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Inhibition of azoxymethane-induced preneoplastic lesions in the rat colon by a stearic acid complexed high-amylose cornstarch using different cooking methods and assessing potential gene targets

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ABSTRACT

Stearic-acid-complexed-high-amylose-cornstarch (SAC) prevented preneoplastic lesions in the colon of azoxymethane (AOM)-treated Fisher344 rats fed cooked SAC for 8 weeks when water-boiling or bread-baking cooking methods were applied. Water-boiled-SAC (w-SAC) diet was compared with water-boiled-control-cornstarch (w-CS) or water-boiled-high-amylose-cornstarch (w-HA), and bread-baked-CS (b-CS) were compared with bread-baked-HA (b-HA) and bread-baked-SAC (b-SAC). Gene expression profiles from rats fed different water-boiled diets were determined. w-SAC or b-SAC markedly reduced pre-noeplasia compared with HA or CS. Increased cecal contents and decreased cecal pH were observed in SAC or HA groups. Total or individual (butyrate, acetate, and propionate) short-chain fatty acids (SCFAs) increased in selected SAC groups. Differentially expressed genes for AOM treatment were observed in rats fed w-CS and injected with AOM or saline (6428 genes), and for diet treatment in rats injected with AOM and fed w-CS or w-SAC (1403 genes). Changes in 7 genes (CACYBP, RhoA, MAPK1, TOPBP1, HSPH1, MCM6, and HSPD1) were confirmed with qRT-PCR.

1. Introduction

Colorectal cancer is one of the leading causes of cancer deaths worldwide. In the USA alone, an estimated 102,900 cases of colon and 39,670 cases of rectal cancer were predicted to occur in 2010, among which 51,370 deaths were expected, accounting for 9% of all cancer deaths (American Cancer Society, 2012). Colorectal cancer was estimated to be the third most common cancer in both men and women. Diet has been reported as an important modifiable factor for controlling the risk of colon cancer (Rogers, Zeisel, & Groopman, 1993).

Resistant starch (RS) is the sum of starch and products of starch degradation that is not digested and absorbed in the small intestine of healthy individuals. Traditionally, RS was

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Abbreviations: ACF, aberrant crypt foci; AOM, azoxymethane; CS, control cornstarch diet; FC, fold change; FDR, false discovery rate; HA, high amylose cornstarch diet; MDF, mucin depleted foci; RS, resistant starch; SAC, stearic acid complexed high-amylose cornstarch diet; SCFAs, short chain fatty acids; w-, water-boiled; b-, bread-baking

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classified into four categories: RS1, physically inaccessible starch (e.g. coarsely ground whole grains and legumes); RS2, crystalline (uncooked) starch granules with the B- or some C-type crystalline structure (e.g. raw potato, banana, and high-amylose maize starch); RS3, retrograded amylose, which can be found in cooked and chilled potatoes; and RS4, chemically modified starch. A newly developed type 5 RS consisting of amylose–lipid complex has been introduced (Hasjim et al., 2010).

RS cannot be digested in the small intestine, and it then enters the large intestine where it is fermented by the anaerobic microflora to produce SCFAs. Thus, RS can increase cecal and large intestinal contents, alter microbial populations and increase large intestinal SCFAs (Scheppach, Bartram, & Richter, 1995; Sengupta, Muir, & Gibson, 2006). These physiological properties produced by RS have been proposed to prevent colorectal cancer development (Scheppach et al., 1995). Of the three main SCFAs (butyrate, acetate, and propionate), butyrate has been extensively studied and has been considered to be the most potent for protection against colon carcinogenesis in a number of animal models and some human studies (Hijova & Chmelarova, 2007; Topping & Clifton, 2001).

SAC, a RS5, was found to reduce postprandial plasma glucose and insulin levels in humans (Hasjim et al., 2010). Twenty male human subjects were fed bread made from 60% (dry basis) SAC or white bread. Subjects fed SAC had their postprandial plasma glucose and insulin levels reduced to 55% and 43%, respectively, compared with those fed control white bread (100%). The results suggested that SAC can be used to stabilize blood glucose levels after meals, which may be important for control of diabetes.

In our previous study, we discovered that water-boiled SAC had a better inhibitory impact on colon carcinogenesis in Fisher 344 rats than uncooked forms. This discovery was notable because humans generally consume cooked cornstarch. In this study, we further explored the impact of different cooking methods on colorectal carcinogenesis. To our knowledge, this is the first time the influences of different cooking methods on the ability of RS to prevent preneoplastic lesions have been reported. We also conducted global gene expression analysis by microarray and identified several potential genetic targets using qRT-PCR that may be important in colon cancer prevention by water-boiled SAC.

2. Materials and methods

2.1. Diets

Three starches were incorporated into diets and fed to the rats, CS (Cargill Gel™ 03420; Cargill Inc., Minneapolis, MN), HA (AmyloGel 03003; Cargill Inc., Minneapolis, MN) and SAC (processed by Dr. Jane's laboratory in the Department of Food Science and Human Nutrition, Iowa State University) (Hasjim et al., 2010) as the negative control, positive control, and experimental diet, respectively. These are all food grade starches and SAC was previously tested in humans (Hasjim et al., 2010) and rodents (Zhao et al., 2011) without any evidence of adverse effects. The diets were formulated based on the standard diet recommended by the American Society for Nutritional Sciences report for mature rats (AIN-93M), except that 5% cellulose was not included, and starch content was set at 55% (Reeves, Nielsen, & Fahey, 1993). All three starches were cooked by two different methods: water-boiled method and bread-baked method (Supplement Table 1). For the water-boiled diets, the starches were mixed with water gradually and cooked on the range top of a stove. The starch–water mixture was slowly stirred throughout the cooking process until a paste was formed. The paste was then cooled to room temperature and added to AIN-93M diet ingredients. For the bread-baked diets, each starch was mixed with bread flour in the proportion of 5:2. First 2.5 g yeast were mixed with 100 g warm water and held for at least 10 min, and then 300 g of starch and bread flour mixture were mixed together. Next, 2.0 g salt was dissolved with 65 g hot water and 12.5 g shortening, and heated on a boiling water bath until melted. The melted shortening, salt solution and yeast were then added into the starch–bread flour mixture and mixed slowly for 3.5 min to form dough. The dough was put in a greased pan, covered with a moist towel and let rise for 1 h. The dough was then put in a loaf pan and let rise for another hour. The raised dough was baked in an oven for 25 min at 400 °F, and then cooled for 1 h at room temperature. The bread was then dried at room temperature with a fan, ground with a food processor and mixed with other ingredients of the modified AIN-93M diet. Diets were prepared and fed to rats every two days.

Notably, a key strategy was to keep the CS, HA and SAC at 55% of the diet and thus in the bread-baking diets, the composition of vitamins and minerals were less than the standard AIN-93M rat diet (Supplement Table 1B). In the bread-baking diet, the vitamin mix was included at 56% of the recommended amount in the AIN-93M diet, and mineral mix was contained at 61% of the amount in the AIN-93M diet. L-Lysine, DL-tryphophan, L-histidine, and DL-threonine were added to the diet since they were needed by rats and not sufficiently obtained from the wheat protein and other ingredients of the bread-baked diet (Supplement Table 1C). Eighteen amino acids from each diet were listed and compared, among which 7 are essential amino acids (Supplement Table 1C). All of the amino acids in the water-boiled diet exceeded the recommended amount for the AIN-93M diet. All of the essential amino acids were present in the bread-baked diet, but isoleucine and leucine were somewhat low compared with the AIN-93M ingredient (86% and 79% of the recommended amount in AIN-93M diet, respectively). There were another 6 amino acids from the bread-baking diet that were 55–97% of what was recommended for the AIN-93M diet. Comparing the bread-baked diet with water-boiled diet, two amino acids (glutamic acid and glycine) from the bread-baking diet had higher amounts than that contained in the water-boiled diet, and all the other amino acids in the bread-baking diet were 38–96% of the respective amino acid content in the water-boiled diet. The total amount of amino acids were higher in the bread-baked diet than in the AIN-93M ingredient (14.83% of bread-baked diet and 14.05% of the AIN-93M diet), but less than that of the water-boiled diet (20.07%). Although most of the amino acids were sufficient compared with their recommended amount in the AIN-93M formulation, they were mostly less than the respective amount in the water-boiled diet. The reason for the lower amount of vitamins, minerals and selected amino acids was that bread flour, wheat gluten, shortening,