Studies on utilization of sweet potato waste products for feed and food ingredients

（サツマイモ加工残さの飼料および食品利用に関する研究）

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ABTS: 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid

ACC: acetyl-CoA carboxylase

aP2: adipocyte protein

A-SPW: air-dry-processed sweet potato waste

BWG: body weight gain

c/EBP: CCAAT-enhancer-binding proteins

CAT: catalase

CP: crude protein

CPE: crude polyphenol extract

CPT1a: carnitine Palmitoyltransferase 1a

CPT1b: carnitine Palmitoyltransferase 1b

DFD: dark, firm, dry

DMEM: Dulbecco’s modified Eagle’s medium

DPPH: 1,1′-diphenyl-2-picrylhydrazyl

D-SPW: dry-heat-processed sweet potato waste

DW: dried-basic weight

EGCG: (-)-Epigallocatechin gallate

FAS: fatty acid synthase

FBS: fetal bovine serum

FCR: feed conversion ratio

FI: feed intake

GAE: gallic acid equivalent

GAPDH: glyceraldehydes-3-phosphate dehydrogenase

GE: gross energy

GLU: glucose

GLUT4: glucose transporter

GOT: glutamic oxalacetic transaminase

GPT: glutamic pyruvate transaminase

GPx: glutathione peroxidase

GSE: grape skin extract

GSH: glutathione

HFD: high fat diet

HLD-C: high-density lipoprotein cholesterol

HS: horse serum

IBMX: 3-isobutyl-1-methylxanthine

INS-R: insulin receptor

LPL: lipoprotein lipase

MDA: malondialdehyde

ME: metabolizable energy (

MTT: 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide

PPARγ: peroxisome proliferator activated receptor γ

PSE: pale soft exudative

RNS: reactive nitrogen species

ROS: reactive oxygen species

SOD: superoxide dismutase

SPP: sweet potato polyphenol

SPRM: sweet potato root meal

SPW: sweet potato waste

SREBP1c: sterol regulatory element-binding transcription factor 1

TBARS: thiobarbituric acid reactive substances

TC: total cholesterol

TCA: tricarboxylic acid cycle

TG: triglyceride

TNF-α: Tumor necrosis factor-α

ABSTRACT

In Kagoshima prefecture, there are approximately 300 kilotons of sweet potatoes are produced per year, and more than 2 kilotons of sweet potato waste (unused pieces mainly composed of the peel of tuberous roots) are estimated to be generated per year in the process of making Shochu, starch and confectioneries. The purpose of the study is to make full reuse of the sweet potato wastes products to be valuable ingredient for food/feed industry. In this thesis, on one hand, the polyphenols extracted from sweet potato waste were determined mainly on the effects of antioxidant and anti-obesity of cells *in vitro* and mice *in vivo*. On the other hand, the sweet potato wastes were fed for broilers to investigate the effect of growth performance, metabolizability, antioxidant status and meat quality.

First, polyphenols were extracted and purified from sweet potato waste products and its antioxidant properties were determined. The SPP had effective radical scavenging abilities including hydroxyl, DPPH and ABTS. Then, *in vitro* experiments showed the SPP extracts improved the antioxidant status in C2C12 cells and 3T3-L1 cells, due to it resulting in a reduction of the TBARS level and overlapping increased the activities of antioxidant enzymes. In addition, the SPP extracts also showed strong dose-dependent inhibition of accumulation of triglyceride formation by decreasing the insulin-mediated Akt phosphorylation and fatty acid synthesis while the mRNA expression of CPT1a was increased during the differentiation of 3T3-L1 preadipocytes into adipocytes.

Furthermore, the body weight gain and fat tissue weight of mice fed with high-fat diet were reduced by activating the long-chain fatty acid β-oxidation pathway in mitochondria. In addition, the SPP extract improve the antioxidant status of HFD mice due to increasing the mRNA expression of GPx1 gene in liver.

Finally, in the feeding experiments of broiler, we found there were no significant differences in body weight gain, feed intake and feed conversion ratio between the D-SPW meal and normal corn diet of broiler during the whole period. The metabolizabilities of crude protein and gross energy of the D-SPW meal were increased compared with those of the A-SPW meal. The D-SPW also improved the antioxidant status and modified the meat quality of broilers.

In conclusion, these results strongly indicated the value of sweet potato waste products to be recycled and reused. The SPP extracts was potential to be antioxidant and anti-obesity drugs in feed/food industry. Moreover, the sweet potato waste after dry-heat processing was available to be broiler feed partially substitute for using corn.

要　　約

鹿児島県では年間約 30 万トン以上のサツマイモが生産され、サツマイモ塊根を主原料とした焼酎・デンプン・菓子等の製造も盛んであるが、その製造工程において塊根の表皮や小断片から成るサツマイモ加工残さ（SPW）が年間 2,000 トン以上発生する。一部の SPW は工場近隣の養豚場が引き取り、未加工のまま自家飼料として利用しているが、大部分は産業廃棄物となっている。SPW はポリフェノール類やビタミン類などの有用成分を高濃度に含有するため、機能性食品・機能性飼料資材としての潜在能力が非常に高いと考えられる。そこで本研究では、SPW を食品および飼料産業における有用な資材として完全に利活用する方法の確立を目指した。

最初に、減圧乾燥サツマイモ加工残さ（D-SPW）からポリフェノール画分（SPP）の調整を行い、その抗酸化特性を Hydroxy、DPPH および ABTS の各ラジカル消去活性測定法によって検証したところ、いずれの測定法においても SPP は高い抗酸化能を示した。

次に、*in vitro* 評価系として C2C12 筋細胞および 3T3-L1 脂肪細胞を用いて SPP 投与試験（0, 30, 90, 180 mg/L）を行った。その結果、SPP（90, 180 mg/L）は細胞の抗酸化関連酵素（グルタチオンペルオキシダーゼ：GPx およびスーパオキサイドジムスターゼ：SOD）の活性を高め、一方で細胞の脂質過酸化の指標であるチオバルビツール酸反応物価を用量依存的に減少させた。2 つの細胞間で応答特性は異なったが、カタラーゼ、GPx、SOD の各 mRNA 発現量は総じて SPP に応答して上方調節された。また 3T3-L1 細胞において、SPP は用量依存的にトリグリセリド蓄積を強く阻害したが、インスリンシグナル伝達および脂肪酸合成関連因子の遺伝子発現の下方調節が原因と考えられた。

さらに、*in vivo* 評価系として C57BL/6 マウスを用いた SPP 投与試験を行った。その結果、SPP（飼料に 1%添加）は高脂肪食給与時の体重および脂肪組織の増加を抑制したが、その際脂肪組織のカルニチンパルミトイルトランスフェラーゼ 1b mRNA 発現量が増加したことから、ミトコンドリアにおける長鎖脂肪酸β酸化経路の活性化が示唆された。また SPP は、肝臓の GPx1 mRNA 発現量を増加し、高脂肪食給与時の生体内抗酸化状態を改善することが示された。

最後に、飼料資材としてのサツマイモ加工残さの評価を行うため、Ross308 チャンキー系ブロイラーを用いた飼養試験を実施した。飼料のトウモロコシの 55%（飼料への配合量としては 26.4%）を D-SPW に置換して 2 から 4 週齢での飼養試験を行った結果、通常

の基礎飼料で飼養したブロイラーと比較して、増体量、飼料摂取量および飼料要求率に差は無かった。室温乾燥サツマイモ加工残さ（A-SPW）給与区と比較して、D-SPW 給与区の飼料タンパク質・エネルギーの代謝効率は高い値を示し、また生体内の抗酸化状態は A-SPW および D-SPW で同等であったため、減圧乾燥法は SPW の飼料化に有効な加工方法であると考えられた。2 から 7 週齢までの長期間の試験でも飼養成績に差は現れず、D-SPW はトウモロコシの部分的な代替に適した飼料資材であることが確認された。一方で肉質には変化が現れ、脂肪含量が高く黄色度の低い鶏肉が生産できることが示された。

以上の結果より、サツマイモ加工残さ中の SPP は高い抗酸化能を示し、機能性食品あるいは抗肥満剤として高度利用できる可能性が示された。また D-SPW は、ブロイラー用配合飼料の資材として、トウモロコシの部分的な代替に適した飼料資材であることが確認され、D-SPW を飼料として大量消費すれば、サツマイモ加工残さの完全な利活用が可能となることが示された。

CHAPTER 1: General Introduction

1.1. Sweet potato world production

Diverse sweet potato varieties are widely cultivated between 40°N and 32°S, up to an altitude of 2000 m (and up to 2800 m in equatorial regions) (FAO, 2012). Sweet potato is ranked the most important food crop after rice, wheat, potato, maize, and cassava (Shekhar et al., 2015). The main sweet potato producers are China, Indonesia, Vietnam, India, Philippines, and Japan in Asia; Brazil and USA in the Americas; and Nigeria, Uganda, Tanzania, Rwanda, Burundi, Madagascar, Angola, and Mozambique in Africa (FAO, 2012). Sweet potato is cultivated extensively for its nutritious and health-promoting values (FAO, 2012; Lee et al., 2012).

In 2016, global production of sweet potatoes was 105 million tons, led by China with 67% of the world total. The following countries are Nigeria 3.9, Tanzania 3.8, Indonesia 2.3, Uganda 2.1 and Ethiopia 1.9 million tons. In 2016, the world average annual yield for sweet potato crop was 13 tons per hectare (Wikipedia website). Sweet potatoes are cultivated throughout tropical and warm temperate regions wherever there is sufficient water to support their growth, which became common as a food crop in the islands of the Pacific Ocean, South India, Uganda and other African countries (Stephen, 2017).

1.2. The nutrients composition in sweet potato

Ipomoea batatas, commonly called sweet potato, is herbaceous perennial vine belonging to the *Convolvulaceae* family. The edible tuberous root is long and tapered, with a smooth skin whose color ranges between yellow, orange, red, brown, purple, and beige. Its flesh ranges from beige through white, red, pink, violet, yellow, orange, and purple. Sweet potato cultivars with white or yellow flesh are less sweet and moist than those with red, pink or orange flesh (Loebenstein & Thottappilly, 2009).

Carbohydrates are the predominant component of sweet potato roots, which are followed by protein, ash, and fat. The starch, crude fiber, protein, ash, and fat of tubes from 80 sweet potatoes varieties had ranges of 42.4–77.3, 1.9–6.4, 1.3–9.5, 1.1–4.9, and 0.2–3.0/100 g of dry matter, respectively (Wang et al., 2016). Sweet potato contains 20.4–31.8% starch (Noda et al., 2001) and 0.29–2.24% crude protein (CP) on a fresh weight basis (Purcell et al., 1972). Essential amino acids make up approximately 40.7% of the sweet potato protein, which is especially rich in aspartic acid (18.5%) and glutamic acid (9.30%) (Mu et al., 2009) but deficient in lysine (Sun, et al., 2012). It has been reported that the digestibility of autoclaved sweet potato protein is 95.1% *in vivo*, which is markedly higher than that of native sweet potato protein (50.4%) (Sun et al., 2012). In addition, sweet potatoes are rich in dietary fiber, minerals, vitamins and antioxidants, such as phenolic acids, anthocyanins, α-tocopherol and β-carotene, which are considered beneficial to human and animal health (Woolfe, 2008).

Various parts of the sweet potato (i.e., leaf, root, root peel, stem) in various forms (extracts, anthocyanin fractions, flours, powders) have their unique chemical compositions. The chemical constituents possibly have unique physiological responses *in vitro* and *in vivo*, and sweet potato thus has great potential to influence human health. Health-promoting bio-functions of sweet potatoes include antioxidation, antibacterial, anti-inflammation, antidiabetic, anticancer, antihepatotoxicity, and antiaging (Wang et al., 2016).

Table 1. Nutrient content (per 100 g portion) of sweet potato

Nutrients		Minerals		Vitamins	
Water, g	77.00	Potassium, mg	337.00	Vitamin C, mg	2.40
Carbohydrates, g	20.00	Sodium, mg	55.00	Thiamin (B_1), mg	0.08
Fiber, g	3.00	Phosphorus, mg	47.00	Riboflavin (B_2), mg	0.06
Protein, g	1.60	Calcium, mg	30.00	Niacin (B_3), mg	0.56
Fat, g	0.05	Magnesium, mg	25.00	Pantothenic acid (B_5), mg	0.80
Sugar, g	4.18	Iron, mg	0.61	Vitamin B_6, mg	0.21
		Selenium, µg	0.60	Folate total (B_9), µg	11.00
Energy, kJ	360.00	Zinc, mg	0.30	Vitamin A, IU	14187.00
		Manganese, mg	0.26	Vitamin E, mg	0.26
		Copper, mg	0.15	Vitamin K_1, µg	1.80
				β-carotene, µg	8509.00
				Lutein + zeaxanthin, µg	0.00

"Nutrient data laboratory". United States Department of Agriculture. Retrieved 10 August 2016.

Undesirable/anti-nutrient components

Phytic acid and tannins represent anti-nutrients in sweet potato roots. Phytic acid is involved in mineral chelation and protein complexation, thus reducing mineral and protein bioavailability (Wang et al., 2016). The antinutrient composition for phytic acid, cyanide, tannins and total oxalate were 1.44 ± 0.01, 30.24 ± 0.02, 0.21 ± 0.02 and 308.00 ± 1.04 mg/100g respectively (Antial et al., 2006). It has been also reported that sporamin, a storage protein found in the tubers of sweet potato, has strong inhibitory activity to trypsin *in vivo*, in addition to its role as a nutritional resource for tuber re-growth (Yeh et al., 1997). Trypsin inhibitors in sweet potato storage roots account for about 60% of the total water-soluble proteins (Lin, 1989). It was reported that dietary uncooked sweet potato roots meal reduced the growth rate of finishing pigs (Pietrosemoli et al., 2016). There are more anti-nutritional factors, such as phosphorus, accumulated in the outer skin layer of sweet potato root than that of inner flesh layer (Dako et al., 2016).

1.3. Polyphenols in sweet potato

The total phenolic content in sweet potato of flour from roots has been much reported, including 10.68–15.69 g gallic acid equivalent (GAE)/100 g DW for maltodextrin and α-amylase-treated purple sweet potato flour (Ahmed et al., 2010a), 13.78–57.23 g GAE/100 g DW for encapsulated and non-encapsulated flours (Ahmed et al., 2010b), and 14.9– 36.2 g GAE/100 g DW for steamed sweet potato flours (Rumbaoa et al., 2009). Sweet potatoes (*Ipomoea batatas*) also contain 0.24 mmol antioxidants per 100 g fresh weight of roots (Halvorsen et al., 2002), with the phenolic components and storage proteins (33 kDa trypsin inhibitors) showing strong inhibition of linoleic acid peroxidation, radical scavenging, electron donation and metal chelation (Hou et al., 2001; Rumbaoa et al., 2009). Carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols, etc. are among the antioxidants produced by plants for their own sustenance. The ability of antioxidants to prevent food deterioration and extend the shelf life of the food causes it to be used widely in the food industry (Ghasemzadeh 2012; Fresco et al., 2006).

Peel, leaf and vine, discarded root and residues of various sweet potato processing activities can possibly be transformed into food, feed, and market materials. A beverage prototype using sweet potato leaf and peel extract was developed and reported to have a quality similar to the commercial vegetable and fruit beverages (Anastacio & Carvalho, 2015). Increasing the commercial value of the major leftover peels has been studied for its phenolic content (Anastácio et al., 2016). Further, Oluyori et al. (2016) suggested a feasible isolation method for several anti-cancer constituents from sweet potato peels for their use as dietary supplements. Organic acid (e.g., limonenic acid) used in polymeric, medicine and cosmetics applications have also been isolated from sweet potato peels (Patricia Folakemi & Eunice Aderonke, 2014).

1.4. Application of sweet potato as feed

Global warming and the growth in the population are both causing the price of foodstuffs to increase. A reduction in the availability of corn and an increase in the price of feed have a direct impact on the animal feed industry worldwide (Mourão et al., 2008). It has been suggested that plants with a high content of protein and starch from various sources should be widely developed and used as energy feed for animals, to help meet the challenge of the growing global population. Sweet potato, the sixth most important food crop in the world, could help solve the global issues of food, energy, natural resources and environment in the 21st century (Kozai et al., 1996).

Many researchers pay attention to the utilization of sweet potato as animal feed. It has been suggested that 75% of the concentrate mixture can be replaced by sweet potato meal in the ration of indigenous growing pigs without any adverse effect on the growth performance and nutrient utilization (Malsawmthangi et al., 2016). Etela I et al. (2008) reported that the sweet potato forage could be used as sole feed or supplement for dry-season Green panic grass on stall-fed lactating White Fulani cows and growing calves. The sweet potato forage also appears to be a cheap nitrogen source and support growth in goats (Aregheore, 2004). Edache et al. (2018) research showed that the maize could completely be replaced by peeled, cooked and sundried sweet potato tubers without adverse effect on performance for growing Japanese quail diet. In addition, the tubers and stems of sweet potato in feed can be adjusted to improve the digestion and growth of sea cucumber A. japonicas in marine farms (Zhao et al., 2018).

There were several researches have indicated that sweet potato root meal (SPRM) can replace corn in the diet of broilers to some extent. For example, Ayuk & Essien (2009) included SPRM in the diets of broilers and reported an increased body weight gain for SPRM inclusion up to 40%, but a significant reduction at the 50% level. Maphosa et al. (2003) found that a diet including SPRM up to 50% had no negative effect on broilers during the finisher period, but adversely influenced the body weight gain, feed intake, and feed conversion during the starter phase. Mozafari et al. (2013) reported that sweet potato meal (sun dried raw or cooked then sun dried) can be substituted for up to 25% of the maize in broiler diets without any significant decrease in performance and growth rate. Moreover, the carcass quality in broilers improved with sweet potato feeding as a result of lower abdominal fat content. Importantly,

increasing the proportion of sweet potato in the starter diet negatively influenced the growth and feed conversion ratio, but had no effect on body weight gain when included at up to 50% in finisher diets. Higher mortality was recorded for broilers when sweet potato meal inclusion levels were greater than 25% (Maphosa et al., 2003). Similarly, a recent study also reported that diets containing 250 g/kg sweet potato flour led to poor growth of broilers (Pandi et al., 2018). Therefore, processed sweet potato or its waste has potential for use in poultry feed, but their inclusion levels need to be considered.

1.5. Sweet potato waste products in this study

Although the leaves and shoots are also edible, the starchy tuberous roots are by far the most important industry product. Purple sweet potato color is also used as a ‘natural’ food coloring. Peeling is often used in processing for food and starch industry. During the industry production, the recycle and reuse of the by-products and the waste pieces of sweet potato with high fiber content and pigment should be considered.

In Kagoshima, on the southern part of Kyushu Island of Japan, approximately 300 kilotons of sweet potatoes are produced per year, and more than 2.3 kilotons of sweet potato waste (unused pieces mainly composed of the peel of tuberous roots) are estimated to be generated per year in the process of making Shochu, starch and confectioneries, in which sweet potatoes are processed as a main ingredient.

In this study, these sweet potato wastes are the raw material. The purpose of the study is to make full reuse of the sweet potato wastes products, which is benefit for the recycling of resources and protecting the environment. The use of sweet potato waste is one of the options being considered to reduce the cost of energy supplements in animal feed. Furthermore, the rich content of polyphenols and bioactive substances in the sweet potato waste has potential to be valuable ingredient for food/feed industry.

CHAPTER 2: Extraction and purification of polyphenols from sweet potato waste products and its antioxidant properties

2.1. Summary

It is well known that the reactive oxygen species are damage to biomolecules (e.g., lipid, protein, amino acids, and DNA) in different conditions. Antioxidants and antioxidant enzymes exert synergistic actions in scavenging free radicals. Due to the high activity of antioxidant of polyphenol compounds, in this chapter, the free radicals scavenging ability of polyphenols from sweet potato waste was investigated. Firstly, the best condition of extraction of crude polyphenols from sweet potato wastes was confirmed: concentration: 80% ethanol; temperature: 80°C; time: 3 h; sample to solvent ration: 10 g/300 mL. Then, the crude polyphenol was purified by HP-20 resin. The result showed as: when the concentration of SPP were 100, 200, 300, 400, 500 μg/mL, the hydroxyl radical scavenging ability were 5.28±0.39%, 13.89±3.14%, 21.94±2.75%, 30.00±3.93%, 29.72±4.32%, respectively; the DPPH radical scavenging ability were 29.52±2.94%, 51.52±2.31%, 76.88±0.74%, 89.14±0.84%, 92.79±0.53%, respectively; the ABTS radical scavenging ability were 63.97±0.49%, 95.48±0.12%, 99.62±0.06%, 99.62±0.15%, 99.29±0.06%, respectively. Therefore, the SPP had effective radical scavenging abilities including hydroxyl, DPPH and ABTS, indicating its potential to impart antioxidant activity when consumed and possible to be regarded as a valuable source of antioxidants.

2.2. Introduction

The normal biochemical reactions in our body, increased exposure to the environment, and higher levels of dietary xenobiotic's result in the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Nimse & Pal, 2015). It is well known that the reactive oxygen species are damage to biomolecules (e.g., lipid, protein, amino acids, and DNA) in different conditions. Therefore, it is very important and necessary to understanding free radical biology. Antioxidants (e.g., glutathione, arginine, citrulline, taurine, creatine, selenium, zinc, vitamin E, vitamin C, vitamin A, and tea polyphenols) and antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidases) exert synergistic actions in scavenging free radicals. Consumption of fruits and vegetables has been shown to be effective in the prevention of chronic diseases. These benefits are often attributed to the high antioxidant content of some plant foods.

In the extracted material from the tuberous root of sweet potato, polyphenolic compounds mainly include caffeic acid, chlorogenic acid, 4,5-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, and 3,4-di-O-caffeoylquinic acid, 4-O-caffeoylquinic acid, 1, 3-di-O-caffeoylquinic acid. Chlorogenic acid constituted about 40% to 60% of the total phenolics in the sweet potato root samples (Truong, et al., 2007).

As widely admitted that polyphenol compounds have high activity of antioxidant, including free radicals scavenging, metal chelation, inhibiting redox-sensitive transcription factors and the activation of “pro-oxidant” enzymes (Chiva-Blanch & Visioli, 2012). Several reports have indicated that the extract from sweet potato (*Ipomoea batatas* Lam. cv. Koganesengan) showed potential effects of cancer-preventing, antiproliferation activities and dietary polyphenol-rich food lead to beneficial effects for health (Rabah et al.,2004; Konczak-Islam et al., 2003). Another study showed that the ethanolic extracts from Sava potato peel showed strong antioxidant activity in *in-vitro* systems and prevented lipid oxidation in emulsion and in oil (Koduvayur Habeebullah et al., 2010). The phenolic content could be served as a useful indicator for the antioxidant activities of sweet potatoes (Ghasemzadeh, 2012). Sweet potato peels usually considered as waste in food service operations may be upgraded into value added functional foods with high phenolic content and antioxidant activity (Anastacio & Carvalho, 2013). The antioxidative compounds extracted from potato peels had potential value to be used as natural antioxidants for feed and

food industries.

Thus, a valuable source of nutrition maybe removed from our food chain. We have showed that sweet potato peels possess high contents of phenolic compounds compared to other edible parts of the sweet potato.

The method of extracting polyphenols from sweet potato waste products was determined by surface response method of four impact factors (ethanol concentration, temperature, time and sample/liquid). The total phenolic content was determined using Folin–Ciocalteu reagent, as described by Singleton & Rossi (1965). The result was expressed as gallic acid equivalents. According to the results of surface response experiment, the best condition of extraction was:

Concentration : 80% ethanol Temperature : 80℃

Time : 3 h Sample/liquid : 10 g/300 mL

In this study, the method of extract polyphenol samples from sweet potato waste products was showed as above.

The purpose of this chapter was to investigate the antioxidant properties of polyphenols from sweet potato waste products *in vitro*.

2.3. Materials and methods

Extraction and purification

The dried sweet potato waste samples were ground into powder by grinder. The sweet potato waste powder sample was extracted with 80% ethanol at 80℃ water bath for 3 hours. The crude polyphenol extract (CPE) solution was vacuum distillation and filtered. Ten gram of pre-prepared resin (HP-20) were put in an Erlenmeyer flask with a lid and 5 mL of CPE in 200 mL diluted water added to the flask. The flasks were sealed tightly and shaken using the shaking incubator (180 rpm) at 25℃ for 5 hours. Then wash the resin and put into 200 mL 95% ethanol in the flask. The flasks were sealed tightly and shaken using the shaking incubator (180 rpm) at 25℃for 5 hours. After adsorption and desorption, the solutions (SPP) were filtered and vacuum distillation and finally kept in 4 ℃.

Measurement of total phenolic acid

The total phenolic content was determined using Folin–Ciocalteu reagent, as described by Singleton & Rossi (1965). The result was expressed as gallic acid equivalents.

Hydroxyl radical scavenging ability assay

The depletion of free radical OH^- was measured. Briefly, 1 mL of 9 mM salicylic acid-ethanol solution, 1 mL of 9 mM $FeSO_4$, 1mL of 8.8 mM H_2O_2, then filled with distilled water to 5 mL, finally add 1mL sample solution. Then the mixture was kept at room temperature for 30 min and the absorbance was read at 510 nm. Ascorbic acid and (-)-Epigallocatechin gallate were employed as positive control. The ability to scavenge hydroxyl radical was calculated by the following equation:

Scavenging ability of OH^- (%) = $[(A_0 - As)/ A_0] \times 100$, where A_0 was the absorbance of the blank and As was the absorbance of the sample.

DPPH radical scavenging ability assay

The DPPH (1,1′-diphenyl-2-picrylhydrazyl) radical-scavenging activity was determined as described by Yuan et al. (2012). Briefly, add 0.2 mL sample solution into 2 mL DPPH solution (0.1 mM) and kept at room temperature for 30 min. Then the absorbance was read at 517 nm. Ascorbic acid and (-)-

Epigallocatechin gallate were employed as positive control. The ability to scavenge DPPH radical was calculated by the following equation:

Scavenging ability of DPPH (%) = [(A_0- As)/ A_0] ×100, where A_0 was the absorbance of the blank and As was the absorbance of the sample.

ABTS radical scavenging ability assay

The ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS or ABTS+) scavenging ability was measured as described by Re et al. (1999). ABTS was produced by reacting 0.0768 g ABTS salt with 0.0132 g potassium persulphate in 20 mL deionized water, stock solution was kept in dark for 14–16 h at room temperature. Prior to use, ABTS stock solution was diluted with PBS (4 mM, pH = 7.4) to obtain the absorbency of 0.700 ± 0.004 measured at 734 nm. After 0.1 mL sample was mixed with 1.9 mL ABTS solution and kept for 5 min at room temperature, the absorbances of reaction mixtures were read at 734 nm. Ascorbic acid and (-)-Epigallocatechin gallate were employed as positive control. The ability to scavenge ABTS radical was calculated by the following equation:

Scavenging ability of ABTS (%) = [(A_0- As)/ A_0] ×100, where A_0 was the absorbance of the blank and As was the absorbance of the sample.

2.4. Results

Hydroxyl radical scavenging ability assay

The hydroxyl radical scavenging curves of SPP were shown in Figure 1A. The hydroxyl radical scavenging ability were 5.28±0.39%, 13.89±3.14%, 21.94±2.75%, 30.00±3.93% and 29.72±4.32% when the concentration of SPP were 100, 200, 300, 400 and 500 μg/mL, respectively. The scavenging ability of hydroxyl radical showed the order as follow: Ascorbic acid > SPP> (-)-Epigallocatechin gallate.

DPPH radical scavenging ability assay

The DPPH radical scavenging curves of SPP were shown in Figure 1B. The DPPH radical scavenging ability were 29.52±2.94%, 51.52±2.31%, 76.88±0.74%, 89.14±0.84% and 92.79±0.53% when the concentration of SPP were 100, 200, 300, 400 and 500 μg/mL, respectively. The DPPH radical scavenging ability of SPP reached the same level of Ascorbic acid and (-)-Epigallocatechin gallate when the concentration was higher than 400 μg/mL.

ABTS radical scavenging ability assay

The ABTS radical scavenging curves of SPP were shown in Figure 1C. As a general rule, the same trend observed in DPPH assay was obtained again. ABTS radical scavenging ability was increasing with the increase of SPP concentration. The ABTS radical scavenging ability were 63.97±0.49%, 95.48±0.12%, 99.62±0.06%, 99.62±0.15% and 99.29±0.06% when the concentration of SPP were 100, 200, 300, 400 and 500 μg/mL, respectively. The ABTS radical scavenging ability of SPP reached the same level of Ascorbic acid and (-)-Epigallocatechin gallate when the concentration was higher than 200 μg/mL.

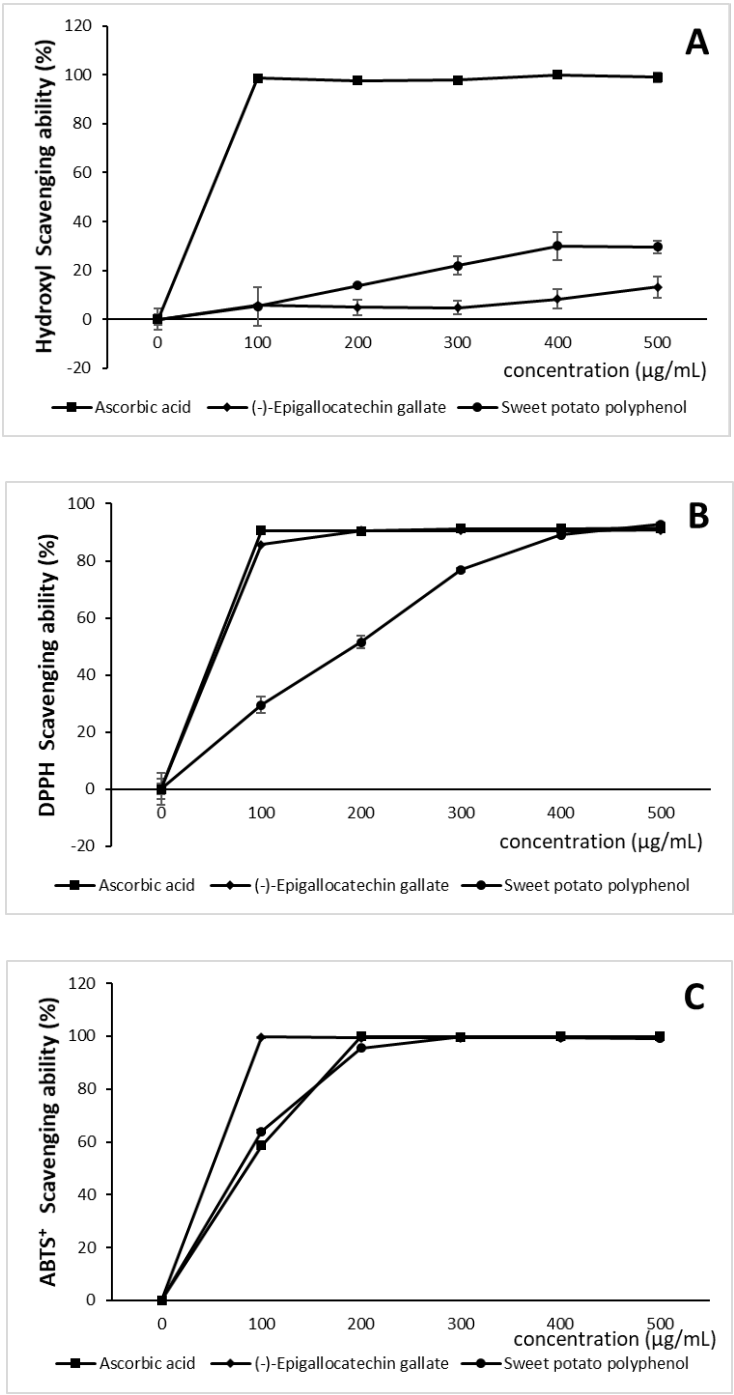


Figure 1. Radical scavenging abilities of sweet potato polyphenol

Ascorbic acid and (-)-Epigallocatechin gallate were employed as positive control. (A) Hydroxyl radical scavenging assay; (B) DPPH radical scavenging assay; (C) ABTS radical scavenging assay.

Values were mean ± SD (n=3).

2.5. Discussion

Free radicals are fundamental on any biochemical process and represent an essential part of aerobic life and metabolism. Various metabolic processes, UV radiations, smoke etc trigger the production of free radicals (Halliwell & Gutteridge, 2015). Hydroxyl radicals are highly reactive and undergo chemical reactions that make them short-lived. When biological systems are exposed to hydroxyl radicals, they can cause damage to cells, including those in humans, where they can react with DNA, lipids, and proteins. DPPH is a well-known radical and a trap ("scavenger") for other radicals. The rate of reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction. DPPH is widely used to test an antioxidant activity as a scavenger of free radicals of food and herbal extract. DPPH can also be used to quantify antioxidants in complex biological systems in recent years (Sudewi et al., 2017). The DPPH was chosen because this method is a simple, easy, fast and sensitive method and requires only a small sample to know the antioxidant activity of natural compounds (Koleva et al., 2002). ABTS is a chemical compound used to observe the reaction kinetics of specific enzymes, which is commonly used as a substrate with hydrogen peroxide for a peroxidase enzyme (such as horseradish peroxidase) or alone with blue multicopper oxidase enzymes (such as laccase or bilirubin oxidase). Its use allows the reaction kinetics of peroxidases themselves to be followed. In this way it also can be used to indirectly follow the reaction kinetics of any hydrogen peroxide-producing enzyme, or to simply quantify the amount of hydrogen peroxide in a sample.

In this study, the sweet potato polyphenol had good performances of DPPH and ABTS radical scavenging, which reached the same level of the effects of Ascorbic acid and (-)-Epigallocatechin gallate. This is agreed with the Donado-Pestana's research, the phenolics from orange-fleshed sweet potato had high activity of scavenging (DPPH, ABTS) ability *in vitro* experiment (Donado-Pestana, et al.,2012). Another study evaluated and compared the antioxidant substance content and antioxidant activities among white (Superior) and colored (Hongyoung, Jayoung, Jasim, Seohong, and Jaseo) potatoes. All colored potato extracts, except for Jaseo and Seohong, showed higher ABTS radical scavenging activities than the general white potato extract. Hongyoung and Jayoung had the highest ABTS and DPPH radical scavenging activities (Lee, et al., 2016).

Conclusion

The SPP had effective radical scavenging abilities including hydroxyl, DPPH and ABTS, indicating its potential to impart antioxidant activity when consumed and possible to be regarded as a valuable source of antioxidants.

CHAPTER 3: Effects of polyphenol extracts on antioxidant in C2C12 cells and 3T3-L1 cells

3.1. Summary

The production of oxidation reaction occurs from normal metabolic processes and they are responsible for damaging cellular macromolecules, such as proteins, lipids, and nucleic acids, causing health impairments. Exogenous antioxidants possess a double role, protecting food from lipid oxidation and organisms by increasing their endogenous antioxidant defense system. Due to the effective radical scavenging abilities of the SPP, including hydroxyl, DPPH and ABTS, the experiment of SPP extracts on the antioxidant status in cells was desired. In the MTT assay, the 90 mg/L SPP significantly increased cell viability at 24 h and the 180 mg/L SPP significantly decreased cell viability during the treatment period in C2C12 cells; the cell viability was increased by 30 mg/L SPP at 24 h and decreased by 90 mg/L and 180 mg/L SPP from 48 h. In the antioxidant determination, the C2C12 myotubes were treated with SPP for 24 hours and the 3T3-L1 adipocytes were treated with SPP for 24 hours. The C2C12 myotubes and 3T3-L1 adipocytes were plated at 6-well plates and divided into 4 groups (6 wells/group): CTRL group (0 mg/L SPP), LSPP group (30 mg/L SPP), MSPP group (90 mg/L SPP), HSPP group (180 mg/L SPP). The results showed the 180 mg/L SPP extracts significantly increased the activity of SOD and GPX and decreased the TBARS level in C2C12 and 3T3-L1 cells, due to it increased the mRNA expressions of antioxidant-related enzymes. The 90 mg/L and 180 mg/LSPP significantly increased the CAT and Cu/Zn-SOD mRNA expression in C2C12 cells and 3T3-L1 cells. The GP-X1 mRNA expression was significantly raised by 180 mg/LSPP in C2C12 cells, however, it was reduced by 30 mg/L SPP and 90 mg/L SPP in 3T3-L1 cells. The 30 mg/L SPP and 90 mg/L SPP also enhanced the mRNA expression of Mn-SOD in C2C12 cells, but not changed in 3T3-L1 cells compared to control. In conclusion, the SPP extracts improved the antioxidant status in C2C12 cells and 3T3-L1 cells, by increasing the activities of antioxidant enzymes, reduced the TBARS level and regulated the mRNA expressions of antioxidant-related enzymes.

3.2. Introduction

The reactive oxygen species occurs from normal metabolic processes and they are responsible for damaging cellular macromolecules, such as proteins, lipids, and nucleic acids, causing health impairments. Exogenous antioxidants possess a double role, protecting food from lipid oxidation and organisms by increasing their endogenous antioxidant defense system (Kouka et al., 2018). Pro-oxidant conditions dominate either due to the increased generation of the free radicals or due to the excessive oxidative stress of the depletion of the dietary antioxidant (Ramakrishna et al.,2012). The most important antioxidant sources are vitamins and polyphenols. Polyphenols have been proposed to be useful as adjuvant therapy for their potential antioxidant activity (Hussain et al., 2016). Polyphenol antioxidant activities are related to their capacity to scavenge a wide range of ROS. Indeed, the mechanisms involved in the antioxidant capacity of polyphenols include suppression of ROS formation by either inhibition of enzymes involved in their production, scavenging of ROS, or upregulation or protection of antioxidant defenses. In addition, Polyphenols may also reduce the catalytic activity of enzymes involved in ROS generation (Kumar et al., 2012).

In the last few years, there is a lot of interest reports regarding the cyto-protective effects of dietary compounds especially the polyphenols from food against oxidative stress and the underlying responsible defense mechanisms, with the purpose of creating potentials therapeutic and preventative agents against ROS/RNS as a tool to struggle to oxidative stress (Halliwell, 2008).

One of the richest sources of antioxidants in the human diet is potato(*Solanum tuberosum* L.) tubers (Lachman et al. 2000). Their antioxidant content decreases a great deal from atherosclerotic processes, and is inhibited from cholesterol accumulation in blood serum and enhances the resistance of the vascular walls (Lachman & Hamouz, 2011)

A better clarification and understanding of the mechanisms presumably involved in the protective role of polyphenols in adverse situations will help to more precisely define the actual situations where polyphenol consumption will prove to be beneficial. Such investigation may in addition prove to be useful for the development of new compounds with antioxidant effects. Thus, due to the determine results of antioxidant activity of SPP in the chapter 2, in the present study, I investigated the following:

1. MTT assay of C2C12 and 3T3-L1 cells

2. Determination of TBARS and antioxidant enzyme activity

3. Gene expressions of antioxidant enzymes

3.3. Materials and methods

Preparation and treatment of C2C12 and 3T3-L1 cell culture

C2C12 mouse skeletal muscle cell line was cultured either as myoblasts (undifferentiated) or as myotubes (differentiated). Cells were grown to confluence in DMEM supplemented with 10% FBS (fetal bovine serum) and 1% (v/v) antibiotic solution (100 U/mL penicillin, 100 U/mL streptomycin) at 37°C in 5% CO_2 in a humidified incubator. When C2C12 cells confluence was 80% reached, differentiation of cells into myotubes was induced by replacing the cells into DMEM supplemented with 2% HS (horse serum) and 1% (v/v) antibiotic solution (100 U/mL penicillin, 100 U/mL streptomycin). Cells were allowed to differentiate for 4 days and the medium was changed every 2 days. 3T3-L1 preadipocytes were plated at 6-well plates and divided into 4 groups (6 wells/group). After cells complete confluent, change the medium to differentiation induction：Day 0-2 10% FBS medium with insulin (5 μg/ml), dexamethasone (1 μM) and 3-isobutyl-1-methylxanthine (500 μM); Day 2-4 10% FBS medium with insulin (5 μg/ml); Day 4-8 10% FBS medium only. Then the cells were divided into 4 groups (6 wells/group) and started the treatment: CTRL group (0 mg/L SPP), LSPP group (30 mg/L SPP), MSPP group (90 mg/L SPP) and HSPP group (180 mg/L SPP). The treatment time was 48 hours. Then all the cells were collected and analyzed.

MTT assay

In this study, the viability of C2C12 and 3T3-L1 cells were measured using the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide) assay. Briefly, C2C12 myoblasts and 3T3-L1 preadipocytes were plated at a density of 2×10^4 cells/well in a 96-well plate. C2C12 myoblasts and 3T3-L1 preadipocytes were both incubated for 24h, 48h and 72h. After the incubation, 20 μL/well of MTT solution (5 mg/mL) was added in the medium and continued incubating for 4h. Aspirate off the medium and add 150 μL/well dimethyl sulfoxide. Absorbance was measured at 490 nm.

TBARS level and antioxidant enzyme activity

C2C12 myotubes and 3T3-L1 adipocytes were incubated for 48h then collected. TBARS was analyzed by previously described method (Ohkawa et al., 1979). Total SOD activity was determined

according to the SOD Assay Kit-WST (Dojindo, Japan) as the manufacturer's protocol. The result of SOD activity was expressed by monitoring the inhibition of reduction of WST/μg protein. Total GPx activity was determined according to the Glutathione Peroxidase Assay Kit (Cayman, USA) as the manufacturer's protocol. The result of GPx activity was expressed by μmol/min/mg protein. Protein content in cells was determined by Lowry's method (Lowry et al., 1951).

Gene expression

Total RNA was isolated from C2C12 myotubes and 3T3-L1 adipocytes using IsogenII reagent (Nippon Gene, Japan) as described in the manufacturer's protocol. RNA (40 ng/μL) was used for reverse transcription with PrimerScript^{TM} RT Master Mix (TAKARA, Japan). The primers used in this study were listed in Table2. Gene expression was determined by real-time PCR using 7300 Real-Time PCR system (Applied Biosystems, USA) with SYBR Select Master Mix (Applied Biosystems, USA). The thermal cycles were showed as follow: 1 cycle at 50℃ for 2 min, 95℃ for 2 min; 60 cycles at 95℃ for 15sec, 60℃ for 15sec, 72℃ for 2 min; 1 cycle at 95℃ for 15sece, 60℃ for 1 min, 95℃ for 15s, 60℃ for 15sec.

Statistical analysis

Results are expressed as means ± standard deviation. The data were analyzed by one-way analysis of variance (ANOVA) using IBM SPSS Statistics 25 software. Dunnett 's test was further performed, when significant differences were found ($p < 0.05$). Significant differences were based on $p < 0.05$.

Table 2. List of antioxidant enzyme primer sequences used for qualitative real time PCR

Gene	Forward	Reverse
CAT	5’-TATTGCCGTTCGATTCTCCACAGT-3’	5’-TTTCCCACAAGATCCCAGTTACCA-3’
GPx1	5’-TGCAATCAGTTCGGACACCAG-3’	5’-CATTCACTTCGCACTTCTCAAACA-3’
Mn-SOD	5’-GCACATTAACGCGCAGATCA-3’	5’-AGCCTCCAGCAACTCTCCTT-3’
Cu/Zn-SOD	5’-AAGGCCGTGTGCGTGCTGAA-3’	5’-CAGGTCTCCAACATGCCTCT-3’
GAPDH	5’-AAATGGTGAAGGTCGGTGTG-3’	5’-TGAAGGGGTCGTTGATGG-3’

3.4. Results

MTT assay

Effect of SPP on viability of C2C12(A) and 3T3-L1(B) cells were shown in Figure 2. In C2C12 cells, there were statistically differences from the 24h, the 90 mg/L SPP significantly increased cell viability by 27.3% ($p<0.05$) and 180 mg/L SPP significantly decreased cell viability by 21.1% ($p<0.05$) compared to the CTRL group. When the cells were treated in SPP medium for 48 h, the cell exhibited 15.8% ($p<0.05$) increase and 62.6% ($p<0.05$) decrease by 30 mg/L SPP and 180 mg/L SPP compared to the CTRL group. However, 30 mg/L SPP treatment still increased cell viability by 7.1% ($p<0.05$) while the cell viability was 22.5% ($p<0.05$) and 71.6% ($p<0.05$) decreased significantly in 90 mg/L SPP group and 180 mg/L SPP group compared to the CTRL group at 72 h. In 3T3-L1 cells, the cell viability was 5% ($p<0.05$) increased by 30 mg/L SPP compared to the CTRL group at 24h. However, after 48 h SPP treatment, the cell viabilities were 11.85% ($p<0.05$) and 20.74% ($p<0.05$) decreased by 90 mg/L and 180 mg/L SPP compared to the CTRL group. The cell viabilities were 38.03% ($p<0.05$) and 44.60% ($p<0.05$) decreased compared to the CTRL group, when the cells were treated by 90 mg/L and 180 mg/L SPP for 72h.

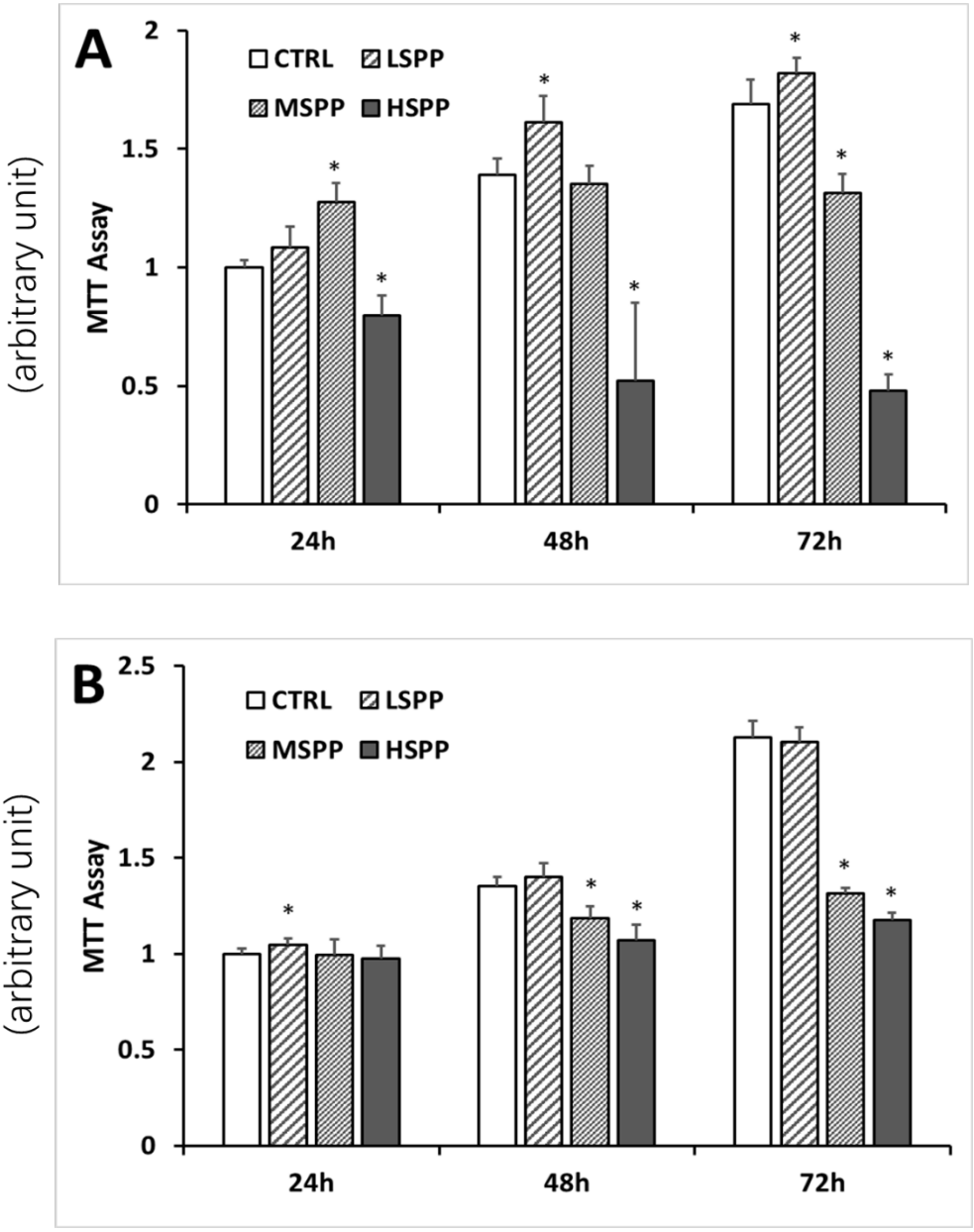


Figure 2. Dose- and time-dependent effects of SPP on cell viabilities

(A) Cells viability of C2C12 myoblasts was evaluated by MTT assay. Myoblasts were treated with four levels (0, 30, 90, 180 mg/L) of SPP for 24h, 48h and 72h.

(B) Cells viability of 3T3-L1 preadipocytes was evaluated by MTT assay. Preadipocytes were treated with four levels (0, 30, 90, 180 mg/L) of SPP for 24h, 48h and 72h.

Data were expressed relative to untreated control cells treated for 24h and values were mean ± SD (n=6).

*$p<0.05$ vs. control individually.

TBARS and antioxidant enzyme (SOD and GPx) activity

Effect of SPP on TBARS level and antioxidant enzyme activity in C2C12 cells and 3T3-L1 cells were shown in Table 3. In C2C12 cells, the GPx activity significantly increased in the HSPP group compared to the CTRL group, and SOD activity dose-dependently increased ($p<0.05$) in response to 90 and 180 mg/L SPP levels; on the other hand, in TBARS determinations, it showed significant dose-dependent decreases ($p<0.05$) of MDA contents by 90 and 180 mg/L SPP treatment. In 3T3-L1 cells, the activities of GPx and SOD were dose-dependently increased ($p<0.05$) in the MSPP and HSPP group compared to the CTRL group, while the TBARS level was also significantly decreased ($p<0.05$) by 90 and 180 mg/L SPP treatment.

Gene expression

Effect of SPP on antioxidant enzymes [CAT (A), GPx1 (B), Mn-SOD (C) and Cu/Zn-SOD (D)] gene expression in C2C12 cells and 3T3-L1 cells were shown in Figure 3. The CAT mRNA expressions were significantly increased ($p<0.05$) by 90 and 180 mg/L SPP in both C2C12 and 3T3-L1 cells, and the response was greater in C2C12 cells. The GPx1 mRNA expression was significantly raised ($p<0.05$) by 180 mg/L SPP in C2C12 cells, however, it was reduced ($p<0.05$) by LSPP and MSPP in 3T3-L1 cells. The mRNA expression of Mn-SOD was also enhanced($p<0.05$) in LSPP and MSPP groups in C2C12 cells, but not changed in 3T3-L1 cells compared to CTRL group. Similar as CAT mRNA expressions, the Cu/Zn-SOD mRNA expression significantly increased ($p<0.05$) by 90 and 180 mg/L SPP in C2C12 cells and 3T3-L1 cells.

Table 3. Effect of SPP on SOD and GPx Activity and TBARS of C2C12 cells and 3T3-L1 Cells

	CTRL	LSPP	MSPP	HSPP
C2C12				
GPx (nmol/min/mg protein)	6.41 ± 0.37	6.27 ± 0.43	6.79 ± 1.24	7.18 ± 0.42*
SOD (%/ug protein)	0.55 ± 0.33	0.77 ± 0.21	0.97 ± 0.29*	1.43 ± 0.64*
TBARS (uM/mg protein)	28.66 ± 3.71	26.02 ± 1.39	23.91 ± 2.21*	22.29 ± 2.13**
3T3-L1				
GPx (nmol/min/mg protein)	15.40 ± 2.03	15.06 ± 2.37	19.58 ± 2.32*	18.23 ± 2.04*
SOD (%/ug protein)	0.13 ± 0.01	0.13 ± 0.01	0.16 ± 0.01*	0.15 ± 0.02*
TBARS (uM/mg protein)	60.80 ± 8.48	41.51 ± 7.69**	39.82 ± 6.45**	33.10 ± 1.21**

C2C12 Myotubes and 3T3-L1 adipocytes were treated with four levels (0, 30, 90, 180 mg/L) of SPP for 48h.

Data were expressed as mean ± SD (n=6). *$p<0.05$ vs. control individually.

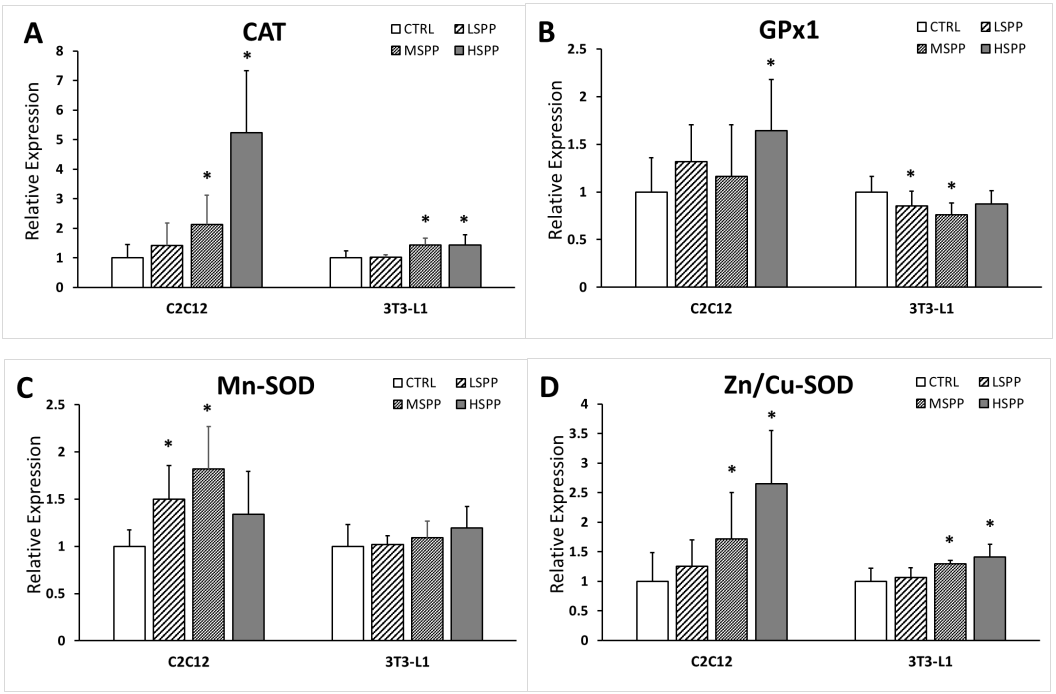


Figure 3. The antioxidant enzymes mRNA expression in C2C12 cells and 3T3-L1 cells

The mRNA expression of antioxidant enzymes [CAT (A), GPx1 (B), Mn-SOD (C) and Cu/Zn-SOD (D)] was measured by qRT-PCR. C2C12 Myotubes and 3T3-L1 adipocytes were treated with four levels (0, 30, 90, 180 mg/L) of SPP for 48h.

The data were monitored by GAPDH and relative to untreated control group.

Values were mean ± SD (n=6).

*$p<0.05$ vs. control.

3.5. Discussion

Reactive oxygen species (ROS) includes superoxide anions (O^{2-}), hydroxyl radical (OH^-), singlet oxygen, hydrogen peroxide (H_2O_2), ferric ion, nitric oxide (NO) etc. Excessive production of free radicals leads to oxidative stress. Free radicals have been implicated in causation of ailments such as cancer, inflammation, diabetes, liver cirrhosis, cardiovascular disease, Alzheimer's, aging and acquired immunodeficiency syndrome (Marx, 1987; Joyce, 1987; Kumar, 2011; Ci et al., 2011).

The diseases associated with the ROS mainly depend on the balance of the pro-oxidant and the antioxidant concentration in the body. Reactive oxygen species (ROS) inactivate enzymes and damage important cellular components causing tissue injury through covalent binding and lipid peroxidation (Geesin et al., 1990). Plants and other organisms have evolved a wide range of mechanisms to contend with this problem, with a variety of antioxidant molecules and enzyme (Pankaj et al., 2007). The polyphenols extracted from plants are widely used in medicine, cosmetics, sunscreens, and food products. In this study, we investigate the effects of SPP on intracellular antioxidant, including TBARS level, antioxidant enzymes (SOD and GPx) activities and the gene expressions of antioxidant-related enzymes. The SPP shows high activities of antioxidant properties in both C2C12 cells and 3T3-L1 cells.

The significance of antioxidant enzyme upregulation is widely studied, such as, increase in catalase and glutathione peroxidase (GPx) activities can keep H_2O_2 concentration in check, whereas upregulation of superoxide dismutase (SOD) reduces the possibility of forming hydroxyl radical through Haber–Weise reaction (Ji, 2007). Catalase is an antioxidant enzyme abundant in various tissues, and it protect cells against potentially harmful effects of the hydrogen (Deisseroth & Dounce, 1970). Okuno et al. (2008) reported that adipose tissue expresses catalase on the level comparable to liver and kidney.

In this study, the SPP (90mg/L and 180mg/L) significantly increased the mRNA expression of catalase in both C2C12 cells and 3T3-L1 cells. This result is also agreed with Kim et al. (2011), reported that the polyphenol-enriched red ginseng extracts at 1.0 mg/ml increased the mRNA expression of catalase in C2C12 cells.

SOD plays a role in modulating ROS and highly sensitive to SPP. Faraci & Didion (2004) reported

that the SOD is a major antioxidant enzyme, which protects cells from the harmful effects of superoxide by accelerating the dismutating superoxide. Our result indicated that the SPP (90 mg/L and 180 mg/L) treatments led to significantly increases in SOD activity in C2C12 cells and 3T3-L1 cells. The MSPP and HSPP significantly increased the Cu/Zn-SOD mRNA expression in C2C12 cells and 3T3-L1 cells, while the LSPP and MSPP also enhanced the mRNA expression of Mn-SOD in C2C12 cells, but not changed in 3T3-L1 cells.

Another *in vitro* experiment revealed that apple polyphenols treatment strongly suppressed the production of hydrogen peroxide induced by reactive oxygen species (ROS) in C2C12 myoblast cells and endogenous superoxide production in Mn-SOD-deficient cells (Sunagawa et al., 2012).

GPx plays an important role in muscle cells to remove H_2O_2 and lipid peroxide. In our study, the HSPP group showed significant increase in GPx activity in C2C12 cells and 3T3-L1 cells. The GPx1 mRNA expression was significantly raised ($p<0.05$) by HSPP in C2C12 cells, however, it was reduced ($p<0.05$) by LSPP and MSPP in 3T3-L1 cells.

In the present study, TBARS levels, a marker of lipid peroxidation, were also measured. It showed significant dose-dependent decreases of MDA contents by SPP treatment in both C2C12 cells and 3T3-L1 cells. Goutzourelas et al. (2014) found the polyphenolic grape extract treatment reduced ROS and TBARS significantly in C2C12 cells when treated for 24h. These results were agreed with another study investigated the effects of the extracts of an orange-fleshed sweet potato on oxidative stress biomarkers (glutathione status and lipid peroxidation) and activities of antioxidant enzymes (catalase, CAT and glutathione peroxidase, GPx) in palmitate-induced insulin resistant C2C12 cells. The results showed a significant increase in intracellular GSH level, a significant reduction in the level of malonaldehyde and a significant improvement in the intracellular antioxidant status (Ayeleso et al., 2018).

Conclusion

The SPP extracts improved the antioxidant status in C2C12 cells and 3T3-L1 cells, due to the increased enzymatic activities and gene expression of antioxidant-related enzymes and the reduced TBARS levels.

CHAPTER 4: Effects of polyphenol extracts on lipid metabolism in 3T3-L1 cells

4.1. Summary

Obesity is a condition in which adipocytes accumulate a large amount of fat and become enlarged，which is characterized by an increase in the number and size of adipocytes differentiated from fibroblastic preadipocytes in adipose tissue. In this chapter, the effects of SPP on lipid metabolism in 3T3-L1 cells were investigated. 3T3-L1 preadipocytes were plated at 6-well plates and divided into 4 groups (6 wells/group): CTRL group (0 mg/L SPP), LSPP group (30 mg/L SPP), MSPP group (90 mg/L SPP), HSPP group (180 mg/L SPP). In order to clarify the acting stage of SPP in the development of adipocyte, cell cultures were supplemented with SPP at different stages. Compared to the control cells, the 90 mg/L SPP and 180 mg/L SPP significantly decreased the TG content in 3T3-L1 adipocytes when the treatment period were Day 0-2, Day 4-6 and Day 0-6. To examine the mRNA expressions of lipid metabolism-related genes, cell cultures were supplemented with SPP during all the differentiation period (6 days). The 90 mg/L SPP significantly decreased the mRNA expressions of GLUT4, FAS, ACC and increased the mRNA expression of CPT1a compared to the control group. In addition, the mRNA expressions of INS-R, GLUT4, FAS, ACC and PPAR-γ were significantly decreased and the mRNA expression of CPT1a was significantly increased in 90 mg/L SPP group compared to those in 30 mg/L SPP group. In conclusion, the SPP extracts showed strong dose-dependent inhibition of accumulation of triglyceride formation by decreasing the insulin-mediated Akt phosphorylation and fatty acid synthesis while the transfer of the acyl was activated during the differentiation of 3T3-L1 preadipocytes into adipocytes.

4.2. Introduction

Currently, obesity has become the leading metabolic disease in the world. Obesity is a condition in which adipocytes accumulate a large amount of fat and become enlarged, which is characterized by an increase in the number and size of adipocytes differentiated from fibroblastic preadipocytes in adipose tissue (Furuyashiki et al., 2004). This is a complex process regulated by genetic, endocrine, metabolic, neurological, pharmacological, environmental, and nutritional factors (Fu et al., 2005; Farmer & Auwerx, 2004; Unger & Zhou, 2001).

There was a study suggested the inhibition of lipid accumulation by EGCG treatment during adipocyte differentiation was associated with a tight regulatory cross-talk between early adipogenic marker gene (PPARγ1/2) and late target gene (LXRα). In addition, the early treatment with EGCG (day 7) down-regulated expression of PPARγ1/2, whereas additional treatment (day 12) had an inhibitory effect on expression of the PPARγ target gene (LXRα). The inhibitory effect of EGCG on expression of these genes reflects the subsequent attenuation of lipid accumulation in mature adipocytes (Moon et al., 2007).

The adipocyte is the primary site for energy storage and accumulates triglycerides during nutritional excess. Many epidemiological studies have indicated that consumption of some foodstuffs and drinks with high phenolic content is associated with the prevention of some diseases (Hertog, 1995; Salah, 1995; Scalbert & Williamson, 2000). For example, a crude apple polyphenol fraction partially purified from unripe apples was found to inhibit adipose differentiation of preadipocytes into adipocytes in mouse fibroblast 3T3-L1 cells (Shoji et al., 2000).

In vivo studies have indicated that intake of phenolic compounds can be beneficial for the inhibition of a high fat diet-induced obesity in overweight and genetically obese laboratory animals (Meydani & Hasan, 2010). Results from such studies may be related to the mechanism by which phenolic compounds prevent obesity in humans. These studies also provide initial evidence for phenolic compounds possibly being useful in the treatment of obesity and raise the possibility of a new application of phenolic compounds as a health supplement (Hsu & Yen, 2008). Obesity has been also shown to be one of the conditions that decrease antioxidant capacity (Asayama et al., 2001; Carmiel-Haggai et al., 2005) and it seems to accomplish this by lowering the levels of antioxidant enzymes (catalase, glutathione peroxidase,

and glutathione reductase).

Thus, due to the good performance of SPP as antioxidant in chapter 2 and chapter 3, in the present study, I investigated as following:

1. The triglyceride (TG) content of different periods in 3T3-L1 cell differentiation;
2. The 3T3-L1 cell staining experiment;
3. The gene expression related to adipocyte differentiation in 3T3-L1 cell.

4.3. Materials and methods

Preparation and treatment of 3T3-L1 cell culture

3T3-L1 preadipocytes were plated on 6-well plates and divided into 4 groups (6 wells/group): CTRL group (0 mg/L SPP), LSPP group (30 mg/L SPP), MSPP group (90 mg/L SPP), HSPP group (180 mg/L SPP). After cells complete confluent, change the medium to differentiation induction: Day 0-2 10% FBS medium with insulin (5 μg/mL), dexamethasone (1 μM) and 3-isobutyl-1-methylxanthine (500 μM); Day 2-4 10% FBS medium with insulin (5 μg/mL); Day 4-6 10% FBS medium only and the medium was changed every 2 days. The CTRL group was maintained in the basic maintenance medium without SPP. In order to clear the acting stage of SPP in the development of adipocyte, the TG contents of stage-SPP-supplemented (Day 0-2, Day 2-4, Day 4-6, Day 0-6) cells were determined. The cell cultures were supplemented with SPP at different stages as shown in Figure 4.

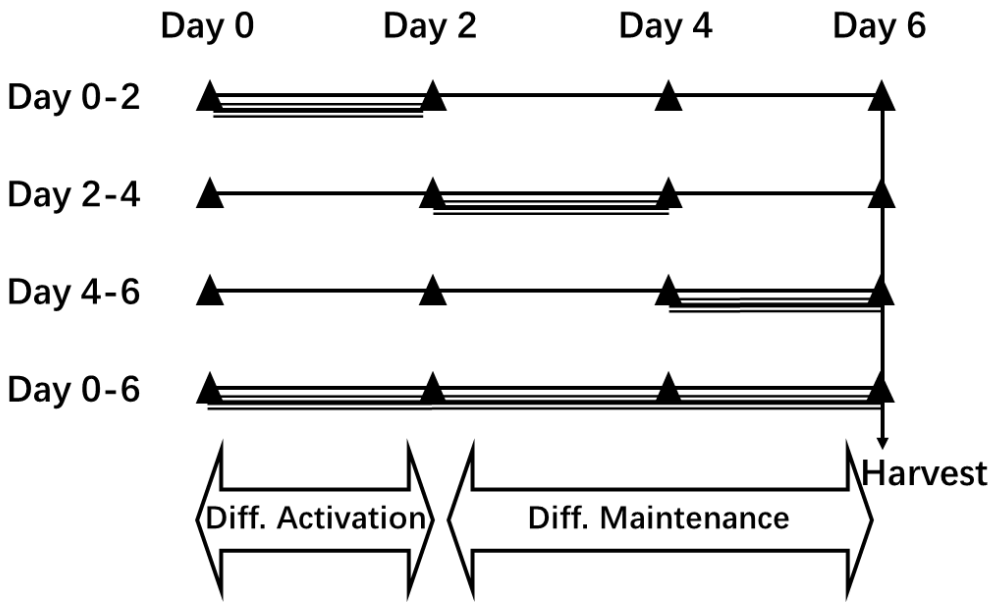


Figure 4. Treatment schedule of SPP in 3T3-L1 cells

Triglyceride (TG) analysis and staining in 3T3-L1 cell

In the end of each treatment period, the cells were washed with PBS solution and harvested into tubes. TG content was determined using a commercial kit (Triglyceride Colorimetric Assay Kit, Cayman Chemical, USA). Total cellular protein content was determined by Lowry's method (Lowry et al., 1951).

Oil-Red-O staining: cells were washed with PBS solution and fixed in 4% paraformaldehyde for 30 min. Then cells were washed twice with ddH_2O and once with 60% isopropanol. The plates were dried for 30 min and then cells were stained with filtered Oil-Red-O working solution (stock solution: Oil-Red-O 3 mg/mL in isopropanol; working solution: 60% Oil-Red-O stock solution and 40% ddH_2O for 8 min and washed 4 times with ddH_2O, finally plates were dried and scanned for images.

RNA extraction and quantitative real-time PCR

Total RNA was isolated from 3T3-L1 adipocytes using IsogenII reagent (Nippon Gene, Japan) as described in the manufacturer's protocol. RNA (40 ng/μL) was used for reverse transcription with PrimerScript^{TM} RT Master Mix (TAKARA, Japan). The primers used in this study were listed in Table 4. Gene expression was determined by real-time PCR using 7300 Real-Time PCR system (Applied Biosystems, USA) with SYBR Select Master Mix (Applied Biosystems, USA). The thermal cycles were shown as follow: 1 cycle at 50℃ for 2 min, 95℃ for 2 min; 60 cycles at 95℃ for 15sec, 60℃ for 15sec, 72℃ for 2 min; 1 cycle at 95℃ for 15sec, 60℃ for 1 min, 95℃ for 15sec, 60℃ for 15sec.

Statistical analysis

Results are expressed as means ± standard deviation. The data were analyzed by one-way analysis of variance (ANOVA) using IBM SPSS Statistics 25 software. Tukey's test was further performed, when significant differences were found ($p < 0.05$). Significant differences were based on $p < 0.05$.

Table 4. Primer sequences

Gene	Description	Primer sequence (5'-3')
GAPDH	Glyceraldehydes-3-phosphate dehydrogenase	F: 5'-AAATGGTGAAGGTCGGTGTG-3' R: 5'-TGAAGGGGTCGTTGATGG-3'
18S	18S ribosomal RNA	F: 5'-ACTCAACACGGGAAACCTCACC-3' R: 5'-CCAGACAAATCGCTCCACCAAC-3'
INS-R	Insulin receptor substrate	F: 5'-CAGCTCGAAACTGCATGGTTG-3' R: 5'-GGTGACATCCACCTCACAGGAA-3'
GLUT4	Glucose transporter type 4	F: 5'-GATGCCGTCGGGTTTCCAGCA-3' R: 5'-TGAGGGTGCCTTGTGGGATGG-3'
FAS	Fatty acid synthase	F: 5'-GGAGGTGGTGATAGCCGGTAT-3' R: 5'-TGGGTAATCCATAGAGCCCAG-3'
ACC	Acetyl-CoA carboxylase	F: 5'-ATGGGCGGAATGGTCTCTTTC-3' R: 5'-TGGGGACCTTGTCTTCATCAT-3'
PPAR-γ	Peroxisome proliferator activated receptor γ	F: 5'-AGGCCGAGAAGGAGAAGCTGTTG-3' R: 5'-TGGCCACCTCTTTGCTGTGCTC-3'
c/EBPα	CCAAT/enhancer binding protein	F: 5'-TGGACAAGAACAGCAACGAGTAC-3' R: 5'-GCAGTTGCCCATGGCCTTGAC-3'
SREBP1c	Sterol regulatory element binding protein-1c	F: 5'-GATGTGCGAACTGGACACAG-3' R: 5'-CATAGGGGGCGTCAAACAG-3'
CPT1a	Carnitine plamitoytransferase 1-a	F: 5'-CTCCGCCTGAGCCATGAAG-3' R: 5'-CACCAGTGATGATGCCATTCT-3'
CPT1b	Carnitine plamitoytransferase 1-b	F: 5'-CAGCACAGCATCGTACCCA-3' R: 5'-TCCCAATGCCGTTCTCAAAAT-3'

4.4. Results

Effects of SPP on TG content in 3T3-L1 cells

The MSPP and HSPP decreased the amount of lipid in 3T3-L1 adipocytes. This observed reduction in lipid accumulation was confirmed by TG content assay. As shown in Table 5, the TG contents showed dose-dependent lipidosis inhibition by SPP treatments. Compared to the CTRL group, the LSPP did not affect the lipid accumulation in the different SPP treatment period. However, compared to the CTRL cells, the MSPP and HSPP significantly decreased ($p<0.05$) the TG content in 3T3-L1 adipocytes by 40.0% and 80.0%, 19.2% and 42.3%, 11.9% and 54.8%, respectively when the treatment period were Day 0-2, Day 4-6 and Day 0-6. The effect of SPP on the storage of intracellular lipid in 3T3-L1 adipocytes was visualized by Oil Red O staining as shown in Figure 5.

Effects of SPP on lipid metabolism related gene expressions in 3T3-L1 cells

After 6 days treatments of SPP, the mRNA expressions of INS-R, GLUT4, FAS, ACC, PPAR-γ, C/EBPα, SREBP-1c, CPT1a and CPT1b were determined and presented in Figure 6. These mRNA expressions were not affected by LSPP, whereas the MSPP significantly decreased ($p<0.05$) the mRNA expressions of GLUT4, FAS, ACC and increased ($p<0.05$) the mRNA expression of CPT1a compared to the CTRL group. In addition, the mRNA expressions of INS-R, GLUT4, FAS, ACC and PPAR-γ were significantly decreased ($p<0.05$) and the mRNA expression of CPT1a was significantly increased ($p<0.05$) in MSPP group compared to those in LSPP group.

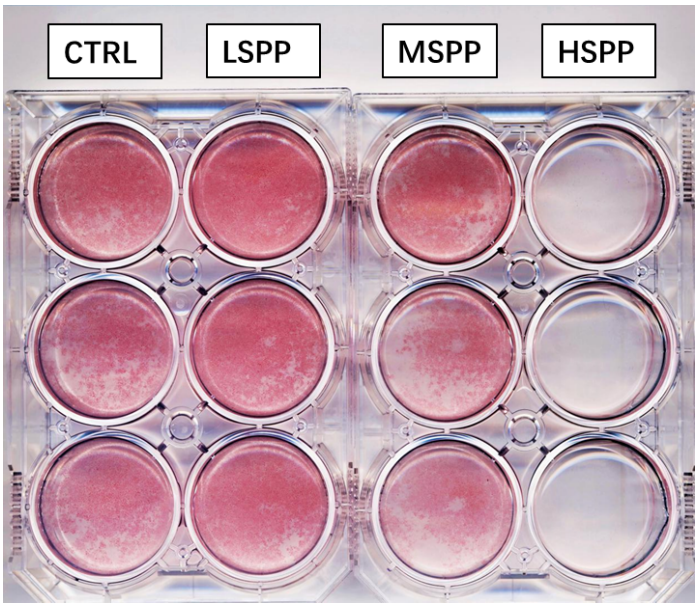
Table 5. TG (mg/g protein) content in 3T3-L1 cells

Treatment period	CTRL	LSPP	MSPP	HSPP
Day 0-2	0.25 ± 0.03[a]	0.28 ± 0.04[a]	0.15 ± 0.03[b]	0.05 ± 0.02[c]
Day 2-4	0.25 ± 0.10	0.25 ± 0.03	0.22 ± 0.05	0.20 ± 0.02
Day 4-6	0.26 ± 0.02[a]	0.23 ± 0.03[ab]	0.21 ± 0.02[b]	0.15 ± 0.03[c]
Day 0-6	0.25 ± 0.03[a]	0.26 ± 0.02[a]	0.22 ± 0.01[b]	0.11 ± 0.02[c]

TG content corrected by total cellular protein as mean ± standard deviation (n =6).

a–c Mean values that do not share a common superscript letter are significantly different ($p < 0.05$).

(A)



(B)

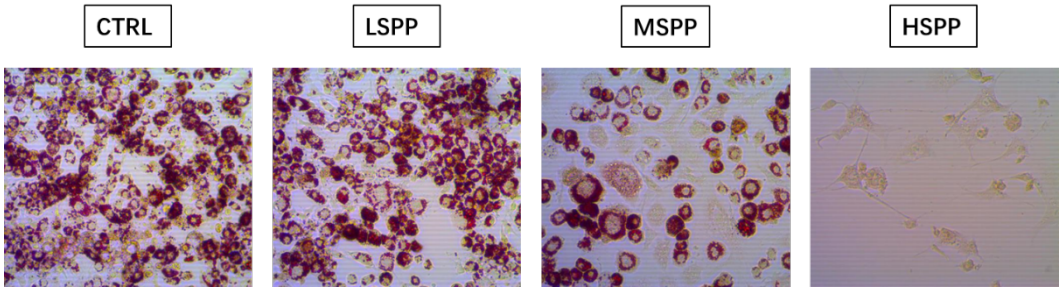


Figure 5. 3T3-L1 cells staining with Oil-Red O

(A) 3T3-L1 cells were treated with SPP for 6 days and stained with Oil-Red O on day 6.

(B) 3T3-L1 cells stained with Oil-Red O under the microscope (20×).

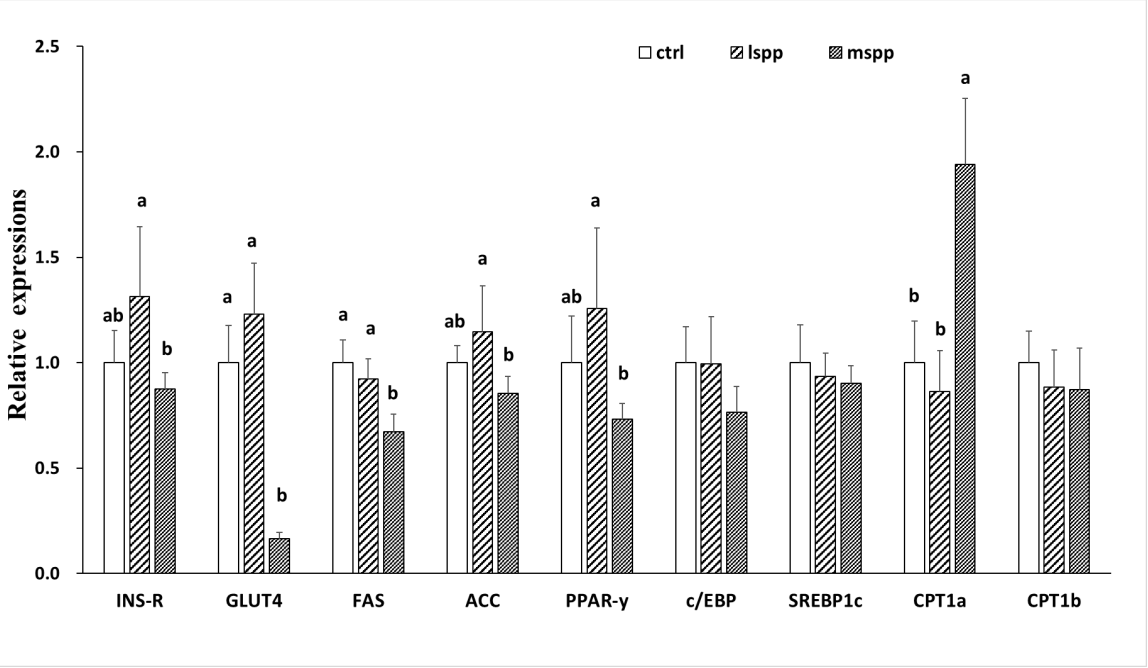


Figure 6. Lipid metabolism-related gene expression in 3T3-L1 cells

The mRNA expressions of lipid metabolism-related genes was measured by RT-PCR. The cells were treated with three levels (0, 30, 90 mg/L) of SPP for 6 days.

The data were monitored by 18S and relative to untreated control group.

Values were mean ± SD (n=6).

a–b Mean values that do not share a common superscript letter are significantly different ($p < 0.05$).

4.5. Discussion

From a reductionist's point of view, obesity in mammals results from an imbalance between the rates of fat synthesis and fat catabolism in white adipose tissue (Schweiger et al., 2017). This concept finds strong support in studies with stable isotopes showing that expansion of fat mass in obese individuals results from increased TG synthesis and decreased TG breakdown in white adipose tissue (Arner et al., 2011).

In this study, the TG contents showed strong dose-dependent lipidosis inhibition by SPP treatments in 3T3-L1 cells compared to those in the control cells. Especially in the differentiation activation period (Day 0-2), the 90 mg/L SPP and 180 mg/L SPP significantly decreased ($p<0.05$) the TG content by 40.0% and 80.0% in 3T3-L1 adipocytes. However, in the differentiation maintenance period (Day 2-6), the TG contents were decreased significantly 19.2% and 42.3% by 90mg/L and 180mg/L SPP treated on Day 4-6, while there was no effect when the SPP treatment kept during Day 2-4. When the cells cultured with SPP for the whole differentiation period (Day 0-6), 11.9% and 54.8% TG content were inhibited by 90mg/L and 180mg/L SPP treatments.

An increasing number of studies showing similar results reported that the effects of polyphenols extracted from plants on the triglyceride accumulation in 3T3-L1 cells. Rejman & Kozubek (2004) mentioned the 5-n-alkylresorcinols isolated from wheat and rye bran inhibit glycerol-3-phosphate dehydrogenase and prevent triglyceride accumulation in 3T3-L1 cells.

Mosqueda-Solís et al. (2017) analyzed the antiadipogenic effect of fifteen phenolic compounds from various chemical groups in 3T3-L1 pre-adipocytes, in which the cells treated with 25 μM, 10 μM or 1 μM of apigenin, luteolin, catechin, epicatechin, epigallocatechin, genistein, daizein, naringenin, hesperidin, quercetin, kaempferol, resveratrol, vanillic acid, piceatannol and pterostilbene for 8 days. The results showed that at 25 μM lipid accumulation was reduced by all the compounds, except for catechin, epicatechin and epigallocatechin. At a dose of 10 μM apigenin, luteolin, naringenin, hesperidin, quercetin and kaempferol induced significant reductions, and at 1 μM only naringenin, hesperidin and quercetin were effective.

Lipid accumulation and adipocyte differentiation are associated with the occurrence and development of obesity (Jeon et al. 2004). A reduction of adiposity is related to the inhibition of angiogenesis along with a reduction of adipocyte numbers and the lipid content of adipocytes. The differentiation of preadipocytes into adipocytes is regulated by a complex network of coordinated regulation of gene expression. Adipogenic transcription factors such as the CCAAT/enhancer binding protein-β (C/EBPβ), nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ), and CCAAT/enhancer binding protein-α (C/EBPα) play a key role in the complex transcriptional cascade that occurs during adipogenesis (Cristancho & Lazar, 2011).

It is well established that the activation of the Akt pathway plays a major role in adipocyte differentiation in which insulin and certain growth factors stimulate adipogenesis. Additionally the overexpression of constitutively active Akt increases glucose uptake and adipocyte differentiation in 3T3-L1 adipocytes (Xu & Liao, 2004). Akt phosphorylates and regulates a number of substrates involved in a diverse array of biological processes and is essential to induce PPAR expression (Green et al., 2008; Peng et al., 2003). The C/EBPα gene and PPAR gene are necessary for the expression of adipocyte-specific genes, such as adipocyte protein (aP2), lipoprotein lipase (LPL), leptin, adiponectin and fatty acid synthase (FAS), which lead to morphological changes and lipid accumulation within the cells (Gregoire et al.,1988).

In the present study, the 3T3-L1 cells were treated with SPP in the whole differentiation period (Day 0-6), then the mRNA expressions of INS-R, GLUT4, FAS, ACC, PPAR-γ, C/EBPα, SREBP-1c, CPT1a and CPT1b were determined. We found the mRNA expressions of GLUT4, FAS and ACC were significantly higher in the 90 mg/L SPP group than those in the CTRL group. Moreover, the mRNA expressions of INS-R, GLUT4, FAS, ACC and PPAR-γ were significantly lower and the mRNA expression of CPT1b was significantly higher in 90 mg/L SPP group than those in 30mg/L SPP group.

CPT1 is a mitochondrial enzyme responsible for the formation of acyl carnitines by catalyzing the transfer of the acyl group of a long-chain fatty acyl-CoA from coenzyme A to L-carnitine, which can be inhibited by malonyl CoA, the first committed intermediate produced during fatty acid synthesis. In this study, the mRNA expression of CPT1b was significantly increased by 90 mg/L SPP compared to control and 30mg/L SPP.

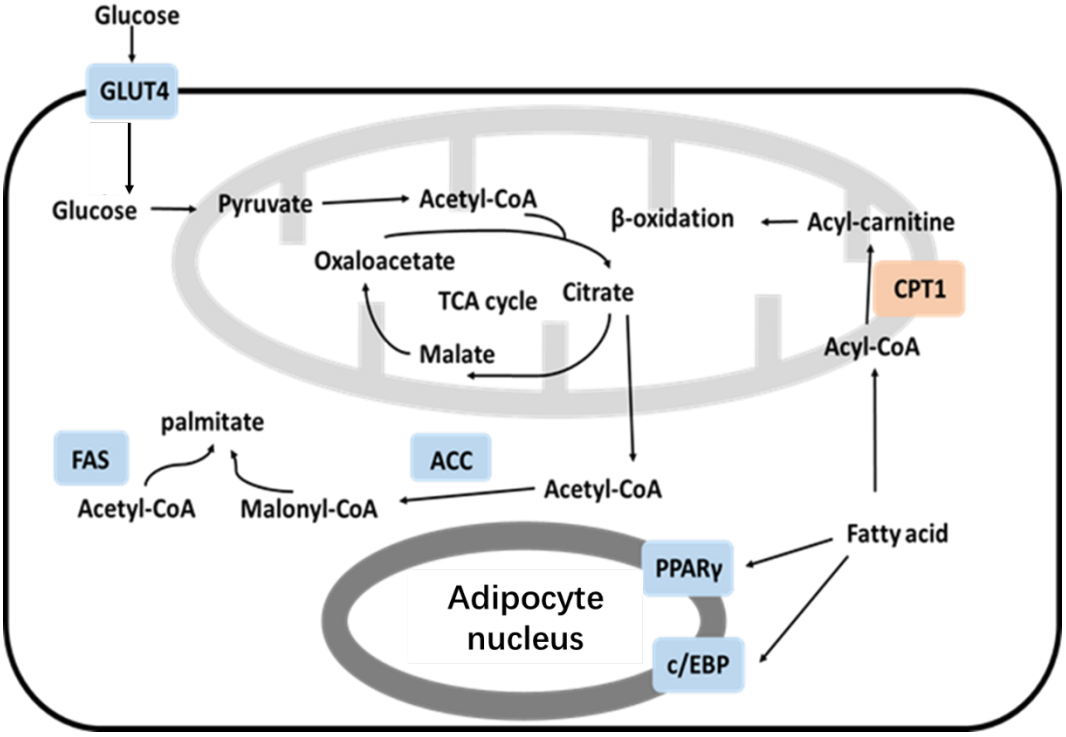


Figure 7. Lipid metabolism pathway

Therefore, our results strongly demonstrated that insulin-mediated Akt phosphorylation and fatty acid synthesis were inhibited while the transfer of the acyl was activated by SPP treatment, which mainly affected the reduced accumulation of triglyceride formation during the differentiation of 3T3-L1 preadipocytes into adipocytes.

Many researches supported that plant polyphenols were benefit to inhibit the adipocyte accumulation. Anthocyanin fractions extracted from purple sweet potato (at doses of 50, 100, 200 μg/mL) were nontoxic, but dose-dependently reduced the accumulation of the intracellular lipid, the triglyceride and total cholesterols in glucose-treated HepG2 cells and, at the molecular level, anthocyanin fractions reduced levels of lipid metabolism-related proteins (FAS and SREBP-1), which was associated with adenosine monophosphate-activated protein kinase (AMPK) signaling pathways (Hwang et al., 2011). Lao et al. (2015) found the green tea polyphenols exerted greater inhibitory effects than purified EGCG on adipogenesis through down-regulating the adipogenic factor C/EBPα, SREBP-1c and PPAR-γ expression which is safer and more effective than EGCG alone for preventing obesity and obesity-related chronic diseases. Blueberry extracts decreased lipid accumulation and adipogenic gene expression by inhibition of the PI3K/Akt/GSK3β pathway during 3T3-L1 preadipocytes differentiated into adipocytes (Song et al., 2013). The polyphenol extract (mainly contains chlorogenic acid) from the dried leaves was the most effective (50 μg/mL) in the inhibition of triglyceride accumulation in 3T3-L1 adipocytes, and rutin (100 μg/mL) likely accounted for a large portion of this activity. Additionally, mate extracts had a modulatory effect on the expression of genes related to the adipogenesis as PPARγ2, leptin, TNF-α and C/EBPα (Gosmann et al., 2012).

Conclusion

The SPP extracts showed strong dose-dependent inhibition of accumulation of triglyceride formation by decreasing the insulin-mediated Akt phosphorylation and fatty acid synthesis while the transfer of the acyl was activated during the differentiation of 3T3-L1 preadipocytes into adipocytes.

CHAPTER 5: Effects of polyphenol extracts on antioxidant status and lipid metabolism in mouse

5.1. Summary

Lipid metabolism disorder is an important risk factor to obesity. Plant polyphenol plays an important role in maintaining or improving lipid profile in a variety of ways, including regulating cholesterol absorption, inhibiting synthesis and secretion of triglyceride, and lowering plasma low density lipoprotein oxidation, etc. This chapter elucidated whether dietary SPP might ameliorate HFD-induced adiposity in C57BL/6 mice. Twenty-four 3-week old male C57BL/6 mice were divided into 4 groups randomly of 6 animals allocated for each treatment. CTRL was normal diet; HFD was high fat diet; LSPP was high fat diet added 0.5% SPP; HSPP was high fat diet added 1% SPP. The mice fed with HFD significantly induced greater body weight compared with the CTRL mice, however, the mice fed with HFD+1% SPP reduced the body weight gain compared to HFD mice. Similarly, the epididymal fat and omental fat weights from mice in the HFD group were significantly higher than those in the normal diet group and the omental fat weights from mice fed the HFD + 1% SPP exhibited a significant reduction compared to the HFD group. The concentrations of TCHO and HDL-C in plasma of mice fed HFD + 1% SPP group were significantly lower than those of the HFD group and 0.5% SPP group. Moreover, in the HFD + 1% SPP groups, the mRNA expressions of GPx-1 in liver and CPT1b in perirenal fat were significantly upregulated compared to the HFD group. In conclusion, the SPP extract reduced the body weight gain and fat tissue weight of mice fed with HFD meal by activating the long-chain fatty acid β-oxidation pathway in mitochondria. In addition, the SPP extract improve the antioxidant status of HFD mice due to increasing the mRNA expression of GPx1 gene in the liver.

5.2. Introduction

Lipid metabolism disorder is an important risk factor to obesity, hyperlipidemia and type 2 diabetes as well as other chronic metabolic disease, which are the most prevailing nutrition-related issues in the world. It is also a key target in preventing metabolic syndrome, chronic disease prevention. Plant polyphenol plays an important role in maintaining or improving lipid profile in a variety of ways, including regulating cholesterol absorption, inhibiting synthesis and secretion of triglyceride, and lowering plasma low density lipoprotein oxidation, etc. Therefore, one potential dietary strategy to reduce glucose intolerance and inflammation is consumption of polyphenol-rich plants or their by-products (Ali et al, 2014). In human study, high intake of polyphenol-rich foods such as nuts, fruits, vegetables, seasoning with aromatic plants, spices, and virgin olive oil may be the cornerstone of a healthy diet preventing the development and progression of metabolic syndrome, although there is no polyphenol or polyphenol-rich food able to influence all metabolic syndrome features (Chiva-Blanch & Badimon, 2017). There are also animal and human studies showed that polyphenols modulate carbohydrate and lipid metabolism, decrease glycemia and insulin resistance, increase lipid metabolism and optimize oxidative processes (Dragan et al., 2015). For example, Park et al. (2013) showed that the polyphenol-rich grape skin extract (GSE) supplementation significantly suppressed the activities of lipogenic enzymes in both adipose and liver tissues, which was concomitant with β-oxidation activation. Furthermore, GSE reversed the high-fatdiet induced changes of the expression of genes involved in lipogenesis and β-oxidation in the liver.

However, the potential anti-obesity effects of sweet potato polyphenols (SPP) still remain unclear, and no studies have determined the effect of SPP on lipid metabolism of adipose tissue and liver in response to a high-fat diet (HFD). In general, a HFD is widely used in nutritional experiments as a good strategy to induce overweight and fat deposition in animals (Surwit et al., 1995).

In the chapter 4, the effects of polyphenol extracts on lipid metabolism in 3T3-L1 cells were examined in *in vitro* experiments. The inhibition of preadipocyte population growth by some phenolic acids (chlorogenic acid, gallic acid, *o*-coumaric acid and *m*-coumaric acid) might have further implication in *in vivo* anti-obesity effects (Hsu et al., 2006).

Therefore, in this chapter, I elucidated whether dietary SPP might ameliorate HFD-induced adiposity in C57BL/6 mice.

5.3. Materials and methods

Animals and diets

Twenty-four 3-week old male C57BL/6 mice (purchased from Japan SLC, Inc. Hamamatsu, Japan) were divided into 4 groups randomly of 6 animals allocated for each treatment. CTRL was normal diet with 5% corn oil; HFD was high fat diet with 15% corn oil; LSPP was high fat diet (15% corn oil) added 0.5% SPP; HSPP was high fat diet (15% corn oil) added 1% SPP. Adaptation period was one week and the mice were fed with commercial diet. The diets in experimental period were shown in Table 6. Mice were kept in plastic cages individually maintained at a 24℃ constant temperature room on 12h light-dark cycle. Mice were food deprived for 6 h prior to killing after 11 weeks of treatment. Mice were weighed and killed by CO_2 overdose. Blood was collected from cardiac puncture using a heparinized syringe. The liver and adipose tissues were also collected: washed by 1.15% (w/v) KCl, weighed, immediately frozen into liquid nitrogen, and finally kept at -80℃ until determine.

Table 6. Composition of experimental diets (g/100g diet).

	CTRL	HFD	LSPP	HSPP
Casein	20.0	20.0	20.0	20.0
Corn starch	25.0	15.0	15.0	15.0
Sucrose	38.0	38.0	37.5	37.0
Corn oil	5.0	15.0	15.0	15.0
Cellulose	7.0	7.0	7.0	7.0
Mineral mixture[1]	3.5	3.5	3.5	3.5
Vitamin mixture[2]	1.0	1.0	1.0	1.0
DL-Met	0.3	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2	0.2
SPP			0.5	1.0

[1]Mineral mixture: $CaCO_3$ 35.7%, KH_22PO_4 19.6%, $K_3C_6H_5O_7 \cdot H_2O$ 7.078%, NaCl 7.4%, K_2SO_4 4.66%, MgO 2.4%, $FeC_6H_5O_7 \cdot H_2O$ 0.606%, $5ZnO \cdot 2CO_2 \cdot H_2O$ 0.165%, $MnCO_3$ 0.03%, $CuCO_3Cu(OH)_2 \cdot H_2O$ 0.03% KIO_3 0.001%, Na_2SeO_4 0.001025%, $(NH_4)6Mo_7O_{24} \cdot 4H_2O$ 0.000795%, $Na_2SiO_3 \cdot 9H_2O$ 0.145%, $CrK(SO_4)_2 \cdot 12H_2O$ 0.0275%, H_3BO_3 0.00815%, NaF 0.00635%, $NiCO_3 \cdot 2Ni(OH_2) \cdot 4H_2O$ 0.00318%, LiCl 0.00174%, NH_4VO_3 0.00066%, granulated sugar 22.1026%.

[2]Vitamin mixture: nicotinic acid 0.30 %, Dl-Calcium pantothenate 0.32%, vitamin B6 0.07%, vitamin B1 0.06%, vitamin B2 0.06%, folic acid, 0.02%, D-biotin (2%) 0.10%, vitamin B12 (0.1%) 0.25%, vitamin E (50%) 1.50%, vitamin A (500,000 IU/g) 0.08%, vitamin D3 (500,000 IU/g) 0.02%, vitamin K1 (phylloquinone) 0.0075%, granulated sugar 97.2125%.

Measurement of plasma parameters related to lipid and glucose metabolism

The plasma concentrations of TG, TC, HLD-C, GLU, GOT and GPT were measured by Fuji DRY-CHEM 3500 (Fuji Medical Systems, Tokyo, Japan) following the manufacturer's instructions.

TBARS level in mice liver

Malondialdehyde (MDA) in liver was determined colorimetrically as a 2-thiobarbituric acid-reactive substance as previously described (Ohkawa et al., 1979).

Gene expression

Total RNA was isolated from liver and perirenal fat tissue using Isogen II reagent (Nippon Gene, Japan) as described in the manufacturer's protocol. RNA (40 ng/μL) was used for reverse transcription with PrimerScript^{TM} RT Master Mix (TAKARA, Japan). The primers used in this study were listed in Table 4. Gene expression was determined by real-time PCR using 7300 Real-Time PCR system (Applied Biosystems, USA) with SYBR Select Master Mix (Applied Biosystems, USA). The thermal cycles were shown as follow: 1 cycle at 50℃ for 2 min, 95℃ for 2 min; 60 cycles at 95℃ for 15sec, 60℃ for 15sec, 72℃ for 2 min; 1 cycle at 95℃ for 15sec, 60℃ for 1 min, 95℃ for 15sec, 60℃ for 15sec.

Statistical analysis

Results are expressed as means ± standard deviation. The data were analyzed by one-way analysis of variance (ANOVA) using IBM SPSS Statistics 25 software. Tukey's test was further performed, when significant differences were found ($p < 0.05$). Significant differences were based on $p < 0.05$.

5.4. Results

Effect of dietary SPP on feed intake and body weight of mice fed HFD meal.

The feed intake and body weight of mice were shown in Table7. After 11-week experiment, the mice fed with HFD induced greater body weight and the body weight gain was 35.48% higher ($p<0.05$) than that of the CTRL mice. Furthermore, the mice fed with HFD + 1% SPP (HSPP group) reduced the body weight gain by 18.77% ($p<0.05$) compared to that in the HFD group while there was no significant difference between the HSPP group and the CTRL group. The feed intake and FCR of three HFD groups were significantly lower ($p<0.05$) than that in the CTRL group.

Table 7. Feed intake and body weight

	CTRL	HFD	LSPP	HSPP
IBW (g)	17.71 ± 0.96	17.69 ± 0.92	17.70 ± 0.87	17.70 ± 0.85
FBW (g)	32.93 ± 1.84	38.32 ± 2.02	36.67 ± 2.59	34.64 ± 1.43
BWG (g)	15.22 ± 1.30^{c}	20.62 ± 2.21^{a}	18.97 ± 2.40^{ab}	16.75 ± 0.89^{bc}
FI (g)	313.30 ± 47.99^{a}	258.13 ± 25.43^{b}	235.88 ± 13.42^{b}	227.43 ± 10.98^{b}
FCR[1]	20.71 ± 3.62^{a}	12.62 ± 1.73^{b}	12.55 ± 1.21^{b}	13.60 ± 0.82^{b}

Data are means ± standard deviation (n =6).

[1] Feed intake: body weight gain.

a–c Mean values that do not share a common superscript letter are significantly different ($p < 0.05$).

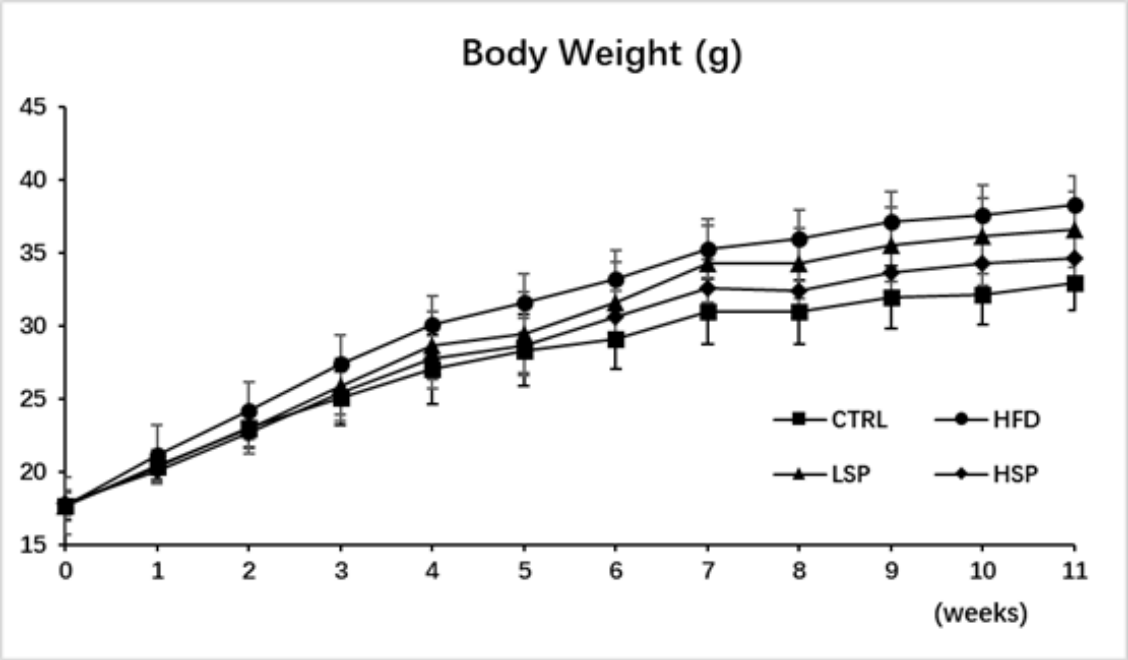


Figure 8. Changes in body weights of mice during the experimental period (11 weeks)

Data are means ± standard deviation (n =6).

Effect of dietary SPP on the organ weights of mice fed HFD meal.

The effects of SPP on the weights and the relative weights to body weight of liver, heart, leg, epididymal fat, perirenal fat, omental fat, subcutaneous fat and brown fat were shown in Table8. The epididymal fat and omental fat weights from mice in the HFD group were significantly higher ($P < 0.05$) than those in the normal diet group. However, the omental fat weights from mice in the HSPP group exhibited a significant reduction ($P < 0.05$) compared to the HFD group. There were no significant differences of the relative organ weights to body weight among the four groups.

Table 8. Organ weights

	CTRL	HFD	LSPP	HSPP
Organ weights (g)				
Liver	1.19 ± 0.15	1.30 ± 0.15	1.17 ± 0.09	1.10 ± 0.10
Heart	0.12 ± 0.01	0.13 ± 0.02	0.12 ± 0.01	0.12 ± 0.01
Leg	2.96 ± 0.48	2.95 ± 0.36	2.69 ± 0.32	2.82 ± 0.42
Epididymal fat	1.60 ± 0.33^{b}	2.22 ± 0.23^{a}	2.04 ± 0.42^{ab}	1.94 ± 0.32^{ab}
Perirenal fat	0.75 ± 0.17	1.05 ± 0.12	1.12 ± 0.44	0.88 ± 0.11
Omental fat	0.72 ± 0.12^{b}	1.05 ± 0.23^{a}	0.93 ± 0.16^{ab}	0.75 ± 0.16^{b}
Subcutaneous fat	0.89 ± 0.26	1.37 ± 0.58	1.44 ± 0.30	1.34 ± 0.11
Brown fat	0.26 ± 0.08	0.56 ± 0.56	0.22 ± 0.03	0.24 ± 0.07
Relative organ weights (percentages of final body weights) (%)[1]				
Liver	3.56 ± 0.31	3.35 ± 0.37	3.19 ± 0.21	3.19 ± 0.19
Heart	0.36 ± 0.03	0.33 ± 0.03	0.34 ± 0.03	0.35 ± 0.02
Leg	8.84 ± 1.33	7.65 ± 1.11	7.31 ± 0.88	8.13 ± 0.96
Epididymal fat	4.75 ± 0.78	5.73 ± 0.52	5.52 ± 0.93	5.61 ± 0.85
Perirenal fat	2.23 ± 0.42	2.70 ± 0.23	3.02 ± 1.10	2.54 ± 0.29
Omental fat	2.16 ± 0.32	2.70 ± 0.53	2.51 ± 0.32	2.15 ± 0.40
Subcutaneous fat	2.66 ± 0.77	3.51 ± 1.33	3.87 ± 0.59	3.87 ± 0.39
Brown fat	0.77 ± 0.21	1.44 ± 1.46	0.60 ± 0.07	0.70 ± 0.19

Data are means ± standard deviation (n =6).

[1]Relative weights are calculated as a percentage of final body weight (weight of liver, heart, leg, epididymal fat, perirenal fat, omental fat, subcutaneous fat, brown fat /final body weight×100).

a–b Mean values that do not share a common superscript letter are significantly different ($p < 0.05$).

Effect of dietary SPP on plasma biochemical parameters percentages of final body weights

Table 9 shows the effects of SPP on the plasma biochemical parameters. In the plasma biochemical analysis, the concentration of TCHO and HDL-C levels in the HSPP group were significantly lower ($P < 0.05$) than those in the HFD group and LSPP group. Meanwhile, compared to the normal control group, the concentration of TG, glucose, GOT and AST levels were not significantly affected by the high-fat diet and the SPP treatments.

Effect of dietary SPP on the hepatic TBARS levels of mice fed HFD meal.

There was no significant difference of TBARS level in the liver among the four groups (Figure 9).

Table 9. Biochemical parameters in the plasma

	CTRL	HFD	LSPP	HSPP
TG (mg/dL)	125.0 ± 14.2	118.5 ± 13.1	118.8 ± 17.9	108.8 ± 13.2
TCHO (mg/dL)	150.8 ± 22.0^{ab}	166.8 ± 16.8^{a}	159.3 ± 13.9^{a}	137.3 ± 15.7^{b}
HDL-C (mg/dL)	170.0 ± 21.2^{a}	186.0 ± 11.5^{a}	167.0 ± 17.8^{a}	145.5 ± 18.3^{b}
GLU (mg/dL)	347.3 ± 62.8	353.8 ± 33.4	326.7 ± 46.1	314.7 ± 59.6
GOT (U/L)	481.2 ± 238.3	419.8 ± 192.7	397.8 ± 156.4	441.0 ± 123.9
GPT (U/L)	126.3 ± 93.9	132.7 ± 84.1	79.5 ± 72.3	83.8 ± 36.8

Values are expressed as means± standard deviation (n=6).

a–b Mean values that do not share a common superscript letter are significantly different ($p < 0.05$).

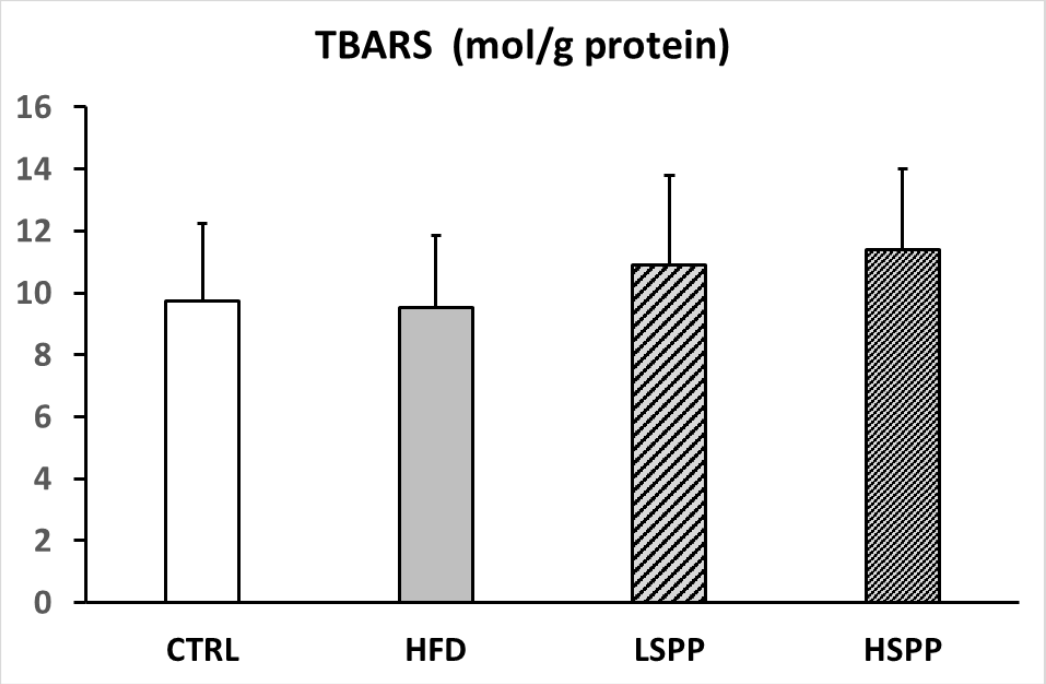


Figure 9. Effect of dietary SPP on the hepatic TBARS levels of mice fed HFD meal

Data are means ± standard deviation (n =6).

Effect of dietary SPP on the antioxidant-related gene expressions in the liver of mice fed HFD meal.

The results of antioxidant-related gene expressions were shown in Figure 10. The high concentration of SPP significantly increased ($P<0.05$) the mRNA expression of GPx1 gene in liver. The mRNA expressions of CAT, Zn-SOD and Mn-SOD gene showed the same trend as GPx1 gene among the four groups.

Effect of dietary SPP on gene expressions of lipid metabolism-related factors in the liver

To determine the mode of action of SPP on HFD induced obesity, we examined the mRNA expressions of lipid metabolism-related factors in the liver (Figure 11.). We found that the mRNA expressions of FAS, ACC, SREBP-1c, C/EBPα, CPT1a and CPT1b were not affected by SPP.

Effect of dietary SPP on the gene expression of lipid metabolism-related factors in the perirenal adipose tissues

The effects of SPP on the gene expression of lipid metabolism-related factors were evaluated by analyzing the mRNA expression levels in the perirenal adipose tissues using quantitative real-time PCR. As shown in Figure12, similar to the results on the liver, SPP had no effect on the mRNA expression of FAS, ACC, SREBP-1c, C/EBPα and CPT1a in perirenal fat among the four groups. In the HSPP groups, the mRNA levels of CPT1b was significantly upregulated ($P<0.05$) compared to HFD group.

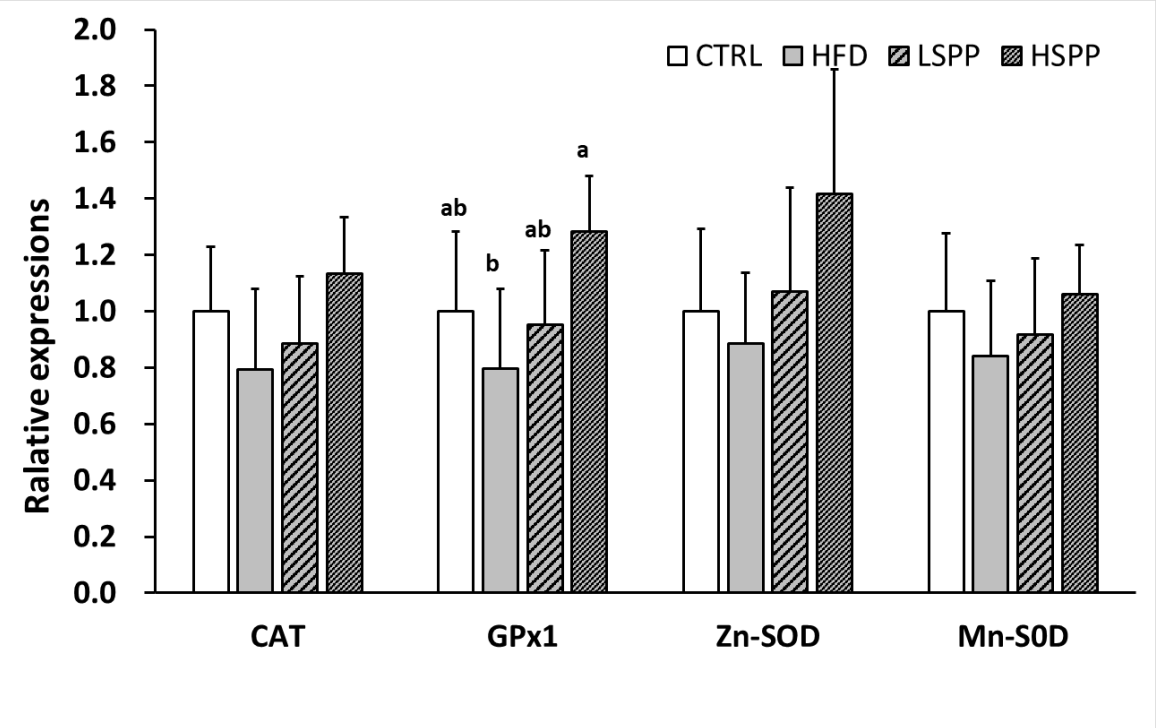


Figure 10. Antioxidant-related gene expressions in the liver

Data are means ± standard deviation (n =6).

The related gene expressions of the four treatments are expressed in arbitrary unit (/18S).

a–b Mean values that do not share a common superscript letter are significantly different ($p < 0.05$).

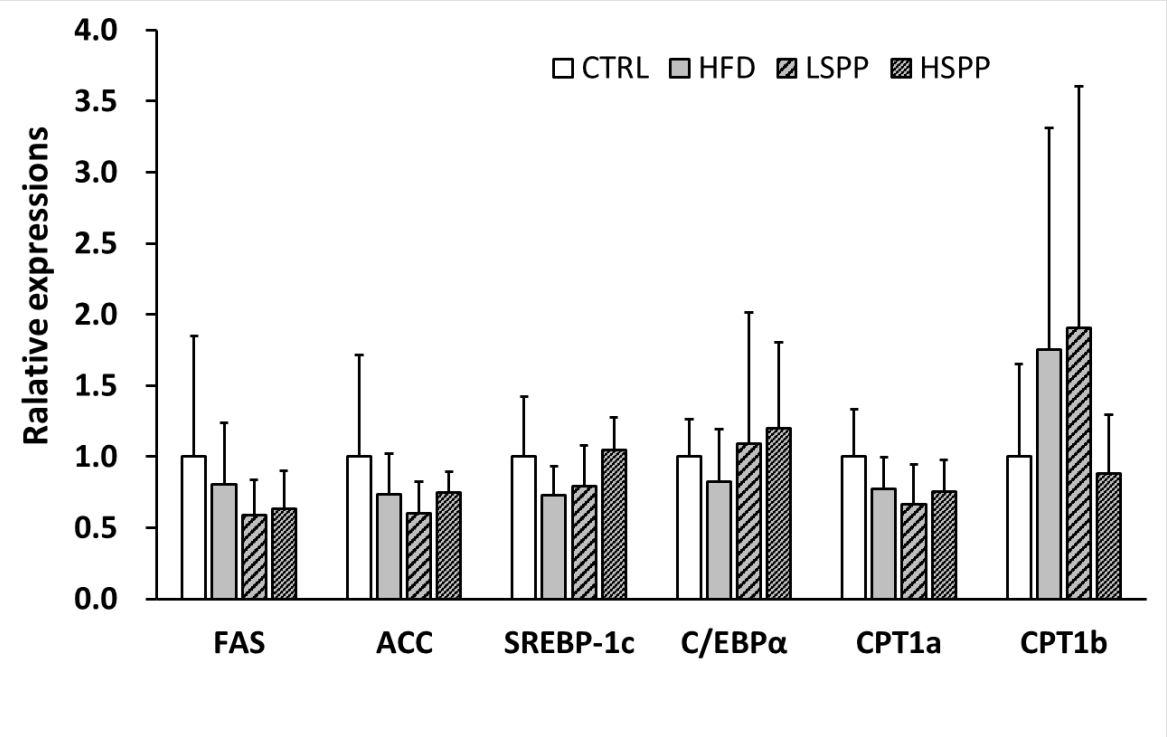


Figure 11. Gene expressions of lipid metabolism-related factors in the liver

Data are means ± standard deviation (n =6).

The related gene expressions of the four treatments are expressed in arbitrary unit (/18S).

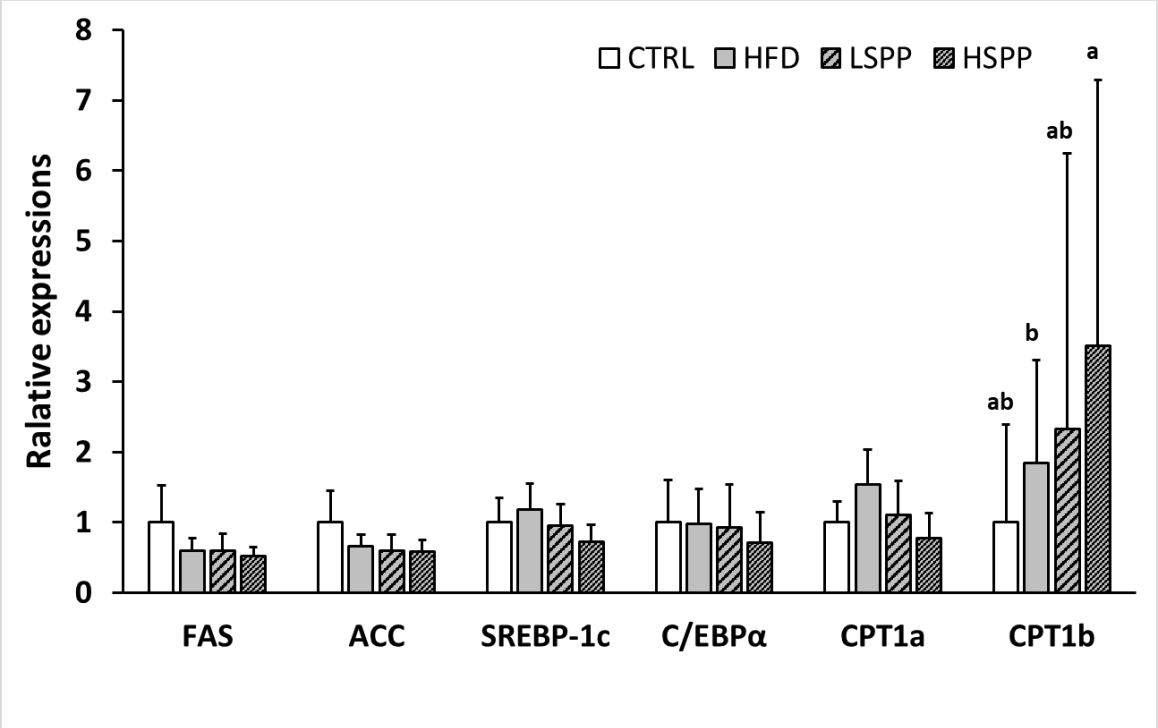


Figure 12. Gene expressions of lipid metabolism-related factors in perirenal adipose tissues

Data are means ± standard deviation (n =6).

The related gene expressions of the four treatments are expressed in arbitrary unit (/18S).

a–b Mean values that do not share a common superscript letter are significantly different ($p < 0.05$).

5.5. Discussion

SPP extracts inhibited adipocyte differentiation in 3T3-L1 preadipocytes in chapter 4, suggesting that SPP might suppress HFD-induce obesity. To examine whether SPP has an anti-obesity effect on obese mice raised on HFD meal, we supplemented the HFD with SPP extracts at 0.5% and 1% level. After 11 weeks, the body weight gain of mice on the HFD supplemented with 0.5% and 1% SPP were 8.0% and 18.8% lighter than those fed with only HFD, although there was no significant difference in food intake among the three HFD groups during the experimental period. In addition, the omental fat weights in the HSPP group exhibited a significant reduction compared to the HFD group. In order to clarity the principles for the lower body weight gain on SPP diet, the gene expressions of lipid metabolism-related factors in adipose tissue were determined. The mRNA expression of CPT1b was significantly increased by HSPP compared to the HFD group, which is an enzyme required for the net transport of long-chain fatty acyl-CoAs from the cytoplasm into the mitochondria. These results indicated that SPP effectively inhibited the body weight gain and fat tissue weight of mice fed with high-fat diet by activating the long-chain fatty acid β-oxidation pathway in mitochondria. Agreed with another study, sweet potato extracts decreased the occurrence of hepatic steatosis (fatty liver) in obese mice by multiply suppressing the expression of lipogenesis-related genes (Sterol Regulatory Element-Binding Protein-1, Acyl-CoA synthase, Glycerol-3-Phosphate Acyltransferase, HMG-CoA reductase, and Fatty Acid Synthase) in liver tissue (Shin et al., 2013). In addition, Han et al.(2003) reported the inhibitory effects of polyphenol fraction of *S. matsudana* leaves on high-fat diet-induced obesity mouse and rat might be due to the inhibition of carbohydrate and lipid absorption from the small intestine through the inhibition of α-amylase and palmitic acid uptake into small intestinal brush border membrane or accelerating fat mobilization through enhancing norepinephrine-induced lipolysis in fat cells. This is a possible and potential reason that SPP reduce the body weight gain of mice in this study, which also needs to be clarified in the future.

In general, a high-fat diet is associated with obesity-mediated insulin resistance (Black et al.,2013) which is related to the elevated total cholesterol and triglyceride levels in plasma. In this study, SPP diet tended to decrease the total cholesterol level in plasma on HFD mice. However, the HDL concentration was also decreased by SPP diet, although HDL is considered to be a good cholesterol benefiting for heart

and blood vessels health.

GPx1 gene, as a glutathione peroxidase, functions in the detoxification of hydrogen peroxide, catalyzing the reduction of hydrogen peroxide to water and other organic hydroperoxides, such as lipid peroxides to the corresponding alcohols (Tan et al.,2013). In the present study, the SPP added in the HFD significantly increased ($P<0.05$) the mRNA expression of GPx1 gene in liver. Wu et al. (2016) also reported the consumption of cherry anthocyanin and mulberry anthocyanin at 200 mg/kg food reduced bodyweight gain by 29.6 and 32.7%, respectively, effectively improve the lipid profiles, reduce MDA production, increase SOD and GPX activities in HFD-fed C57BL/6 mice. The polyphenol-rich grape skin extract may protect against diet-induced adiposity and hepatic steatosis by regulating mRNA expression and/or activities of enzymes that regulate lipogenesis and fatty acid oxidation in the adipose tissue and liver (Park et al., 2013).

Conclusion

In conclusion, the SPP extract reduced the body weight gain and fat tissue weight of mice fed with high-fat diet by activating the long-chain fatty acid beta-oxidation pathway in mitochondria. In addition, the SPP extract improve the antioxidant status of HFD mice due to increasing the mRNA expression of GPx1 gene in the liver.

CHAPTER 6: Effects of sweet potato waste products on broiler performance

6.1. Summary

Recently a method for dry-heat processing under reduced pressure has been developed. A relatively mild temperature (60 to 80 ºC) reduces the carbonization and oxidation of the material thus reducing the loss of nutritional value and function compared with conventional high temperature dry-heating. In the broiler feeding experiments, experiment1 was to compare the effect of feeding dry-heat-processed (D-SPW) and air-dry-processed sweet potato waste (A-SPW) on the growth performance, nutrient metabolizability and oxidative status of broilers. Twenty-four 14-d-old chicks were assigned to three groups (14-28 d): control, A-SPW and D-SPW. The feed conversion ratio (feed/gain) of the D-SPW group was greater than that of the A-SPW group. The relative weight of abdominal fat and the muscle lipid content of the D-SPW group were increased compared with those of the A-SPW group. The metabolizabilities of crude protein and gross energy of the D-SPW group were increased compared with those of the A-SPW group. The plasma α-tocopherol concentrations of the A-SPW and D-SPW groups were greater than that of the control group. Plasma malondialdehyde was decreased in the A-SPW and D-SPW groups, and muscle MDA was decreased in the D-SPW group, compared with the control group. The experiment2 evaluated dry-heat-processed sweet potato waste (D-SPW) as a broiler feed on the growth performance, metabolizability and meat quality from starter period (14-28 d) to grower-finisher period (28-42 d). Except for lipids, the nutrient metabolizabilities of the D-SPW group in the starter and grower-finisher periods were lower than those of the control group. There were no significant differences in growth performance between the two groups. The pH values of breast and leg muscles were higher in the D-SPW group than those in the control group at both 1 and 48 h postmortem. Regarding surface color parameters, the L* values of the breast muscle were higher in the D-SPW group at 1 h postmortem than in the control group but the a* and b* values of the leg muscle in the D-SPW group were lower than those in the control group at 48 h postmortem. The b* value of the abdominal fat in the D-SPW group was also lower than that in the control group at 1 h postmortem. Therefore, the D-SPW is a suitable feed ingredient to partially substitute for corn as it achieved the same growth performance as corn-based diet without loss of its antioxidant capacity and even modified the meat quality of broilers.

6.2. Introduction

In general, sweet potatoes are dried at air temperature for three days before use as feed for broilers. Recently a method for dry-heat processing under reduced pressure has been developed. In the dry-heat processing method, a relatively mild temperature (60 to 80 ºC) reduces the carbonization and oxidation of the material thus reducing the loss of nutritional value and function compared with conventional high temperature dry-heating. In our study, we applied this dry-heat processing method to dry sweet potato waste and used the dry-heat-processed sweet potato waste (D-SPW) to be feed for chickens. In a previous experiment, broilers fed the diet 50% of the corn replaced with D-SPW exhibited a better body weight gain (618 vs. 550 g/14-30 d, $p < 0.05$) than those fed with diet 100% of the corn replaced with D-SPW, while the difference of feed conversion ratio (1.82 vs. 2.03, $p > 0.05$) was not significant between two groups. Therefore, we chose the diet approximately 50% level of the corn replaced with dry-processed sweet potato wastes in this study.

In Kagoshima, in the southern part of Kyushu Island, Japan, approximately 300,000 tons of sweet potatoes are produced annually for the manufacture of shochu, starch and confectionery, leading to more than 2,300 tons of waste (mainly the peel of the tuberous roots). Sweet potato waste contains a high content of starch and antioxidant substances such as tocopherols and polyphenols. However, because of the presence of anti-nutritional components such as amylopectin and protease inhibitors, the nutritional value of sweet potato and sweet potato waste as animal feed is often lower than expected. Recently a method for dry-heat-processing under reduced-pressure for sweet potato waste has been developed (Tsusue et al., 2014). During this processing method, the sweet potato waste is dried at 72°C for 6 h under reduced-pressure which improves the digestibility of the starch and protein in these tuberous roots without losing antioxidant capacity. As Takahata et al. (1994) reported, the heated sweet potato possessed a large amount of maltose produced from the inner starch by β-amylase hydrolysis. Heat processing can also sharply reduce the activity of trypsin inhibitors in sweet potato (Obidairo & Akpochafo, 1984; Zhang & Corke, 2001).

Similarly, in another study (Beckford & Bartlett, 2015) evaluating a diet where corn was substituted with SPRM at levels of 0%, 10%, 20%, and 30%, the final body weight was significantly lower in birds

fed the 20% SPRM diet compared with those fed the 30% diet but did not differ significantly from those fed the 0% and 10% SPRM diets.

Therefore, the objective of the present study:

Experiment 1: to compare the effect of feeding dry-heat-processed and air-dry-processed sweet potato waste on the growth performance, nutrient metabolizability and oxidative status of broiler chickens.

Experiment 2: to investigate the effect of feeding a D-SPW diet on the growth performance, metabolizability and meat quality of broiler chickens during both the starter and grower-finisher periods.

6.3. Experiment 1：Evaluation of effects of the dry-heat processed sweet potato waste as broiler feed

6.3.1. Materials and methods

Preparation of the dry-processed sweet potato wastes

For the air-dry-processed sweet potato waste (A-SPW) meal, the waste epidermal pieces of sweet potato, *Ipomoea batatas* (L), were collected from a shochu liquor factory, spread on a stainless-steel wire mesh, air dried by blowing with an electric fan for 3 days at room temperature, and crushed into mash using a pulverizer (DD-2-3.7, Makino MFG Co., Tokyo, Japan). The moisture content in the A-SPW powder was about 12% which was equivalent as those in other ingredients such as corn and soybean meal. For the dry-heat-processed sweet potato waste (D-SPW) meal, the waste fraction described above was dried using a vacuum dryer (SC-200U, SE-Biomasstechno Co., Yamaguchi, Japan) for 6 h at 72 ºC and 15 kPa absolute reduced pressure, cooled, and then crushed into mash.

Measurement of starch and sugar in the dry-processed sweet potato wastes

The dried sweet potato wastes were smashed into fine powder using a pulverizer (EU6820, Panasonic, Osaka, Japan). For starch determination, samples were washed with water and 40% ethanol, centrifuged, and the precipitates transferred with 5 mL of DMSO into an erlenmeyer flask. Then, 5 mL of 8 M HCl was added and the samples were incubated at 60 ºC for 30 min. After cooling, the pH of the solution was adjusted to 4–5 with NaOH, and the volume was made up to 100 mL with H_2O. The concentration of starch in the solution was determined using a commercial kit (Roche Diagnostics Maltose/Sucrose/D-Glucose, R-biopharm, Darmstadt, Germany). For sugar determination, samples were washed with H_2O, centrifuged, and the supernatant was collected. Subsequently, 5 mL Carrez-I-solution [potassium hexacyanoferrate (II) (85 mM ferrocyanide)] and 5mL Carrez-II-solution (250mM zinc sulfate) were added. After adjusting the pH to 7.5–8.5 with NaOH, the volume of solution was made up to 100 mL with H_2O, and the solution was mixed and filtered. The concentration of maltose, sucrose and glucose in the solution were determined using the kit described above.

Animals, diets and management

The animal experiment was conducted in accordance with the guidelines of Kagoshima University. Eighty 1-d-old male broiler chicks (Ross 308) were obtained from a commercial hatchery (Kumiai Hina Center, Kagoshima, Japan). The chicks were housed in an electrically-heated battery brooder and provided with water and a commercial starter diet (23% CP and 3.1 Mcal/kg metabolizable energy (ME), Nichiwa Sangyou Company, Kagoshima, Japan) until they were 11 d old, and with water and a basal diet from 11 to 14 d old. Then, 24 chicks of similar body weight (380 ± 16 g) were selected and randomly divided into three groups (n = 8 per group); i.e. control, A-SPW, and D-SPW. The birds were individually housed. The control group was fed a corn-soybean meal-based diet, the A-SPW group was fed this basal diet with 55% of the corn replaced with A-SPW, and the D-SPW group was fed the basal diet with 50% of the corn replaced with D-SPW. The CP and ME of the three diets were adjusted to 20% and 3.1 Mcal/kg, respectively. The ingredients and nutrient compositions of the experimental diets are shown in Table 1. For the determination of nutrient metabolizability, an indigestible marker (Cr_2O_3) was included at a level of 0.3% in the diets. The animal experiment was conducted in a temperature-controlled room with 24 h light. The room temperature was kept at 25 ºC with a relative humidity of 50–70% throughout the experiment. Body weight was recorded every three days and feed intake was recorded daily during the experimental period. Excreta was collected for 48 h on days 26 and 27, frozen and stored at −30 ºC until analysis. At the end of experimental period (28 d), birds were slaughtered and then dissected to measure the weight of breast muscle (*M. pectoralis superficialis*), legs (with the lower part of the heel removed), abdominal fat, heart and liver. The muscle samples were stored at −30 ºC until analysis. Blood was collected into heparinized tubes (28 d), quickly centrifuged at 5,900 × *g* for 10 min at 4 ºC, and the separated plasma was stored at −30 ºC until analysis.

Table 10. Composition of the experimental diets

Ingredient	Basal diet	A-SPW[a] diet	D-SPW[b] diet
Corn, %	55.1	22.7	26.0
Alfalfa meal, %	2.9	1.2	1.4
A-SPW meal, %	-	28.3	-
D-SPW meal, %	-	-	26.4
Soybean meal, %	33.5	37.2	36.0
Corn oil, %	4.7	6.8	6.5
DL-Met, %	0.138	0.140	0.140
$CaHPO_4$, %	2.00	2.00	2.00
$CaCO_3$, %	0.66	0.66	0.66
NaCl, %	0.50	0.50	0.50
Mineral and vitamin mixture[c], %	0.50	0.50	0.50
Nutrient composition[d]			
Metabolizable energy, Mcal/kg	3.1	3.1	3.1
Crude protein, %	20.0	20.0	20.0
Calcium, %	1.0	1.0	1.0
Available phosphorus, %	0.62	0.62	0.62
Sodium, %	0.21	0.21	0.21

[a]A-SPW, metabolizable energy: 2.82 Mcal/kg, crude protein: 4.0% (measured values).

[b]D-SPW, 2.82 Mcal/kg, crude protein: 5.4% (measured values).

[c] Provided per kilogram of diet: 1,500 IU of vitamin A (all-trans retinol); 200 IU of vitamin D3; 9.1 IU of vitamin E (α-tocopherol acetate); 1.1 mg of vitamin K3 (menadione); 1.8 mg of thiamin; 3.6 mg of riboflavin; 11 mg of calcium D-pantothenate; 35 mg of nicotinic acid; 3.5 mg of pyridoxine; 0.15 mg of biotin; 0.55 mg of folic acid; 0.01 mg of cyanocobalamine; 1,498 mg of choline chloride; 600 mg of magnesium oxide (Mg); 60 mg of manganese sulfate (Mn); 40 mg of zinc sulfate (Zn); 80 mg of iron sulfate (Fe); 8 mg of copper sulfate (Cu); 0.35 mg of calcium iodinate (I).

[d]Calculated values.

Plasma triacylglycerol and muscle lipids

Plasma triacylglycerol concentrations were measured using a Fuji DRY-CHEM 3500 analyzer (Fuji Medical Systems, Tokyo, Japan) in accordance with the manufacturer's instructions. Muscle samples were homogenized and the lipid fraction was extracted using the method of Bligh and Dyer (1959).

Metabolizability

The CP and gross energy (GE) of the diets and excreta were measured using a Macrocorder JM1000CN analyzer (J-Science Lab Co., Ltd, Kyoto, Japan) and a bomb calorimeter (Yoshida, Tokyo, Japan), respectively. The Cr_2O_3 in feed and excreta were measured in 0.5 g (dry weight) samples. These samples were heated in a porcelain melting pot at 600 ºC for 2 h, then 0.3 g cornstarch and 1 mL alkaline reagent solution (50 g K_3PO_4 and 25 g KOH in 100 mL H_2O) were added, and the pot was heated at 800 ºC for 30 min. After cooling, the contents of the pot were transferred to a flask with H_2O and the absorbance of the solution was determined at 370 nm. Nutrient metabolizability was defined as the percentage of apparent feed nutrients that was not excreted into feces or urine (Yamamoto et al., 2007). The calculations for metabolizability were as follows:

CP metabolizability (%) = 100 − [100 × (dietary Cr_2O_3 / excretal Cr_2O_3) × (excretal CP / dietary CP)];

GE metabolizability (%) = 100 − [100 × (dietary Cr_2O_3 / excretal Cr_2O_3) × (excretal GE / dietary GE)]

Malondialdehyde and α-tocopherol in muscle and plasma

Malondialdehyde (MDA) in muscle and plasma was determined colorimetrically as a 2-thiobarbituric acid-reactive substance as previously described (Ohkawa et al., 1979). The α-tocopherol concentration in muscle and plasma was determined using the LC-2000Plus HPLC system (Jasco Co., Tokyo, Japan) with an Inertsil ODS-3 column (4.6 × 250 mm; GL Sciences, Inc., Tokyo, Japan) in accordance with the method described by Faustman et al. (Faustman et al., 1989).

Statistical analysis

Results are expressed as means ± standard deviation. The data on the contents of starch and sugar in dry-processed SPW meals were analyzed using Student's t-test. The data on growth performance, the

relative weights of tissues and organs, muscle lipid and plasma triacylglycerol, nutrient metabolizability and the α-tocopherol and MDA content of plasma and muscle were analyzed by one-way analysis of variance (ANOVA) using IBM SPSS Statistics 25 software. Tukey's test was further performed, when significant differences were found ($p < 0.05$). Significant differences were based on $p < 0.05$.

6.3.2. Results

The contents of starch and sugar in dry-processed SPW meals are presented in Figure 13. Compared with A-SPW meal, D-SPW meal had a significantly lower starch content (37% vs. 54%, $p < 0.05$), a higher maltose content (9.0% vs. 5.8%, $p < 0.05$), a lower sucrose content (5.1% vs. 8.1%, $p < 0.05$), and no change in glucose content.

Data for growth performance, the relative weights of tissues and organs, muscle lipid and plasma triacylglycerol, and nutrient metabolizability in broilers are summarized in Table 11. There were no differences in body weight gain or feed intake among the three groups; however, the feed conversion ratio of the D-SPW group was significantly greater than that of the A-SPW group ($p < 0.05$). No differences were seen in the relative weights (% body weight) of organs or tissues among the three groups, except for a decrease in abdominal fat in the A-SPW group. Total lipid content in breast muscle and abdominal fat weight were significantly greater in the D-SPW group compared with the A-SPW group ($p < 0.05$), but there were no significant differences in plasma triacylglycerol concentration. Metabolizabilities of CP and GE in the D-SPW group were significantly greater than those in the A-SPW group ($p < 0.05$). However, the CP and GE metabolizabilities in the A-SPW and D-SPW groups were significantly lower than those in the control group ($p < 0.01$).

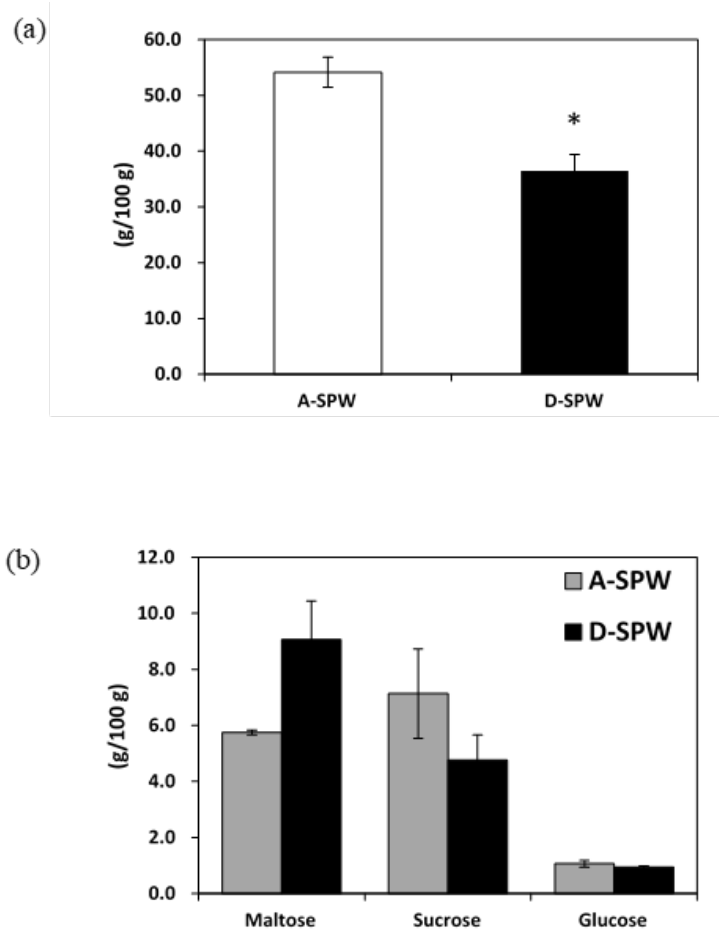


Figure 13. Effects of dry processing on the contents of starch and sugars in sweet potato waste meal

Figure 13a, Starch content of air-dry-processed sweet potato waste (A-SPW) and dry-heat-processed sweet potato waste (D-SPW) meals;

Figure 13b, maltose, sucrose and glucose contents in A-SPW and D-SPW meals.

Values are means (n = 3), and standard deviations are represented by vertical bars.

* Mean values between A-SPW and D-SPW are significantly different ($p < 0.05$).

Table 11. Effects of dry-processed sweet potato wastes on growth performance, the relative weights of tissues and organs, muscle lipid and plasma triacylglycerol, and metabolizability in broilers

	Control	A-SPW	D-SPW
Growth performance			
Initial body weight, g/14 d	381 ± 17	381 ± 16	380 ± 16
Body weight gain, g/14-28 d	684 ± 165	514 ± 151	679 ± 169
Feed intake, g/14-28 d	1052 ± 145	914 ± 219	1043 ± 191
Feed conversion ratio[2], g:g	1.51 ± 0.12[b]	1.81 ± 0.22[a]	1.56 ± 0.14[b]
Relative weights of tissues and organs[3], % body weight			
Breast muscle	19.7 ± 0.8	18.8 ± 1.4	19.4 ± 0.9
Legs	17.2 ± 0.6	17.2 ± 0.5	17.2 ± 1.2
Liver	2.03 ± 0.16	2.00 ± 0.17	2.09 ± 0.18
Heart	0.5 ± 0.07	0.52 ± 0.06	0.53 ± 0.03
Abdominal fat	0.45 ± 0.24[ab]	0.29 ± 0.11[b]	0.54 ± 0.23[a]
Lipids			
Lipid in breast muscle, % tissue	1.48 ± 0.46[ab]	1.18 ± 0.21[b]	1.72 ± 0.25[a]
Plasma triacylglycerol, mg/dL	22.8 ± 4.1	20.5 ± 2.8	22.3 ± 7.2
Metabolizability			
Crude protein, %	65.1 ± 2.5[a]	47.6 ± 5.4[c]	53.0 ± 3.6[b]
Gross energy, %	76.9 ± 1.9[a]	70.1 ± 0.9[c]	73.2 ± 2.0[b]

[a–c] Means within the same row that do not share a common superscript letter are significantly different ($p < 0.05$).

[1]Data are means for 8 replicates.

[2]Feed intake : body weight gain.

[3]Relative weights are calculated as a percentage of final body weight (weight of breast muscle, legs, liver, heart or abdominal fat /body weight × 100).

The α-tocopherol and malondialdehyde (MDA) content of plasma and muscle in broilers are shown in Figure 14. The plasma α-tocopherol concentrations in the A-SPW and D-SPW groups were significantly ($p < 0.01$) increased compared with that in the control group (Figure 14a), and the same trend was seen in muscle α-tocopherol content (Figure 14b). The plasma MDA concentration was significantly ($p < 0.05$) decreased in the A-SPW and the D-SPW groups (Figure 14c), and the muscle MDA concentration was significantly ($p < 0.05$) decreased in the D-SPW group (Figure 14d), compared with those in the control group.

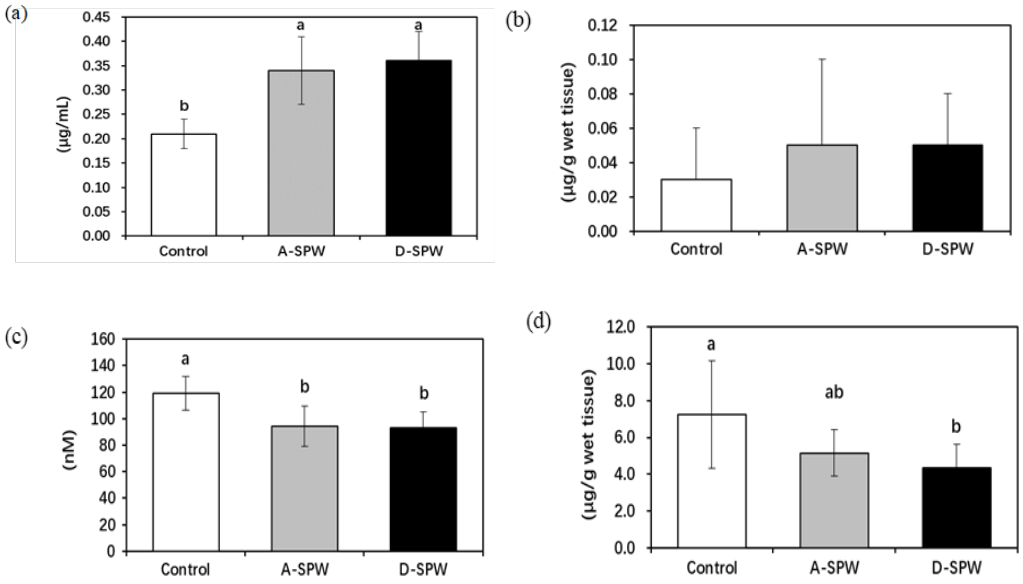


Figure 14. Effect of feeding dry-processed sweet potato wastes on the α-tocopherol and malondialdehyde (MDA) content of plasma and muscle in broilers

Figures 14a, Plasma α-tocopherol concentration; Figures 14b, muscle α-tocopherol content; Figures 14c, plasma MDA concentration; Figures 14d, muscle MDA content.

Values are means (n=8), and standard deviations are represented by vertical bars.

a–b Mean values that do not share a common superscript letter are significantly different ($p < 0.05$).

6.3.3. Discussion

Starch is the main source of carbohydrate in animal diets and the digestibility of starch markedly affects animal growth. The structural characteristics of starches vary with species, and amylopectin has a much larger surface area per molecule than amylose, which makes it a preferable substrate for amylolytic attack (Singh et al., 2010). Sweet potato starches with high amylose content and low paste viscosity show high resistant starch of gelatinized starch. Starches with low pasting temperature and slow retrogradation show high digestible starch content of raw starch (Katayama et al., 2011).

In the present study, the starch of sweet potato waste resulted in better growth performance for chicks when the sweet potato waste had been dry-processed at a higher temperature (72 ºC) than room temperature. Research shows that heated sweet potato possesses a large amount of maltose produced by β-amylase hydrolysis, which has been attributed to the good heat stability of β-amylase and the early gelatinization of starch granules in sweet potato during the heating process (Takahata et al., 1994). Heating also breaks down starch granules to allow amylopectin and amylose to be more readily digested by pancreatic amylase, which may increase the glycemic index of sweet potato (Allen, 2012).

In this study, the metabolizability of CP and GE in broilers was lower for sweet potato waste meal than corn meal, probably because of the remaining high content of trypsin inhibitors in sweet potato waste, which could limit its nutritional value. It has been reported that sporamin, a storage protein found in the tubers of sweet potato, has strong inhibitory activity to trypsin *in vitro*, in addition to its role as a nutritional resource for tuber re-growth (Yeh et al., 1997). The activity of trypsin inhibitors could be greatly affected by the heating process. Flouring after drying for 24 h at 70 ºC is the most effective way to degrade the activity of trypsin inhibitors, followed by microwave baking. Higher temperatures led to further loss of trypsin inhibitor activity, with the activity completely lost after drying for 4 h at 100 ºC, and moist heat treatment resulted in better inactivation of trypsin inhibitors than dry heat treatment (Sasi et al., 2003; Zhang & Corke, 2001).

Lipids are not only an important part of energy storage, but are also intimately related with meat quality. A majority of studies indicate that a high content of intramuscular fat improves the tenderness and flavor of raw muscle and cooked muscle (DeVol et al., 1988; Narciso-Gaytan et al., 2010; Wood & Enser,

1997). The muscle bundle can be dissolved by oxidating intramuscular fat, and intramuscular fat is rich in aroma precursors (fatty acids and phospholipids), which increase muscle flavor by lipid degradation. The results of the present study show that the D-SPW group had a higher lipid content in breast muscle, which may thus be indicative of better meat quality.

Our study also shows that plasma and muscle MDA, measured as indicators of oxidative stress statues in the living body, were significantly decreased in the A-SPW and D-SPW groups compared with the control group. It is probable that this was related to the high concentration of tocopherols seen in the plasma of the A-SPW and D-SPW groups. Thus, the antioxidative capacity of sweet potato waste appears not to have been affected by dry processing at a higher temperature (72 ºC). Research on dietary α-tocopherol for broiler chickens has showed that 200 mg/kg dietary α-tocopherol acetate is required to optimise the muscle content of α-tocopherol and for stability against lipid peroxidation (Morrissey et al., 1997). In recent years, several reports have indicated that the phytochemicals in sweet potatoes display antioxidative or radical-scavenging activity and have several health-promoting functions in humans (Konczak-Islam et al., 2003; Rabah et al., 2004). Xu (2007) found that the scavenging capacity of DPPH (di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium) radicals, superoxide radicals and hydroxyl radicals was 80.3, 66.2 and 53.32%, respectively, from sweet potato pulp, and 86.8, 84.6 and 75.0%, respectively, from sweet potato peel. It has also been reported that sweet potato protein hydrolysates show high antioxidant activities and a protective effect against oxidative DNA damage through both hydroxyl radical-scavenging activity and Fe^{2+} chelating ability, probably because of the increase in several antioxidant amino acids, such as His, Met, Cys, Tyr and Phe, as well as the hydrophobic amino acids (Zhang et al., 2012). Our findings both strengthen the understanding of the natural antioxidant properties of sweet potato in the growth of broiler, and increase the potential for sweet potato waste as broiler feed.

6.4. Experiment 2：Effect of feeding a dry-heat-processed sweet potato waste on the growth performance and meat quality of broilers

6.4.1. Materials and methods

Preparation of D-SPW

The sweet potato (Ipomoea batatas(L)) waste was collected from a shochu liquor factory, dried using a vacuum dryer (SC-200U, SE-Biomasstechno Co., Yamaguchi, Japan) for 6 h at 72°C and 15 kPa absolute reduced-pressure, then after cooling it was crushed into mash using a pulverizer (DD-2-3.7, Makino Corp., Tokoname, Japan).

Animals, diets and management

The animal experiments were conducted in accordance with the guidelines of Kagoshima University. A total of eighty 1-day-old male broiler chicks (Ross 308) were obtained from a commercial hatchery (Kumiai Hina Center, Kagoshima, Japan). The chicks were housed in an electrically-heated battery brooder and provided with water and a commercial starter diet (23% CP and 13.0 MJ/kg metabolizable energy (ME), Nichiwa Sangyou Co., Kagoshima, Japan) for 14 days. Then sixteen 14-day-old chicks of similar body weight (446 g) were selected and randomly divided into a control group and a D-SPW group (n = 8 per group). The chicks received a starter diet (20% CP, 13.0 MJ/kg ME) from 14 to 28 d and a grower-finisher diet (18% CP, 13.0 MJ/kg ME) from 28 to 42 d of age. In the D-SPW group, 49% of the corn in the starter diet and 42% of the corn in the grower-finisher diets were replaced with D-SPW. The ingredients and nutritional compositions of the experimental diets are shown in Table 12. The animal experiments were conducted in a temperature-controlled room with a 23 h light and 1 h dark cycle. The room temperature was kept at 24°C with a relative humidity of 50 to 70% throughout the experiment. Body weights were recorded every three days and feed intake was recorded daily during the experimental period.

At the end of the experimental period (42 d), the chickens were slaughtered then dissected to measure the weights of the breast muscles (M. pectoralis major and M. pectoralis profundus), legs (with the lower

part of the heel removed), liver, heart, gizzard and abdominal fat. The muscle samples were stored at 4°C until analysis of their meat quality. The blood was collected in heparinized tubes, quickly centrifuged at 5,900×g for 10 min at 4°C, then the separated plasma was stored at -30°C until analysis.

Table 12. Composition of the experimental diets

Ingredient	Starter period[3]		Grower-finisher period[3]	
	Control diet	D-SPW diet	Control diet	D-SPW diet
Corn, %	58.0	27.4	65.0	35.0
D-SPW meal, %	-	26.4	-	25.5
Soybean meal, %	33.5	36.0	28.3	30.5
Corn oil, %	4.7	6.5	2.9	5.2
DL-Met, %	0.138	0.140	0.150	0.160
$CaHPO_4$, %	2.00	2.00	2.00	2.00
$CaCO_3$, %	0.66	0.66	0.66	0.66
NaCl, %	0.50	0.50	0.50	0.50
Mineral and vitamin mixture[1], %	0.50	0.50	0.50	0.50
Nutrient composition[2]				
Crude protein, %	20	20	18	18
Metabolizable	13.0	13.0	13.0	13.0

[1] Provided per kilogram of diet: 1,500 IU of vitamin A (all-trans retinol); 200 IU of vitamin D3; 9.1 IU of vitamin E (α-tocopherol acetate); 1.1 mg of vitamin K3 (menadione); 1.8 mg of thiamin; 3.6 mg of riboflavin; 11mg of calcium D-pantothenate; 35mg of nicotinic acid; 3.5 mg of pyridoxine; 0.15 mg of biotin; 0.55 mg of folic acid; 0.01 mg of cyanocobalamine; 1,498 mg of choline chloride; 600 mg of magnesium oxide (Mg); 60 mg of manganese sulfate (Mn); 40 mg of zinc sulfate (Zn); 80 mg of iron sulfate (Fe); 8 mg of copper sulfate (Cu); 0.35 mg of calcium iodinate (I).

[2] Calculated values.

[3] Broilers received the starter diets from 14 to 28 d of age and the grower-finisher diets from 28 to 42 d of age.

Metabolizabilities of nutrients and gross energy

An indigestible indicator (Cr_2O_3) was added at level of 0.3% to the diets of the two groups (control and D-SPW). The excreta were collected for 48 h from 26 to 27 d and from 41 to 42 d then dried at 105°C. The Cr_2O_3 in the feed and excreta were measured using 0.5-g (dry weight) samples. These samples were heated at 600ºC for 2 h, then 0.3 g cornstarch with 1 mL alkaline reagent solution (50 g K_3PO_4 and 25 g KOH in 100 mL H_2O) were added and finally heated at 800ºC for 30 min. After cooling, the contents were transferred to a flask with H_2O and the absorbance of the solution was determined at 370 nm. The nutrient metabolizabilities were defined as the percentage of apparent nutrients from the diet that were not excreted into the feces or urine (Yamamoto et al., 2007).

For the diets and excreta samples, the CP was determined using a Macrocorder (JM1000CN, J-Science Lab Co., Ltd, Kyoto, Japan), GE by a bomb calorimeter (Yoshida Manufacturing Co. Ltd., Tokyo, Japan), and ether extracts (lipid) and crude ash according to the AOAC Official Methods.

The calculations were made as follows:

Nutrient metabolizability (%) = 100 − [100 × (dietary Cr_2O_3 / excretal Cr_2O_3) × (excretal CP / dietary CP)];

GE metabolizability (%) = 100 − [100 × (dietary Cr_2O_3 / excretal Cr_2O_3) × (excretal GE / dietary GE)]

Meat quality

The breast and leg muscle samples were kept at 4°C. The muscle pH was determined using an electronic pH meter at 1 h and 48 h postmortem. The surface color parameters of the meat samples, lightness (L*), redness (a*), and yellowness (b*), were evaluated using a CR-400 Chroma Meter (Konica Minolta Inc., Osaka, Japan) at 1 h and 48 h postmortem at 4°C. The drip loss was calculated using the equation:

Drip loss (%) = [(sample weight at 1 h - sample weight at 48 h) / sample weight at 1 h] ×100.

For the instrumental sensor tests, chicken meat soups were prepared as follows: 15 g minced meat samples with 110 mL dd H_2O were kept in a water bath (80°C, 60 min). After filtration, the taste attributes

of the cooked meat soups (umami, aftertaste of umami and aftertaste of bitterness) were determined and analyzed using a TS-5000Z Insent Sensing System (Insent Intelligent Sensory Technology Inc., Kanagawa, Japan). The lipid and protein contents in the breast and leg muscles were measured as described by Bligh and Dyer (1959) and by the Folin-phenol reagent method as described by Lowry et al. (1951), respectively.

Statistical analysis

Results are expressed as means ± standard deviation. The data were analyzed using the Student's t-test to determine whether there was a significantly difference ($p < 0.05$) between control group and D-SPW group. Analysis of variance (ANOVA) was conducted to assess the effect of *feed*, *time* and their interaction (*feed*×*time*) on the meat color value, pH value and drip loss using JMP ver. 14 (SAS Institute Inc., Cary, NC).

6.4.2. Results

Effect of feeding D-SPW on the growth performances of broilers

The growth performances of the broilers are presented in Table 13. There were no significant differences in body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) between the control and D-SPW groups during the whole period. The organ and tissue weights as a percentage of the live body weight are shown in Table 2. There were no significant differences between the control and D-SPW groups.

Table 13. Effect of feeding D-SPW on their growth performance and relative weights of their tissues and organs of broilers

	Control	D-SPW	*P*-value
Starter period (14-28d)			
Body weight gain, g/14d	773 ± 171	828 ± 145	NS
Feed intake, g/14d	1,366 ± 225	1,514 ± 210	NS
Feed conversion ratio[1], g:g	1.79 ± 0.15	1.85 ± 0.18	NS
Grower-finisher period (29-42d)			
Body weight gain, g/14d	946 ± 216	925 ± 297	NS
Feed intake, g/14d	1,933 ± 335	1,936 ± 308	NS
Feed conversion ratio, g:g	2.08 ± 0.22	2.28 ± 0.77	NS
Whole period (14-42d)			
Body weight gain, g/28d	1,709 ± 372	1,753 ± 387	NS
Feed intake, g/28d	3,283 ± 545	3,450 ± 496	NS
Feed conversion ratio, g:g	1.94 ± 0.13	2.00 ± 0.21	NS
Relative weights of tissues and organs[2], % body weight			
Musculus pectoralis major	18.63 ± 0.83	19.06 ± 1.85	NS
Musculus pectoralis profundus	3.97 ± 0.31	4.12 ± 0.39	NS
Legs[3]	19.42 ± 1.00	18.49 ± 1.06	NS
Liver	1.54 ± 0.12	1.44 ± 0.16	NS
Heart	0.33 ± 0.03	0.34 ± 0.03	NS
Gizzard	0.82 ± 0.15	0.75 ± 0.14	NS
Abdominal fat	0.81 ± 0.37	0.88 ± 0.30	NS

Data are means ± standard deviation (n =7 or 8).

[1] Feed intake: body weight gain.

[2] Relative weights are calculated as a percentage of final body weight (weight of breast muscles, legs, liver, heart, gizzard or abdominal fat /final body weight×100).

[3] Legs with lower part of heel removed

Effect of feeding D-SPW on the nutrients and GE metabolizabilities of broilers

The metabolizabilities of nutrients and GE of broilers are presented in Table 14. During the starter period (14 to 28 d), the D-SPW diet significantly increased CF metabolizability ($p < 0.05$) and decreased the metabolizabilities of CP, ash and GE ($p < 0.05$) compared with the control group. During the grower-finisher period, the metabolizability of CP, ash and GE were significantly lower ($p < 0.05$) in the D-SPW group than in the control group while there were no significant differences in CF metabolizability between the two groups.

Table 14. Effect of feeding D-SPW on their nutrient and energy metabolizabilities of broilers

	Control	D-SPW	*P*-value
Starter period (14-28d)			
Protein, %	64.7 ± 3.6	58.4 ± 4.5	0.01
Fat, %	77.7 ± 2.3	81.4 ± 2.4	0.01
Mineral, %	21.9 ± 3.1	17.6 ± 4.0	0.05
Gross energy, %	74.8 ± 1.1	73.1 ± 1.2	0.01
Grower-finisher period (28-42d)			
Protein, %	66.9 ± 2.7	60.9 ± 4.5	0.01
Fat, %	83.3 ± 2.1	84.4 ± 2.5	NS
Mineral, %	20.8 ± 3.0	17.3 ± 2.6	0.05
Gross energy, %	79.0 ± 1.6	76.1 ± 2.2	0.05

Data are means ± standard deviation (n=7 or 8).

Effect of feeding D-SPW on the meat quality of broilers

The surface color results from the breast muscle, leg muscle and abdominal fat are presented in Table 15. For the breast muscle, the L* value was significantly higher at 1 h postmortem ($p < 0.05$) and the b* value significantly lower at 48 h postmortem ($p < 0.05$) in the D-SPW group than those in the control group. For the leg muscle, there were no significant differences between the two groups at 1 h postmortem, but at 48 h postmortem the a* and b* values of the leg muscle in the D-SPW group were significantly lower ($p < 0.05$) than those in the control group. For the abdominal fat, the b* value in the D-SPW group was significantly lower ($p < 0.05$) than that in the control group while the L* and a* values were not significantly different.

The pH value and drip loss of the breast and leg muscles are presented in Table 16. The pH values of the breast and leg muscles of the D-SPW group were significantly higher ($p < 0.05$) than those of the control group at both 1 h and 48 h postmortem. There were no significant differences in the drip loss of the breast and leg muscles between the D-SPW and control groups. Figure 15 shows that the instrumental taste measurements of the cooked meat soup made using the breast and leg muscles were not significantly different between the control and D-SPW groups.

The compositions of the breast and leg muscles are shown in Table 17. The protein content of the breast muscle was significantly lower ($p < 0.05$) and the lipid content significantly higher ($p < 0.05$) in the D-SPW group than of those in the control group. The results were similar in the leg muscle, with the lipid content being significantly higher ($p < 0.05$) in the D-SPW group than those in the control group.

Table 15. Effect of feeding D-SPW on the color parameters of their tissues of broilers

Tissue	Color parameters[1]	Time	Control	D-SPW	ANOVA[2]		
					feed	*time*	*feed*×*time*
Breast muscle	L*	1h	50.84 ± 1.12	54.20 ± 2.48†	NS	<0.001	NS
		48h	56.44 ± 2.42	56.54 ± 3.19			
	a*	1h	2.18 ± 0.70	2.21 ± 0.95	NS	NS	NS
		48h	1.90 ± 0.72	1.73 ± 0.90			
	b*	1h	5.41 ± 1.32	5.05 ± 0.71	0.005	<0.001	0.028
		48h	11.21 ± 1.84	8.54 ± 1.38†			
Leg muscle	L*	1h	53.94 ± 2.02	54.63 ± 1.32	0.017	0.003	NS
		48h	55.13 ± 2.03	56.78 ± 1.75			
	a*	1h	3.95 ± 0.63	3.16 ± 0.83	0.002	NS	NS
		48h	3.69 ± 0.75	2.58 ± 0.84†			
	b*	1h	5.34 ± 1.37	4.25 ± 0.83	<0.001	<0.001	NS
		48h	9.53 ± 1.20	7.00 ± 0.79†			
Abdominal fat	L*	1h	69.38 ± 2.49	71.08 ± 2.05	NS	—	—
	a*	1h	5.71 ± 2.45	4.98 ± 1.54	NS	—	—
	b*	1h	14.61 ± 0.60	11.83 ± 2.16†	0.010	—	—

Data are means ± standard deviation (n=7 or 8). [1] L*, lightness; a*, redness; b*, yellowness. † $p < 0.05$ vs. Control. [2] Effects of *feed*, *time* and interaction (*feed*×*time*) were analyzed by two-way analysis of variance. NS, effects were not significant.

Table 16. Effect of feeding D-SPW on the pH values and drip loss of broiler muscles

Parameters	Muscle	Time	Control	D-SPW	ANOVA[1]		
					feed	*time*	*feed*×*time*
pH	Breast	1h	6.10 ± 0.19	6.32 ± 2.48†	0.002	NS	NS
		48h	6.09 ± 0.11	6.25 ± 3.19†			
	Leg	1h	6.23 ± 0.13	6.57 ± 1.32†	<0.001	NS	NS
		48h	6.23 ± 0.05	6.37 ± 1.75†			
Drip loss, %	Breast	48h	6.09 ± 0.89	5.81 ± 0.62	NS	—	—
	Leg	48h	3.73 ± 0.40	3.71 ± .050	NS	—	—

Data are means ± standard deviation (n=7 or 8).

† $p < 0.05$ vs. Control.[1] Effects of *feed*, *time* and interaction (*feed*×*time*) were analyzed by two-way analysis of variance. NS, effects were not significant.

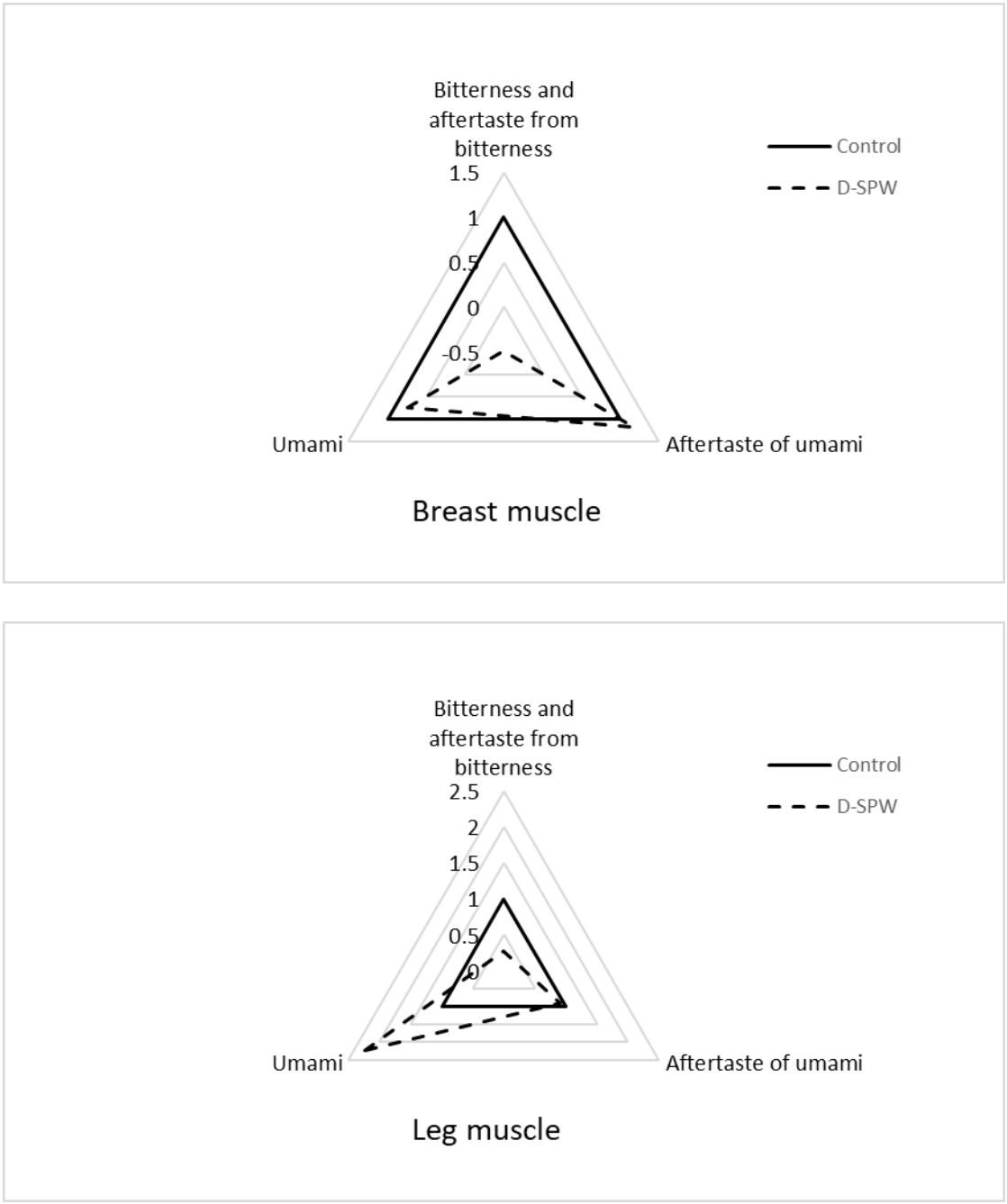


Figure 15. Effect of feeding D-SPW on the taste attributes of soup made from their muscles of broilers

Values are expressed as means (n=7 or 8).

The three tastes are expressed in arbitrary unit.

Table 17. Effect of feeding D-SPW on muscle composition of broilers

Muscle	Composition	Control	D-SPW	*P*-value
Breast	Moisture, %	73.6 ± 1.0	74.0 ± 1.0	NS
	Protein, %	23.0 ± 1.4	19.8 ± 2.8	0.05
	Lipid, %	2.4 ± 0.5	3.5 ± 0.9	0.05
	Ash, %	1.1 ± 0.1	1.1 ± 0.1	NS
Leg	Moisture, %	74.3 ± 2.8	74.7 ± 2.1	NS
	Protein, %	19.1 ± 3.1	16.2 ± 2.1	NS
	Lipid, %	3.2 ± 0.3	4.7 ± 0.8	0.01
	Ash, %	1.0 ± 0.2	0.9 ± 0.1	NS

Data are means ± standard deviation (n=7 or 8).

6.4.3. Discussion

In the present study, we have compared the effect of dietary D-SPW and normal corn on the growth performance of broilers during the starter and grower-finisher periods. There were no significant differences in body weight gain, feed intake or FCR from days 14 to 42. Although the 25% D-SPW feed achieved the same growth performance as the corn-based control diet, the metabolizabilities of the crude fat, crude protein, crude ash and energy utilization were different. During the starter period, the metabolizability of the crude fat from the D-SPW diet was significantly higher than that from the control diet, probably caused by the higher activity of lipase in the intestine. On one hand, the lipase activity could have been increased by the higher content (1.8%) of corn oil in the D-SPW diet than in the control group, because the quantity or activity of lipase is increased by the pancreas when the enzyme efficiency in the digesta is reduced (Schneeman & Gallaher, 1980). On the other hand, pancreatic lipase inhibitors such as polyphenolics and terpenes were present in the feed material (Birari & Bhutani, 2007). However, the changes in the composition and physical structure as the drying process proceeds reduced the overall polyphenol and terpenes contents of the D-SPW feed (McSweeney & Seetharaman, 2015).

In the present study, the crude protein metabolizability and energy utilization in the D-SPW group were significantly lower than those in the control group, probably because there were anti-nutritional factors and resistant starch in the D-SPW diet. Sporamin, the major storage protein in sweet potato tubers, exhibits resistance to pepsin, trypsin, and chymotrypsin, so was most likely to have reduced the crude protein metabolizability and energy utilization in the D-SPW diet (Maloney et al., 2014; Yeh et al., 1997). Starch metabolizability is also an important factor affecting energy utilization. The content of resistant starch in sweet potato ranges from 35.7% to 62.8% (Baek et al., 2014), which hampers the metabolizability of nutrients and GE for broilers. Dhital et al. (2010) also suggested that structural features on starch granules such as surface pores and channels increased the effective surface area, and so facilitated the rapid diffusion of amylases to substrates in maize starch more than in potato starch which lacks such structural features. Although the reduced-pressure-heat-drying process could improve the digestibility of D-SPW to some extent, some deficiencies in sweet potato still need to be solved for use as feed for broilers.

The color of meat is a visible quality attribute which determines its appearance and plays an important role in consumer acceptance. In the present study, the yellowness of the leg muscle and abdominal fat in the D-SPW group was significantly lower than that in the control group. This reduction in yellowness could possibly be associated with the lower concentration of zeaxanthin in the D-SPW diet (35% corn) than in the control diet (65% corn). In agreement with the report of Toyomizu et al. (2001), there was a significant overall correlation between the yellowness of the breast muscle and the spirulina content in diet. Toyomizu et al. (2001) also mentioned that muscles would become darker (lower L*) as the pigment concentrations increased. In the present study, the L* values of the breast muscle in the D-SPW group were significantly higher than in the control group. During storage from 1 to 48 h postmortem, the a* value decreased and the b* value increased, probably due to the oxidation of myoglobin in the muscle samples.

The pH value of meat, another factor closely related to meat quality, is strongly associated with meat color, water-holding capacity and texture (Bihan-Duval et al., 2008). Several studies have determined the relationship between pH value and various quality characteristics of chicken meat, such as a negative correlation with L* values, expressible moisture, drip loss, and cooking loss (Woelfel et al., 2002; Petracci et al., 2004). In general, a low ultimate pH (measured 24 h post-slaughter in poultry) results in "acid meat", which exhibits similar defects to those of PSE meat (Barbut, 1997), while a high ultimate pH leads to DFD (dark, firm, dry) meat with its dark color and rapid loss of quality during storage (Allen et al.,1997). Meat with a low pH has also been reported to decrease its tenderness (Froning et al., 1978; Barbut, 1993) and increase its shelf-life (Allen et al., 1997). We also analyzed the pH values in the muscles using two-way ANOVA in which effects of *feed*, *time* and their interaction on the L*, a* and b* values were examined (Table 16). Significant effects of *feed* were observed on the pH values of the breast and leg muscles. Adam & Abugroun (2010) have reported that the pH in normal meat gradually decreased from 7.4 to 5.3～5.7 after slaughter. Although the pH values were in the normal range in the present experiment, it is unclear how the high pH value due to feeding of D-SPW affects the meat quality. Lower protein content and higher lipid content in the muscles of D-SPW group may be associated with high pH value.

Regarding meat composition, the D-SPW diet significantly increased the lipid contents of the breast and leg muscles compared with the control diet, probably because of the higher content of corn oil in the

D-SPW diet and better fat digestibility during the starter period. While the abdominal fat was not affected by the D-SPW diet, the higher lipid content in the muscle could improve its textural properties. It has been reported (Agbolosu et al., 2014) that the juiciness of meat is directly related to the intramuscular lipid and moisture contents. In combination with water, the melted lipids can form a broth which when retained in the meat is released upon chewing (Hoffman et al., 2003).

Conclusion

Experiment 1. In conclusion, these results demonstrate that the starch of sweet potato waste can be enzymolyzed to maltose by dry-heat processing under reduced pressure, and this processing of sweet potato waste improves its nutrient metabolizability and makes it into an available feed for broilers without loss of its antioxidant capacity.

Experiment 2. In conclusion, this study has indicated that the D-SPW diet achieved the same growth performance as the corn-based control diet and modified the quality of the broiler meat, especially reduction in yellowness of color value and increase in lipid content. Therefore, a feed incorporating D-SPW in the diet is available for broilers instead of using corn. D-SPW is a suitable feed ingredient for broilers to partially substitute for corn as it achieved the same growth performance as broilers fed the corn-based control diet and modified the meat quality of broilers.

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