Toxicology Letters 258 (2016) 168–174

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Short communication

Construction of local gene network for revealing different liver function of rats fed deep-fried oil with or without resistant starch

Zhiwei Wang[a,b], Tianqi Liao[a], Zhongkai Zhou[a,b,c,*], Yuyang Wang[a], Yongjia Diao[a], Padraig Strappe[c], Paul Prenzler[c], Jamie Ayton[d], Chris Blanchard[c]

[a] Key Laboratory of Food Nutrition and Safety, Ministry of Education, Tianjin University of Science and Technology, Tianjin 300457, China
[b] Tianjin Food Safety & Low Carbon Manufacturing Collaborative Innovation Center, 300457 Tianjin, China
[c] ARC Industrial Transformation Training Centre for Functional Grains, Charles Sturt University, Wagga Wagga, NSW 2678, Australia
[d] NSW Department of Primary Industries, Agriculture Institute, Wagga Wagga, NSW 2650, Australia

HIGHLIGHTS

- Up/down regulated genes and corresponding signaling pathways were used to construct LGN.
- Changes in genes induced by DO led to a larger probability of disease or infection.
- Signaling pathways of rats were almost the same between DO-containing diet and DO-RS diet.
- RS in DO-containing diet may mainly regulate the genes that related to DO damage.

ARTICLE INFO

Article history:
Received 3 February 2016
Received in revised form 19 June 2016
Accepted 26 June 2016
Available online 27 June 2016

Keywords:
Deep-fried oil
Resistant starch
Gene network
Signaling pathway

ABSTRACT

To study the mechanism underlying the liver damage induced by deep-fried oil (DO) consumption and the beneficial effects from resistant starch (RS) supplement, differential gene expression and pathway network were analyzed based on RNA sequencing data from rats. The up/down regulated genes and corresponding signaling pathways were used to construct a novel local gene network (LGN). The topology of the network showed characteristics of small-world network, with some pathways demonstrating a high degree. Some changes in genes led to a larger probability occurrence of disease or infection with DO intake. More importantly, the main pathways were found to be almost the same between the two LGNs (30 pathways overlapped in total 48) with gene expression profile. This finding may indicate that RS supplement in DO-containing diet may mainly regulate the genes that related to DO damage, and RS in the diet may provide direct signals to the liver cells and modulate its effect through a network involving complex gene regulatory events. It is the first attempt to reveal the mechanism of the attenuation of liver dysfunction from RS supplement in the DO-containing diet using differential gene expression and pathway network.

1. Introduction

Deep-fried oil is a major dietary component, and increasing concern on its effect on human health has been highlighted (Sebastian et al., 2014). Deep frying in oil is a traditional and popular cooking method used worldwide because of its convenience and its unique effects on food flavor and texture (Saguy and Dana, 2003). However, a number of complex chemical reactions take place during deep frying including hydrolysis, oxidation, cyclization, and polymerization (Li et al., 2008). Most of the reaction products are non-volatile, but they accumulate in the oil, causing modification of the physical and chemical characteristics, such as viscosity, fatty acid (FA) composition, and total polar materials (Gertz 2000; Li et al., 2015). Many of the toxic substances in deep-fried oil, in particular micromolecules, can easily affect body's metabolic system. Study also indicated that, during the deep-frying process, the thermal degradation of the oil

* Corresponding author at: School of Food Engineering and Biotechnology, Tianjin University of Science and Technology, Tianjin 300457, China.
E-mail address: zkzhou@tust.edu.cn (Z. Zhou).

http://dx.doi.org/10.1016/j.toxlet.2016.06.2101
0378-4274/

components is increased in direct proportion to deep-frying temperature and time (Chen et al., 2014). Although the association between deep-frying conditions and the changes in deep-fried oil composition has been well documented, detailed mechanisms that link deep-fried oil to cytotoxic-related metabolic changes, especially to liver tissue, the target organ for detoxification, remain unclear (Aladedunye and Przybylski, 2009; Arem et al., 2013).

In recent years, resistant starch (RS) has drawn increasing interests in that it is not digested in the upper gastrointestinal tract, but is fermented in the large intestine for benefiting gut environment (Lafiandra et al., 2014). Our recent research has shown that the applications of RS in foods can moderate the glycemic response and maintain a proper microorganism profile in the human gut (Zhou et al., 2013a,b). The fermentation of RS in the colon by intestinal microorganisms to produce a range of metabolites including short chain fatty acids, which play an important role in regulating glucose and lipid metabolism (Nugent, 2005).

Analysis of gene expression variation in the liver, a major site of glucose and lipid metabolisms, is a widely used approach to understand body physiology (Kodama et al., 2012). To discover new genes or new molecular networks involved in the diabetes pathogenesis, microarray technology currently offers the fastest and most comprehensive molecular evaluation (Dillies et al., 2013) and many studies have applied microarrays to understand gene expression profiles (Lowe et al., 2007; del Rosario et al., 2014). However, to date there is no study for describing the protective roles of RS on liver dysfunction induced by deep-fried oil. Thus, in this study, genome-wide analysis is performed using liver tissues of rats to further reveal the negative influence on liver metabolism following deep-fried oil consumption, in particular to investigate the changes in the liver function of rats before and after RS supplement using an RNA sequencing method.

2. Materials and methods

2.1. Materials

Non-heat edible oil (fresh canola oil, as control) was purchased from a local supermarket. Deep-fried edible oil (canola oil following deep-frying process. DO) was prepared as described previously (Kuligowski et al., 2011). Briefly, the fresh canola oil was heated at 190 ± 5 °C for 4 intermittent days (8 h each day) for a total of 32 h. Fresh canola oil (7 L) was poured into an iron saucepan with a bore of 45 cm and a depth of 20 cm, and 100 g of chicken nuggets, potato chips, bread pieces, or fish were fried for 4 or 2 min, respectively, in succession for a total of 30 min without replenish. Other chemicals were of reagent grade and used as received.

2.2. Animals and diets

Male, 6 weeks old Wistar rats of 295 ± 10 g weight were purchased from the animal house, Chinese Military Medical Science Academy. After one week's adaptive feeding with the basic diet, the rats were randomly divided into three groups. Group 1: basal diet supplemented with non-heated canola oil (fresh oil, FO group); Group 2: basal diet supplemented with deep-fried canola oil (DO group). Group 3: basal diet supplemented with deep-fried canola oil plus resistant starch (DO-RS group). One point five milliliters (1.5 mL) of non-heated or heated oil was fed by oral gavage using a feeding needle once daily for 6 consecutive weeks before animals were sacrificed for analysis. In particular, for DO-RS group, deep-fried oil and RS were well mixed following the ratio of 1.5:2 (v:g) before oral gavage. Each group contained eight animals housed in plastic cages (4 rats/cage) with free access to food and water. The conditions of humidity (55 ± 5%), light (12/12 h light/dark cycle) and temperature (at 23 °C) were controlled throughout the entire experimental period. The main ingredients of basal diet (standard rodent chow) are shown in Table S1.

2.3. Total RNA extraction and gene expression analysis

After the six week feeding period, rats were dissected immediately with sterile scissors. The liver was removed, weighed, cut into 0.5-cm^3 pieces, immediately frozen in liquid nitrogen, and stored at −80 °C before homogenizing for total RNA extraction.

Total RNA was extracted from each liver sample using Trizol Reagent (Invitrogen, Life Technologies, Carlsbad, CA, USA) following the manufacturer's protocol. The mRNA was sheared into short fragments by adding a fragmentation buffer. First–strand cDNA was synthesized from these short poly (A) + mRNA fragments by adding random primers and Superscript II. Buffer, dNTPs, DNA polymerase I, and RNaseH were then added to generate second–strand cDNA. The double–stranded cDNA was end-repaired by adding T4 DNA polymerase, Klenow Enzyme and T4 polynucleotide kinase. This was followed by a single 'A' base addition using Klenow 3-5′ exo-polymerase, and then sequencing adapters were ligated to the fragments using DNA ligase. For high-throughput sequencing, the cDNA fragments (PE200) were separated by agarose gel electrophoresis and then sequenced on the Illumina Hiseq™ 2000 platform.

2.4. Gene expression comparison analysis

The raw data were first stored as a .DAT file, and then transformed to digital signal data as a CEL file using AGCC software (Affymetrix GeneChip Command Console Software). The fluorescence signal intensity levels were pre-analyzed using the RMA algorithm (Irizarry et al., 2003) for gene expression comparison analysis: 3 or more biological replications were performed and analyzed, using the R language package based on SAM (significance analysis of microarray) to analyze the differential gene expression, the screening standard was: Q-value ≤ 5% and the Fold Change ≥ 2 or ≤ 0.5.

2.5. Statistical analysis

The results were analyzed for statistical significance by one-way analysis of variance (ANOVA) test using the Statistical Package of the Social Science (SPSS) program. All data are expressed as mean ± SD values. In all analyses, a $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Network construction based on differential gene expression between FO and DO groups

Prior to the gene expression analysis, compared to FO diet, the changes of liver function by DO-containing diet were determined in rats. Our previous results revealed that the intake of DO increased liver oxidative stress as reflected by enhanced malondialdehyde, aminotransferase, alkaline phosphatase, and reduced GSH-Px, superoxide dismutase activities and total antioxidation capacity, indicating the liver damaged occurred by DO consumption (data submitted for publication).

Gene expression profiling comparison was carried out from the data collected by RNA sequencing. Among over 30, 000 total genes, 429 were counted as differentially expressed genes (ratio > 2 or <0.5) in the rat livers between FO and DO groups. Among them, 129 genes were up-regulated, whereas 300 genes were