	< 0.0001
PVPP or not	1	109655294.447	109655294.447	2424.691	< 0.0001
Replication	11	621258.859	56478.078	1.249	0.252

PVPP or not / Tukey (HSD) /

Analysis of the differences between the categories with a confidence interval of 95%:

Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
no PVPP vs Corrected by PVPP	1007.634	49.241	1.966	< 0.0001	Yes
Tukey's d critical value:			2.78		

Table 22: Two-way ANOVA & Type I Sum of Squares analysis of mogroside V

content

Analysis of variance					
Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	9	519.712	57.746	202.315	< 0.0001
Error	44	12.559	0.285		
Corrected Total	53	532.271			

Computed against model Y=Mean(Y)

Type I Sum of Squares analysis					
Source	DF	Sum of squares	Mean squares	F	Pr > F
Drying method	1	446.919	446.919	1565.797	< 0.0001
Processing method	2	0.430	0.215	0.752	0.477
Sample variables	4	69.994	17.499	61.307	< 0.0001

Table 23: Two-way ANOVA and Tukey HSD analysis of CAA values

Analysis of variance (CAA (µmol of QE/100 g)):

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	17	667415.782	39259.752	3.366	**0.001**
Error	36	419903.993	11664.000		
Corrected Total	53	1087319.774			

Computed against model Y=Mean(Y)

Type I Sum of Squares analysis (CAA (µmol of QE/100 g)):

Source	DF	Sum of squares	Mean squares	F	Pr > F
Processing	2	250325.284	125162.642	10.731	**0.000**
Sample	5	166486.824	33297.365	2.855	**0.029**
Processing*Sample	10	250603.673	25060.367	2.149	**0.046**

Processing / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (CAA (µmol of QE/100 g)):

Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
Ctrl vs Thml	161.516	4.487	2.444	**0.000**	Yes
Ctrl vs HHP	44.773	1.244	2.444	0.436	No
HHP vs Thml	116.743	3.243	2.444	**0.007**	Yes
Tukey's d critical value:			3.457		

Sample / Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95% (CAA (μmol of QE/100 g)):

Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
A vs Z	153.975	3.024	3.009	**0.048**	Yes
A vs C	132.969	2.612	3.009	0.120	No
A vs Y	55.634	1.093	3.009	0.881	No
A vs X	36.254	0.712	3.009	0.979	No
A vs B	34.739	0.682	3.009	0.983	No
B vs Z	119.236	2.342	3.009	0.204	No
B vs C	98.230	1.929	3.009	0.402	No
B vs Y	20.895	0.410	3.009	0.998	No
B vs X	1.515	0.030	3.009	1.000	No
X vs Z	117.721	2.312	3.009	0.216	No
X vs C	96.715	1.900	3.009	0.419	No
X vs Y	19.380	0.381	3.009	0.999	No
Y vs Z	98.341	1.932	3.009	0.400	No
Y vs C	77.335	1.519	3.009	0.655	No
C vs Z	21.006	0.413	3.009	0.998	No
Tukey's d critical value:			4.255		