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Plasma and hepatic cholesterol-lowering effects of tomato pomace, tomato seed oil and defatted tomato seed in hamsters fed with high-fat diets

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1. Introduction

ABSTRACT

The cholesterol-lowering effects of tomato pomace (TP), tomato seed oil (TSO) and defatted tomato seed (DTS) were determined in male Golden Syrian hamsters. Hamsters fed high-fat diets containing 10% TSO or 18% DTS were compared to a diet containing 10% corn oil and 10% microcrystalline cellulose (control 1), 42% TP were compared to 25% microcrystalline cellulose (control 2). TP, TSO and DTS reduced hepatic total cholesterol (TC) content. DTS also lowered plasma TC and low-density lipoprotein cholesterol (LDL-C) concentrations. Fecal excretion of lipid, bile acid and cholesterol increased in the DTS group compared to control 1. DTS-fed hamsters had higher levels of hepatic CYP7A1, CYP51, ABCB11, and ABCG5 gene expression than control, suggesting both hepatic bile acid and cholesterol synthesis increased due to increased fecal excretion of bile acid and cholesterol. The results suggest that protein, dietary fibre or phenolic compounds in DTS may be responsible for plasma cholesterol decrease.

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The prevalence of chronic obesity related diseases such as cardiovascular disease (CVD) and diabetes once only observed in western populations is becoming a worldwide epidemic. The increase of obesity related diseases are related to lifestyle choices such as diet, smoking and exercise. High caloric intake relative to caloric expenditure, high-fat and particularly high saturated fat intake, and low fibre intake have been related to CVD. Biomarkers for elevated risk of CVD are elevated levels of plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) (Villanueva, Yokoyama, Hong, Barttley, & Rupérez, 2011). As an alternative to pharmacological medicine, dietary means to control risk factors for obesity related diseases have recently received much attention for the reduction of CVD risk and other obesity related diseases. The functions of food components such as fibre, protein, and phytosterols on reducing plasma cholesterol have been extensively studied. The hypocholesterolemic effects of some tomato products were previously reported. Green or red freeze-dried tomato powders (Friedman, Fitch, Levin, & Yokoyama, 2000), tomato pomace (TP) (Rahmatnejad, Bojarpour, Mirzadeh, Chaji, & Mohammadabadi, 2009), tomato juice (Silaste, Alfthan, Aro, Kesaniemi, & Horkko, 2007), and tomato tomatine (Friedman, Fitch, & Yokoyama, 2000) have been reported to lower plasma LDL-C in hamsters or humans. Several mechanisms to explain plasma cholesterol lowering such as increasing fecal cholesterol and bile acid excretion, and/or reducing intestinal cholesterol absorption have been proposed.

TP remaining after juice/pulp extraction represents 3–5% (weight%) of the fresh tomato and consists mainly of peel and seed (Celmaa, Cuadrosb, & López-Rodríguezc, 2009). In 2010, China and the US produced over 54 million tons or over 37% of the world's tomatoes (FAOSTAT, 2010). At least one third of tomatoes are consumed in the form of processed products such as tomato juice, pulp, and paste resulting in about 0.5–1.0 million tons of TP in these two countries alone. Typically, TP is used as livestock feed with low value, applied as a soil amendment or is otherwise dumped in landfills, which contributes to environmental problems (Celmaa et al., 2009). TP has been found to contain significant amounts of potentially valuable components such as protein, dietary fibre, lycopene, unsaturated fats and other nutrients (unpublished results). In one of the few studies of the



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hypocholesterolemic properties of TP, Elliott et al. reported that 10% TP supplementation resulted in significantly higher serum cholesterol than the control diet in rats (Elliott, Mulvihill, Duncan, Forsythe, & Kritchevsky, 1981). Rahmatnejad et al. found the opposite results when supplementation of 24% TP into diet significantly decreased concentrations of TC and LDL-C in the serum of broiler chickens, but no differences were observed with 8% or 16% TP supplementation (Rahmatnejad et al., 2009). It is difficult to determine the cause of the different results since the source of raw materials, addition levels of tomato pomace, different diets and different animals were used. To date, there has been no research reported on the cholesterol-lowering effects of tomato seed oil (TSO) and defatted tomato seed (DTS). DTS contains mainly protein and fibre. Other food processing products high in fibre or protein such as pectin, soy protein, and peptide, etc. and some oils such as flax seed oil have shown hypocholesterolemic properties (Elliott et al., 1981; Tzang et al., 2009; Zhang, Bartley, Mitchell, Zhang, & Yokoyama, 2011). Therefore, it was hypothesised that DTS and TSO may have potential cholesterol-lowering properties.

The Syrian Golden hamster has been extensively used as a model to study cholesterol metabolism because of its similarity to humans in terms of lipid profiles and high susceptibility to dietary cholesterol resulting in hypercholesterolemic plasma profiles (Kim, Bartley, Rimando, & Yokoyama, 2010). The aim of this study was to investigate the cholesterol-lowering effects of TP byproducts including TP, TSO, and DTS using the hypercholesterolemic male Syrian Golden hamster model. The profile of plasma, hepatic, and fecal lipids and the expression of selected hepatic genes relating to cholesterol, bile acid, and fatty acid metabolism were determined to elucidate the potential cholesterol-lowering mechanism of TP byproducts after supplementation in high-fat diets.

2. Materials and methods

2.1. Materials

Tomato pomace by-products preparation:

TP: Fresh tomato pomace was collected after a hot-break process at a local tomato processing plant (Pacific Coast Producers, Woodland, CA, USA) and stored at -18 °C until used. Tomato pomace was thawed at 4 °C, and then dried at 40 °C in a cabinet drier (CPM Wolverine Proctor LLC, Lexington, PA, USA) for 24 h to moisture content of 5.0 ± 0.2%. The dried pomace was ground to powder using a grinder extractor mill (M2 Stein Mill, The Steinlite Corporation, Atchison, KS, USA) and sieved through a Tyler Sieve Shaker

Table 1

Diet composition (grams)

(RO-TAP Testing Sieve Shaker, W.S. Tyler Co., Cleveland, OH, USA) with a 0.42 mm screen.

TSO: The oil was extracted from tomato seed which was separated from dried tomato pomace by an aspirator system (FC2K testing husker, YAMANMOTO, CO., Higashine, Japan). Before extraction, the seed was ground to a powder using the method previously described for the preparation of TP powder. TSO was extracted using hexane (\geq 98.5%, Sigma–Aldrich). It was found that the optimum oil extraction conditions were 8 min of extraction time at temperature of 25 °C, solvent-to-solids ratio of 5/1 (v/w) with stirring speed of 400 rpm (Shao et al., 2012). After extraction, the hexane extract (oil solution) was separated from the solid residue (tomato seed) by centrifugation (Eppendorf 5804 R, Government Scientific Source, Reston, VA, USA) at 5000 rpm for 10 min at 4 °C. The hexane was removed by a rotary vacuum evaporator (Buchi Rotavapor R-205, BUCHI Analytical Inc., New Castle, Penn., USA) at room temperature.

DTS: For preparation of defatted tomato seed, oil in the tomato seed was completely removed by Soxhlet extraction (m 903.09 AOAC) (AOAC, 1990).

2.2. Chemical composition

Proximate composition of TP, DTS, and TSO were determined according to the following analytical methods (AOAC, 1990): Moisture content of the samples was determined by the oven method. Samples were dried to a constant weight at 105 °C (m 934.06 AOAC). The total fat content was determined by the Soxhlet method (m 903.09 AOAC). Total protein was evaluated by the Kjeldahl method and using a protein conversion factor of 6.25 (m 978.04 AOAC). The 6.25 factor was used since 50% of the protein in the TP diet was casein. The soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) were determined using the enzymatic–gravimetric method (m 991.43 AOAC). Samples were placed in a muffle furnace (BF51828C Thermo Scientific Lindberg Blue M Muffle Furnace, Vernon Hills, IL, USA) at 550 °C to measure the ash content (m 930.05 AOAC). Lycopene content was evaluated by a spectro-photometric method (Anthon & Barrett, 2007).

2.3. Animals and diets

Male Syrian Golden hamsters, 70–80 g, were purchased from Charles River Laboratories (Wilmington, MA). The animals were housed individually in wire-bottom cages in a room maintained at 20–22 °C, 60% relative humidity, and a 12 h alternating

Ingredient ^a	Control 1(10% fibre) ^b	TSO	DTS	Control 2 (25% fibre) ^c	TP
Butter	80.0	80.0	80.0	80.0	80.0
Corn oil	100.0	0.0	100.0	100.0	37.5
Fish oil	20.0	20.0	20.0	20.0	20.0
Cholesterol	1.0	1.0	1.0	1.0	1.0
Tomato sample	0.0	100.0	178.6	0.0	416.7
Microcrystalline cellulose	100.0	100.0	0.0	250.0	0.0
Casein	200.0	200.0	132.1	200.0	108.3
Corn starch	448.0	448.0	437.3	298.0	285.5
DL Methionine	3.0	3.0	3.0	3.0	3.0
Choline bitartrate	3.0	3.0	3.0	3.0	3.0
Mineral mix	35.0	35.0	35.0	35.0	35.0
Vitamin mix	10.0	10.0	10.0	10.0	10.0
Total weight	1000.0	1000.0	1000.0	1000.0	1000.0

^a Butterfat, anhydrous (Odell's, Reno, NV); corn oil obtained from local supermarket; fish oil, cholesterol, L-proline (Sigma–Aldrich); Buffalo corn starch (ConAgra foods food ingredients company, Omaha, NE); microcrystalline cellulose. casein, choline bitartrate, mineral mix, and vitamin mix from Dyets, Inc. (Bethlehem, PA); DL-methionine (ICN). ^b Control 1: 10% corn oil and 10% microcrystalline cellulose as oil and fibre control for tomato seed oil (TSO) and defatted tomato seed (DTS), respectively.

^c Control 2: 25% fibre control for tomato pomace (TP).

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