New vinegar produced by tomato suppresses adipocyte differentiation and fat accumulation in 3T3-L1 cells and obese rat model

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ABSTRACT

There is an increasing surplus of tomatoes that are abandoned due to their failure to meet customer standards. Therefore, to allow both value additions and the effective reuse of surplus tomatoes, we developed tomato vinegar (TV) containing phytochemicals and evaluated its anti-obesity effects in vitro and in vivo. TV inhibited adipocyte differentiation of 3T3-L1 preadipocyte and lipid accumulation during differentiation. TV supplementation in rats fed a high-fat diet (HFD) markedly decreased visceral fat weights without changing the food and calories intakes. TV significantly decreased hepatic triglyceride and cholesterol levels compared to the HFD group. Furthermore, TV lowered plasma LDL-cholesterol level and atherogenic index compared to the HFD group, whereas elevated HDL-cholesterol to total cholesterol ratio. These results show that TV prevented obesity by suppressing visceral fat and lipid accumulation in adipocyte and obese rats, and suggest that TV can be used as an anti-obesity therapeutic agent or functional food.

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1. Introduction

Obesity, caused by an imbalance in high energy consumption with low energy expenditure, is a serious global disorder (James, 2008; Kondo, Kishi, Fushimi, & Kaqa, 2009a). Dramatic increase in the occurrence of obesity is associated with several health problems, including type two diabetes mellitus, dyslipidemia, nonalcoholic fatty liver, cardiovascular disease and certain types of cancer (Haslam and James, 2005). Therefore, prevention and treatment of obesity are important in the modern society, and clinical measures are urgently required.

Tomatoes are the most widely consumed fresh fruit in the world. They contain significant amount of carotenoids (lycopene and β-carotene), several polyphenols (caffeic and chlorogenic acid), rutin and naringenin. In addition, tomatoes are a rich source of both vitamins and trace elements such as selenium, copper, manganese, and zinc that act as cofactors for antioxidant enzymes (Beckles, 2012; Tyssandier et al., 2004). Recently, many studies have focused on the health benefits of tomato with respect to obesity and other diseases. For example, tomato juice or extract can lower plasma level of low-density lipoprotein (LDL) cholesterol, and blood pressure (Engelhard, Gazer, & Paran, 2006; Silaste, Alifthan, Aro, Kesaniemi, & Horkko, 2007) and may prevent coronary heart disease (Rao, 2002). Studies have also shown that intakes of tomato and tomato-based products may reduce the risk of types of cancer (Choi et al., 2011; Pannellini et al., 2010; Wang, Ausman, Greenberg, Russell, & Wang, 2010).

During the past decade, average production and yield of tomatoes have been steadily increasing worldwide. However, the amount of fresh tomatoes consumed by Americans was approximately 8% lower than the output in 2008 (USDA foreign agricultural service, 2008). To consume surplus tomatoes, numerous processed-tomato products such as tomato soup, sauce, ketchup, paste, and juice have been developed (Tonucci et al., 1995). Although fresh tomato is commonly ingested and more than half of the tomato intake is attributable to these processed products, we are still unable to use all the surplus tomatoes (Rao and Agarwal, 2000). Therefore, a novel processed-tomato product with additional functional enhancements is required for greater demand for tomato products (Lee, Kim, Shim & Shon, 2011).

Vinegar has long been used as a condiment and traditional medicine worldwide (Horiuchi, Kanno & Kobayashi, 1999). Nowadays, various types of vinegar are produced using different raw
materials and technologies for a variety of uses (Gullo and Giudici, 2008). In addition, vinegar beverages are popularly consumed, since the main component of vinegar, acetic acid (AcOH) (Kondo, Kishi, Fushimi, Ugajin, & Kaga, 2009b), has numerous beneficial effects against hyperglycemia (Sakakibara, Yamauchi, Oshima, Tsukamoto & Kadowaki, 2006), dyslipidemia (Fushimi et al., 2006), and hypertension (Kondo, Tayama, Tsukamoto, Ikeda & Yamori, 2001). Furthermore, Kondo, Kishi, Fushimi, and Kaqa (2009a) have reported that vinegar intake reduces body weight, body fat mass, and plasma triglyceride (TG) levels in obese Japanese patients. Setorki, Asgary, Eidi, Rohani and Khazaei (2010) have reported that high-dose vinegar intake with a cholesterol diet reduces some biochemical risk factors of atherosclerosis in hypercholesterolemic rabbits. It has been shown that supplementing meals with vinegar may be beneficial in not only the recovery of liver and skeletal-muscle glycogen levels but also in enhancing fatty acid utilisation in the liver (Fushimi & Sato, 2005). These data indicate that vinegar or AcOH intake might have an ameliorating effect on obesity and hyperlipidemia.

Many studies on vinegar obtained from the fermentation of natural sources such as onions, grapes, persimmons, and apples have been actively performed (Budak et al., 2011; Fushimi et al., 2006; Kondo et al., 2001; Sakakibara et al., 2006). However, there are few studies on vinegar obtained from the fermentation of tomatoes. Therefore, the novel tomato vinegar (TV) might be expected to use as a functional food for coronary heart disease, hyperglycemia, hypertension, and dyslipidemia (Fushimi et al., 2006; Kondo et al., 2001; Sakakibara et al., 2006). In this study, we produced TV via two step of fermentation and assessed its effectiveness in treating lipid accumulation in 3T3-L1 adipocytes and in rats fed a HFD.

2. Materials and methods

2.1. Materials

Tomatoes (Lycopersicon esculentum) were purchased from a local market (Gangseo-gu, Busan, Korea). The apple extract was obtained from Invertec, Santiago, Chile. It was kept at 4°C for preserving its qualities. Its characteristics were as follows: 70 Brix, pH 3.4–3.8, and 0.7–3.0% acidity. Garcinia cambogia extract (GC) was purchased from Shinwon Chemicals (Seoul, Korea). Saccharomyces cerevisiae KCCM 34709 and Acetobacter sp. KCCM 40085 were obtained from Korea Culture Center of Microorganisms (Seoul, Korea). 3T3-L1 preadipocytes were purchased from ATCC (Manassas, VA, USA).

2.2. Production of TV

Alcohol and acetic acid fermentations using the tomatoes were performed three times by batch culture, in that order. Before starting the alcohol fermentation, tomato juice was obtained as follows. Matured tomatoes were cut and crushed without stems in a mechanical juicer. Then 300 mL of the crushed tomatoes and 540 mL of distilled water were mixed and fortified with 160 mL of apple extract to 13 Brix. This mixture constituted the tomato juice. In the alcohol fermentation step, Saccharomyces cerevisiae KCCM 34709 (5%, v/v) was inoculated to tomato juice as a starter, after which was cultivated in an incubator at 30°C for 2 days. At the end of alcohol fermentation, the tomato wine was filtered through 110 mm pore-size filter paper and developed in a shaking incubator with Acetobacter sp. KCCM 40085 (10%, v/v) at 30°C and 200 rpm for 8 days. To remove Acetobacter, TV was centrifuged at 1700g for 5 min, after which the supernatants were separated. As a result, TV with a total acidity of 5.6% was developed and stored at 4°C.

2.3. Total acidity, alcohol and sugar contents of TV

The alcohol contents of tomato wine and tomato vinegar were measured using a Gay-Lussac hydrometer. Essentially, 100 mL of tomato wine was taken from the fermenter and centrifuged for 5 min at 1700g in order to remove Saccharomyces cerevisiae KCTC 7904. Then, the supernatant was boiled and adjusted to 100 mL with distilled water. The temperature of the sample was lowered until 15°C and then calculated using an alcohol hydrometer. Sugar contents of TV were investigated using a saccharimeter (Atago pocket PAL-3, Atago Co., Japan). Total acidity of TV was analysed by titrating the diluted sample with 0.1 N NaOH until pH 8.3, and total acidity was expressed as a quantity of acetic acid.

2.4. Lycopene, carotenoid and total polyphenol contents of TV

Lycopene contents of TV were assessed by following the hexane extract procedure developed by Wayne, Penelope and Julie (2002). Briefly, 1 mL of TV was added along with 0.05% BHT containing 5 mL of acetone, 5 mL of ethanol, and 10 mL of hexane, after which the sample was shaken in an ice bath at 180 rpm for 16 min. After 16 min of shaking, 3 mL of distilled water was added to the sample, followed by shaking for another 5 min in an ice bath. The sample was then left at room temperature for 5 min to allow phase separation. The absorbance of the hexane (upper) layer was measured using a UV-spectrophotometer (U-1800, Hitachi Co., Ltd, Japan) at 503 nm. Lycopene contents (mg/100 mL) = (absorbance of sample × 31.2)/weight of sample (g) × 100.

Carotenoids were extracted from TV and analysed by Wang's method (Wang et al., 1998). Briefly, 5 mL of TV and 45 mL of acetone were placed in a flask, after which 25 mL each of distilled water and hexane were added. The sample was then extracted on a shaker at 200 rpm at room temperature and centrifuged at 3640g for 5 min. The resulting supernatant was collected and made up to 50 mL with extraction solvent. Absorbance was measured at 450 nm. Total carotenoids (mg/100 mL) = OD (λmax) × volume/E_{1% 1cm} (2400) × weight (mL) × 100.

Total polyphenol contents were identified by the Folin–Ciocalteu method (Slinkard and Singleton, 1977), using gallic acid as a standard. Briefly, 0.1 mL of tomato sample was added to 0.5 mL of Folin–Ciocalteu reagent and 8.4 mL of distilled water. The solution was mixed and allowed to stand for 3 min. Next, 1 mL of 20% Na₂CO₃ was added and incubated for 2 h in the dark at room temperature. The absorbance was read at 725 nm. Polyphenol quantification was based on a standard curve of gallic acid.

2.5. 3T3-L1 cell culture and differentiation

3T3-L1 preadipocytes were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% newborn calf serum (NBCS, Invitrogen, Carsbad, CA, USA) and 10 mg/mL of penicillin/streptomycin at 37°C in a 5% CO₂ atmosphere. Cells were plated at a concentration of 5 × 10⁵ cells/well in six-well plates. Cells were grown to 100% confluence in an initial culture medium of DMEM containing 10% NBCS. At 100% confluence, the initial culture medium was replaced with differentiation medium containing 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 0.25 μM dexamethasone, and 10 μg/mL of insulin in DMEM containing 10% fetal bovine serum (FBS) and 10 mg/mL of penicillin/streptomycin (0 day). The differentiation medium was removed 2 days after incubation. After an additional 2 days of incubation in DMEM supplemented with 10% FBS and 10 μg/mL of insulin (maturation medium), the medium was changed every 2 days with DMEM.
containing 10% FBS. At 8 days post-induction of differentiation, cells were stained with oil-red O and photographed. 0.2 μg/mL of TV and 2 μg/mL of GC were added with regularly medium on days 0–8. The control was treated with an equal volume of distilled water.

2.6. Oil-red O staining

After induction of differentiation, cells were washed with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde for 1 h on ice, then followed by washing with distilled water. Finally, cells were stained with 0.5% Oil Red O in isopropanol for 1 h. Stained cells were then washed with water and viewed using a fluorescence microscope (Olympus Optical Co., Ltd., Japan) at a magnification of 200×. To quantify intracellular lipids, the stained lipid droplets were dissolved with 60% isopropanol for 10 min. The absorbance of extracted dye was then measured at 520 nm.

2.7. TG assay in 3T3-L1 cells

Differentiated 3T3-L1 cells were harvested in PBS, lysed in lysis buffer (50 mM Tris–HCl, 150 mM NaCl, 1 mM EDTA, 50 mM NaF, 30 mM Na3P2O7, Triton X-100, 1 mM PMSF, 2 μg/mL aprotinin) for 1 h on ice, and centrifuged at 272g for 5 min in a microcentrifuge to pellet cell debris. The supernatant was used to analyse TG levels using the TG assay kit (Wako, Osaka, Japan). Briefly, 20 μL of supernatant was mixed with 3 mL of colour formation reagent and incubated at 37°C for 5 min. After incubation, the mixture was measured at 600 nm with a microplate reader (Emax, Molecular Devices, Sunnyvale, USA).

2.8. Animals and diets

Four-week-old male Sprague–Dawley rats were purchased from Da-Mul science, Inc. (Dae-Jeon, Korea). The rats were individually housed in stainless cages at 22 ± 2°C on a 12 h light–dark cycle. All rats were fed pellets of commercial chow for 1 week after arrival. The rats were randomly divided into four groups (n = 6): normal diet fed rats (ND), high-fat diet fed rats with 45% of the calories from fat (HFD), HFD-fed rats treated with TV and HFD fed rats treated with GC. TV and GC were administered to the rats using an oral feeding needle at a dose of 7 mL/kg/day for 5 weeks of treatment. The rats were fed rats treated with GC. TV and GC were administered to the rats using an oral feeding needle at a dose of 7 mL/kg/day for 5 weeks.

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### Table 1

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<tr>
<td></td>
<td>gm (%) kcal (%)</td>
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<td>Lard</td>
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<td>Total</td>
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* Mineral mixture according to AIN-93G.

2.9. Plasma and hepatic lipids

The plasma TG assay was determined using a TG measure kit (Wako, Osaka, Japan). Briefly, 20 μL of supernatant was mixed with 3 mL of colour formation reagent and incubated at 37°C for 5 min. After incubation, the mixture was measured at 600 nm with a microplate reader. The Wako TG assay kit is based on an enzymatic method using N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline sodium salt (DAOS) as a blue pigment. The cholesterol assay and HDL-cholesterol assay were conducted using a total cholesterol measure kit and HDL-cholesterol assay kit (Asan Pharmaceutical, Seoul, Korea), respectively. Briefly, 20 μL of supernatant was mixed with 3 mL of enzymatic colour formation reagent and incubated at 37°C for 5 min. After incubation, the mixture was measured at 500 nm (total cholesterol) or 550 nm (HDL-cholesterol) with a microplate reader. The atherogenic index (AI) was calculated as follows: (TC – HDL-cholesterol)/HDL-cholesterol. The plasma LDL-cholesterol concentrations were determined using the Friedwald method (TC – (HDL-cholesterol-TG/5)) (Friedewald, Levy & Fredrickson, 1972). The hepatic lipid was extracted using a procedure developed by Folch, Lees, and Sloaner Stanley (1957), and the cholesterol and TG concentrations were analysed with the same enzymatic kit as was used in the plasma analysis.

2.10. Histological analysis of liver and adipose tissues

Liver and epididymal white adipose tissues were removed from the rats and fixed in a buffer solution of 10% formalin. The fixed tissues were processed routinely for paraffin embedding, after which 4 μm sections were prepared and dyed with hematoxylin and eosin. The stained areas were then viewed using an optical microscope at a magnification of 200×. The adipocyte area was measured in randomly selected adipose cells using the SPOT INSIGHTTM (Diagnostic Instrument Co., Sterling Heights, MI) software program (version 4.0).

2.11. Statistical analysis

All data are presented as the mean ± S.E. values. Data were evaluated by one-way analysis of variance using SPSS (Chicago, IL) software and by determining differences between the means using Duncan’s multiple-range test for in vivo test and Student’s t-test for in vitro test. Values were considered statistically significant when p < 0.05.
3. Results

3.1. TV production by a two-step fermentation system

TV was prepared by a two-stage fermentation process that included alcohol and acetic acid fermentations. The alcohol fermentation process, in which tomato wine was manufactured from tomato juice, yielded 4.6–5.8% alcohol after incubation at 30 °C for 4 days. The amount of alcohol in the medium reached a maximum after 3 days (Fig. 1A). However, the alcohol rates were similar in 2 and 3 days. Thus, 2 days were selected as the starting point for acetic acid fermentation concerning fermentation cost. In the acetic acid fermentation process, TV was left in a shaking incubator at 30 °C and 200 rpm for 8 days. The acetic acid concentration increased rapidly after 4 days and reached 5.6% after 8 days. On the other hand, the alcohol concentration began to decrease rapidly after 4 days and eventually reached 0% after 8 days (Fig. 1B). Therefore, we determined contents of tomato vinegar (mg/100 mL). Data values are expressed as means ± S.E. (Vallverdú-Queralt et al., 2012). Therefore we determined contents of extractable phenolic compounds in TV, which was determined by a two-stage fermentation process that included alcohol and acetic acid fermentations. The alcohol fermentation process, in which tomato wine was manufactured from tomato juice, yielded 4.6–5.8% alcohol after incubation at 30 °C for 4 days. The amount of alcohol in the medium reached a maximum after 3 days (Fig. 1A). However, the alcohol rates were similar in 2 and 3 days. Thus, 2 days were selected as the starting point for acetic acid fermentation concerning fermentation cost. In the acetic acid fermentation process, TV was left in a shaking incubator at 30 °C and 200 rpm for 8 days. The acetic acid concentration increased rapidly after 4 days and reached 5.6% after 8 days. On the other hand, the alcohol concentration began to decrease rapidly after 4 days and eventually reached 0% after 8 days (Fig. 1B). Therefore, the maximum acidity content (5.6%) was obtained after 8 days by adjusting the initial sugar concentration to 13°Brix. The vinegar with an acidity of 5.6% was obtained through the 8 day acetic acid fermentation process (Fig. 1A and B).

3.2. Lycopene, carotenoid and total polyphenol contents of TV

Lycopene and carotenoids, which have highly beneficial effects, are known as enriched components in tomatoes (Sun et al., 2012). Numerous polyphenols in tomatoes play important protective roles, including antioxidant and enzyme modulatory activities (Vallverdú-Queralt et al., 2012). Therefore we determined contents of lycopene and carotenoid in TV, major bioactive compounds of tomato (Table 2). Contents of lycopene and carotenoid in TV were 3.19 and 6.45 mg/100 mL, respectively. The total content of extractable phenolic compounds in TV, which was determined using the regression equation of the calibration curve (y = 0.3802x + 0.0018; r² = 0.9904) and expressed in gallic acid equivalents (GAE), was 37.10 ± 0.23 mg GAE/100 mL.

3.3. TV inhibits adipocyte differentiation of 3T3-L1 cells

To determine the effects of TV on adipogenic differentiation, we treated confluent 3T3-L1 cells with or without TV or GC for 8 days. Following various treatments, differentiated 3T3-L1 cells in six-well plates were subjected to Oil-Red O staining (Fig. 2A). The anti-adipogenic effects of both TV and GC were investigated at concentrations having no effect on cell viability according to SRB assay (Fig. S1). Representative images of Oil-Red O staining demonstrate that both TV and GC suppressed lipid accumulation. This result was supported by quantitative data obtained by spectrophotometric analysis (OD, 520 nm) of Oil-Red O-stained cells eluted with isopropanol, which revealed that both TV and GC significantly inhibited adipogenic differentiation compared to control 3T3-L1 cells (P < 0.01, Fig. 2A). These observations were confirmed by TG content assay (Fig. 2B). The TG contents of TV- and GC-treated 3T3-L1 cells were lowered by 45.71% and 57.83% respectively, compared to control (P < 0.01). Thus, treatment of 3T3-L1 cells with TV and GC during induction resulted in inhibition of adipocyte differentiation.

3.4. TV prevents visceral obesity in HFD fed rats

Perirenal, abdominal and epididymal adipose tissues are well known as major visceral fat tissues. The accumulation of visceral fat has an important role in the metabolic syndrome with regard to the energy homeostasis and secretion of adipocyte-derived hormones such as leptin, adiponectin and tumor necrosis factor α (TNFα) (Arai et al., 2013; Kim and Park, 2008; Shoji et al., 2008). High-fat diet (45% calories from fat) feeding to rats for 5 weeks significantly caused an increase in visceral fat pad weight (Table 3 and Fig. 3A). At the end of experimental period, the body weight change was not significant in the TV-treated group compared with control or GC-treated groups. However, the weights of perirenal, abdominal and epididymal adipose tissue in rats given TV were significantly reduced by 27.4%, 26.2% and 28.8% compared to those of HFD rats, respectively, while GC only lowered perirenal and abdominal fat pad weights. Furthermore, the weights of perirenal, abdominal and epididymal adipose tissue in rats given TV were significantly reduced by 27.4%, 26.2% and 28.8% compared to those of HFD rats, respectively, while GC only lowered perirenal and abdominal fat pad weights. Furthermore, the adipocyte size was decreased by GC or TV treatment by 51% and 63% compared to the HFD control group, respectively (Fig. 3B). Food intake was lower in the HFD group than in the ND group, whereas calorie intake was higher in the HFD group. Both TV and GC did not affect them in HFD induced obese rats (Table 3).

Table 2

<table>
<thead>
<tr>
<th>Tomato vinegar</th>
<th>Lycopene (mg/100 mL)</th>
<th>Total carotenoid (mg/100 mL)</th>
<th>Total polyphenol (mg/100 mL)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>3.19 ± 0.05</td>
<td>6.45 ± 0.20</td>
<td>37.10 ± 0.09</td>
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</tbody>
</table>

Data values are expressed as means ± S.E. (n = 6).

Fig. 1. Production of TV by two-step fermentation. (A) Changes in sugar and alcohol contents during alcohol fermentation. (B) Changes in alcohol content and titratable acidity during acetic acid fermentation. Data values are expressed as the means ± S.E. (n = 3).

Fig. 2. (A) Changes in lycopene and carotenoid contents during acetic acid fermentation. Data values are expressed as the means ± S.E. (n = 3).
3.5. Anti-artherogenic effect of TV in HFD-fed rats

As shown in Table 4, both the plasma TG and TC levels did not show significant changes with TV and GC treatments in the HFD-fed rats. On the other hand, TV and GC treatment markedly increased the HDL-cholesterol concentration and ratio of HDL-cholesterol to TC, which was reduced in HFD. The LDL-cholesterol concentration and atherosclerosis index (AI) were elevated in the HFD group compared to the ND group, however both TV and GC treatment significantly decreased them.

3.6. TV reduces hepatic fat accumulation

Histological observation of livers indicated a series of morphological changes, notably hepatic lipid accumulation in HFD-fed rats; however, both TV and GC treatment clearly lowered lipid droplet formation compared to the HFD group (Fig. 4A). In addition, HFD for 35 days resulted in increasing hepatic lipid contents (Fig. 4B and C). However, TV treatment significantly lowered hepatic TG and cholesterol levels by 19.8% and 22.68% compared to the HFD group, respectively, whereas GC treatment did not result in a significant reduction in the cholesterol content (Fig. 4B and C). These data demonstrate that TV intake resulted in lower hepatic lipid accumulation in HFD-fed rats than GC intake.

4. Discussion

Recently, many studies have focused on the health benefits of various vinegars, including anti-obesity, anti-atherosclerosis and anti-hyperlipidemic activities (Budak et al., 2011; Kondo, Kishi, Fushimi, Ugajin, and Kaga, 2009b; Setorki et al., 2010). Tomatoes are considered to be a functional vegetable with anti-obesity effects since they are rich in active components such as lycopene, polyphenols and other carotenoids (Hsu et al., 2008; Ibrahim, Ahmed & El-din, 2008; Silaste et al., 2007). Therefore, vinegar made from tomatoes gained interest due to its potential as a functional beverage. In this study, we developed a novel vinegar from surplus tomatoes and investigated its anti-obesity effects in HFD-fed rats.

Since traditional vinegar production is commonly time-consuming and creates an off flavour, many studies have been carried in order to improve the fermentation method in a shorter time period (Horiuchi, Kanno & Kobayashi, 2000). In the present study, TV was produced by a two-stage fermentation process: alcohol fermentation, including conversion of sugars to ethanol, and acetic acid fermentation, including oxidation of ethanol to acetic acid. In the alcohol fermentation process, tomato wine showed a maximum ethanol production rate in medium of 5.8% after 3 days. This result was similar to previous findings in which onion and pomegranate vinegars obtained maximum ethanol contents after 5 days of fermentation (Kim, Park, & Jun, 2008; Yae et al., 2007). In the acetic acid fermentation process, the acetic acid concentration increased rapidly after 4 days and reached 5.6% after 8 days. Horiuchi et al. (2000) reported that a new type of vinegar with higher contents of minerals, amino acids, and organic acids could be produced from worthless onions using a two-step fermentation system. Therefore, vinegar that can be successfully produced from fresh tomatoes is also of interest in terms of its physiological characteristics.
In our sensory test, the drink with 5% of TV was preferred by most of the people. Ten percent of vinegar resulted in vinegar toxicity in SD rats in our previous study. Therefore, we chose 5% of TV as our main concentration, taking into account stability and preference. 

**Garcinia cambogia**, an edible native Southeastern Asian fruit, containing 10–30% hydroxycitric acid (HCA), has been used as a popular spice and is claimed to lower body weight and reduce fat mass (Saito et al., 2005). Many studies have shown that GC is effective at decreasing appetite, inhibiting visceral fat synthesis, and reducing body weight gain (Greenwood et al., 1981; Hayamizu et al., 2003). GC is easily used in diet beverages at 1.3–1.8%, and has been verified for its stability and functionality in Korea and Japan. Therefore, in this study, we used 1.8% of GC containing HCA as a positive control for the anti-obesity effect.

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The present study demonstrated that TV has powerful anti-visceral obesity activity in HFD-induced obese rats. Intra-abdominal deposition of visceral adipose tissue is known as a general type of obesity, and it plays an important role in the development of many diseases, such as type 2 diabetes mellitus, hyperlipidemia, hypertension and coronary heart disease (Hayamizu et al., 2003). Previous studies have reported that the removal of visceral fat diminishes insulin resistance (Barzilai et al., 1999) and reduces adipose tissue-specific expression of pro-inflammatory cytokines in animal models (Foster, Shi, Seeley, & Woods, 2010). In this study, rats fed a HFD significantly displayed increased visceral fat accumulation such as perirenal, abdominal and epididymal adipose tissues compared to the ND rats. However, TV treatment effectively reduced total visceral fat and epididymal adipocyte size compared to the HFD group. Table 4 presents the plasma lipid profiles in rats fed high-fat diet. The data show that TV treatment significantly reduced triglyceride and total cholesterol levels, and increased HDL-cholesterol levels. TV also reduced LDLa amount and improved HTR and AI values.

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**Table 4**

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<td>Triglyceride (mg/dL)</td>
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<td>44.16 ± 4.91bc</td>
<td>35.41 ± 4.00a</td>
<td>47.08 ± 5.79c</td>
<td>40.00 ± 3.16ab</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>44.22 ± 12.27ab</td>
<td>57.67 ± 13.49b</td>
<td>35.30 ± 6.85a</td>
<td>39.90 ± 13.63a</td>
</tr>
<tr>
<td>HTR (%)</td>
<td>58.09 ± 8.80ab</td>
<td>46.10 ± 8.74a</td>
<td>68.59 ± 11.11b</td>
<td>60.66 ± 11.31b</td>
</tr>
<tr>
<td>AI</td>
<td>0.75 ± 0.27a</td>
<td>1.24 ± 0.48b</td>
<td>0.49 ± 0.24a</td>
<td>0.70 ± 0.34a</td>
</tr>
</tbody>
</table>

Data values are expressed as means ± S.E. (n = 6). Data values with different superscripts within a row indicate significant difference (p < 0.05) by Duncan’s multiple range tests. ND, normal diet; HFD, high-fat diet; GC, Garcinia cambogia; TV, tomato vinegar; HTR, HDL-cholesterol/total cholesterol ratio = (HDL-cholesterol/total cholesterol) × 100; AI, atherogenic index = (total cholesterol – HDL-cholesterol)/HDL-cholesterol.
However, supplementation of tomato powder for 9 weeks to TC concentration when administrated to HFD-fed rats for 5 weeks. Study, TV and GC had no significant effect on the plasma TG or similar to our results. Tomato-wine reduces white adipose weight in HFD-fed rats, which is ceral obesity properties. During cell differentiation, which may contribute to their anti-visceral obesity properties.

Tomato and tomato-based products are rich sources of important nutrients and contain several phytochemicals, such as lycopene, carotenoids and polyphenol compounds, which may have beneficial health effects (Stewart et al., 2000). Lycopene, a major compound of tomatoes, is associated with decreased waist circumference and visceral fat mass in elderly men (Slujs, Beulens, Grobbe & van der Schouw, 2009), and it has been shown to inhibit pro-inflammatory cytokine expression in adipose tissue (Gouranton et al., 2011). Kim et al. (2012) reported that lycopene enriched tomato-wine reduces white adipose weight in HFD-fed rats, which is similar to our results.

Although TV significantly reduced visceral fat mass in HFD-induced obese rats, the body weight did not show a significant decrease in the TV-treated group compared with the HFD-fed or GC-treated groups. This result is consistent with the other researches, which have shown that supplementation of tomato-based products for 8–9 weeks has no significant effect of body weight gain in rats in comparison with HFD (Hsu et al., 2008; Ibrahim et al., 2008). Food intakes, calorie intakes, and FER did not show significant changes with either TV or GC treatment in the HFD-fed rats. This result suggests that TV supplementation could not decrease the body weight gain in HFD-fed rats at 5 weeks. HFD generally increases lipid contents, including TG and cholesterol, in the plasma which is linked with elevated incidence of atherosclerotic events (Ghasi, Nwobodo & Ofili, 2000). In the present study, TV and GC had no significant effect on the plasma TG or TC concentration when administrated to HFD-fed rats for 5 weeks. However, supplementation of tomato powder for 9 weeks to hyperlipidemic rats has been shown to decrease plasma level of TG and cholesterol (Alshatwi et al., 2010), and the administration of tomato paste for 8 weeks was found to reduce plasma cholesterol in high cholesterol diet-fed hamsters (Hsu et al., 2008). Taken together, these data suggest that TV can significantly reduce plasma lipids in extended experiments. Additionally, our results regarding HDL- and LDL-cholesterol levels in HFD-fed rats supplemented with TV were similar to the findings of Alshatwi et al. (2010) who found that administration of tomato powder for 9 weeks tended to elevate HDL-cholesterol levels and significantly reduced LDL-cholesterol levels in hyperlipidemic rats. We found that TV or GC significantly increased the HDL-cholesterol to TC ratio (HTR), which represents the percentage of TC contained in HDL-cholesterol, in HFD-fed rats whereas improved LDL-cholesterol levels and AI. These data indicate that TV has anti-atherogenic effects that promote reduction of the plasma LDL-cholesterol level, although the pathway remains to be elucidated.

Many reports have demonstrated that increased consumption of tomato lycopene is coincident with a lower occurrence of cardiovascular disease (Arab & Steck, 2000; Omoni & Aluko, 2005; Ris-sansen, Voutilainen, Nyssonen & Salonen, 2002) and several types of cancers (Fornelli, Leone, Verdesca, Minervini & Zacheo, 2007; Ilic, Forbes, & Hassed, 2011; Palozza et al., 2010). Carotenoids, an important component of fresh tomatoes, are also known to be beneficial in preventing atherosclerosis (Bicanic et al., 2005) and chronic diseases (Markovits, Ben Amotz & Levy, 2009). Besides lycopene and carotenoids, tomatoes include several polyphenols such as caffeic acid and chlorogenic acid. Polyphenols are a major class of secondary metabolites, and more than 100 different compounds are present in fresh tomatoes (Siracusa, Patané, Avola & Ruberto, 2012). In this study, the contents of lycopene, total carotenoids and polyphenols in TV were determined to be 3.19, 6.45, and 37.10 mg/dL, respectively. In addition, acetic acid has been shown to suppress body fat accumulation in mice livers (Kondo, Kishi, Fushimi, and Kaga, 2009a), as well as reduce plasma cholesterol and TG levels in rats (Fushimi et al., 2006). Therefore, our results suggest that active compounds such as lycopene, polyphenol, and acetic acid contribute to the anti-atherogenic effects of TV.
Incidence of obesity can result in increased prevalence of non-alcoholic fatty liver disease (Sofic et al., 2012). Hepatic steatosis is the major pathological stage of non-alcoholic fatty liver disease, which is triggered by hepatic esterification of free fatty acids to TG in the liver (Stringer et al., 2010). In this study, we found that feeding rats a HFD for 5 weeks with TV resulted in significantly decreased hepatic TG and cholesterol contents, whereas GC decreased hepatic cholesterol content only. In addition, TV and GC diminished the hepatic accumulation of lipid droplets in HFD-fed rats (Alshahtwi et al., 2010). On the other hand, it has been reported that tomato wine has no effect on the hepatic TG or cholesterol level in rats fed a cholesterol-rich diet. Blood levels of TG and cholesterol in rats were reduced after treatment with tomato powder (Kanno et al., 2008). The functional role of some tomato products on lipid profile and liver function in adult rats. Journal of Agricultural and Food Chemistry, 59(10), 908–913. Horisuchi, J., Kanno, T., & Kobayashi, M. (2000). Effective onion vinegar production by a two-step fermentation system. Journal of Bioscience and Bioengineering, 89(3), 289–293.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2013.05.126.

References


