

## Supplementary Information

### **Anti-glycation and anti-oxidative effects of a phenolic-enriched maple syrup extract and its protective effects on normal human colon cells**

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### **HPLC- DAD analyses for presence of phenolics**

MSX was evaluated by HPLC-DAD for the presence of peaks indicative of phenolic compounds (see **Fig. S1**) present therein as previously reported by our laboratory (Zhang et al., 2014). Briefly, MSX (dissolved in DMSO; all at equivalent concentrations of 15 mg/mL) was analyzed on a Luna C18 column (250 × 4.6 mm i.d., 5 μM; Phenomenex) with a flow rate of 0.75 mL/min and injection volume of 20 μL for each sample. A linear gradient solvent system consisting of solvent A (0.1% aqueous trifluoroacetic acid) and solvent B (methanol) at room temperature was used as follows: 0–30 min, from 5% to 33.4% B; 30–80 min, from 33.4% to 71% B; 80–85 min, from 71% to 100% B; 85–86 min, from 100% to 5% B; 86–94 min, 5% B.

### **Analyses of phenolic content using MaPLES**

MSX and its purified fractions (MSX-EtOAc and MSX-Aq), were evaluated for total phenolic content based on maple phenolic lignan-enriched standard (MaPLES) by the Folin-Ciocalteu method as previously reported (Liu et al., 2016). A calibration curve using an authentic standard of maple phenolic lignan-enriched standard was prepared and the results were expressed as (mg/100g of MaPLES).

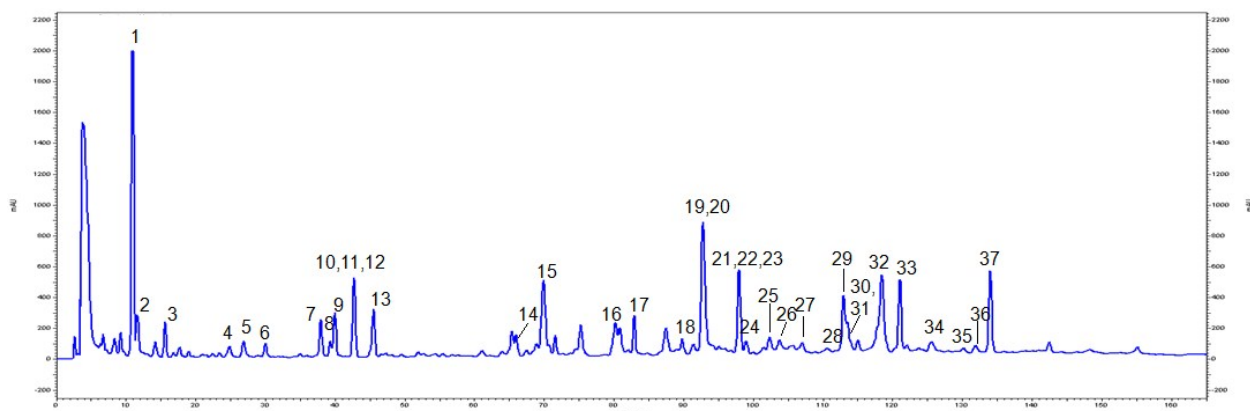
### **Analyses of sugar content**

Sugar content of MSX was analyzed by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) using a Hamilton RCX-30 250/4.6 column (Metrohm AG, Riverview, FL, USA) on a 940 Professional IC Vario system (Metrohm AG, Riverview, FL, USA) and eluted with isocratic 100 mM NaOH at 1 mL/min. Glucose, fructose and sucrose were used as monosaccharide standards.

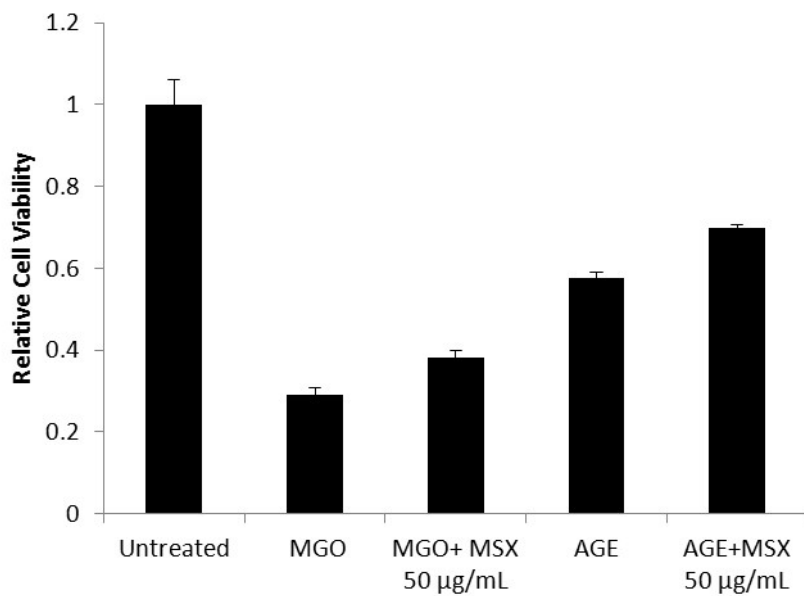
### **Phosphorylation of p38**

Primary antibodies for p38 and phosphorylated-p38 (Thr180/Tyr182) were purchased from Cell Signaling Technology (Danvers, MA, USA). The normal human colon CCD-18Co cells were

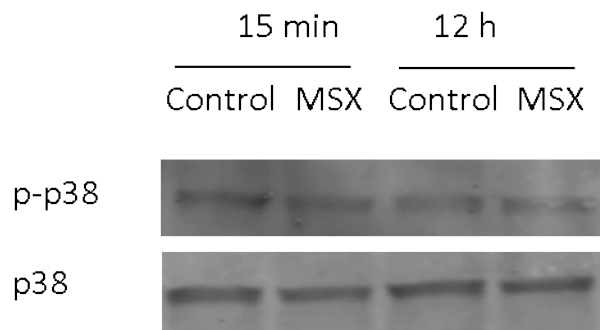
treated with 250 µg/mL MSX for 15 mins or for 12 h. The cell lysates were analyzed for p-p38 and p38 by western blot analyses (see Fig. S3).



**Figure S1.** HPLC-DAD chromatogram of a phenolic-enriched maple syrup extract (MSX) showing 37 compounds identified as follows: (1) 4-hydroxy-2-(hydroxymethyl)-5-methyl-3(2*H*)-furanone, (2) 3,4-dihydro-5-(hydroxymethyl)pyran-2-one, (3) 5-(hydroxymethyl)furfural, (4) 2-hydroxy-3,4-dihydroxyacetophenone, (5) 4-(hydroxymethyl)-1,2-benzenediol, (6) catechol, (7) *C*-veratroylglycol, (8) *threo,threo*-1-[4-(2-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-1-(hydroxymethyl)ethoxy)-3-methoxyphenyl]-1,2,3-propanetriol, (9) 2,3-dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone, (10) 4-acetylcatechol, (11) tyrosol, (12) catechaldehyde, (13) 1,2-diguaiacyl-1,3-propanediol, (14) 3',5'-dimethoxy-4'-hydroxy-2-hydroxyacetophenone, (15) leptolepisol D, (16) 3,4-dihydroxy-2-methylbenzaldehyde, (17) vanillin, (18) fraxetin, (19) syringaldehyde, (20) syringenin, (21) scopoletin, (22) *threo*-guaiacylglycerol- $\beta$ -*O*-4'-dihydroconiferyl alcohol, (23) *erythro*-guaiacylglycerol- $\beta$ -*O*-4'-dihydroconiferyl alcohol, (24) 5-(3'',4''-dimethoxyphenyl)-3-hydroxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-(hydroxymethyl) dihydrofuran-2-one, (25) 1-(2,3,4-trihydroxy-5-methylphenyl)ethanone, (26) *erythro*-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2,6-dimethoxyphenoxy]-1,3-propanediol, (27) icariside E4, (28) 3',4',5'-trihydroxyacetophenone, (29) dehydroconiferyl alcohol, (30) sakuraresinol, (31) secoisolariciresinol, (32) acernikol, (33) (1*S*,2*R*)-2-[2,6-dimethoxy-4-[(1*S*,3*aR*,4*S*,6*aR*)-tetrahydro-4-(4-hydroxy-3,5-dimethoxyphenyl)-1*H*,3*H*-furo[3,4-*c*]furan-1-yl]phenoxy]-1-(4-hydroxy-3-methoxyphenyl)-1,3-propanediol, (34) 2-[4-[2,3-dihydro-3-(hydroxymethyl)-5-(3-hydroxypropyl)-7-methoxy-2-benzofuranyl]-2,6-dimethoxyphenoxy]-1-(4-hydroxy-3-methoxyphenyl)-1,3-propanediol, (35) 4,4'-dihydroxy-3,3',5,5'-tetramethoxystilbene, (36) 4,4'-dihydroxy-3,3',5'-trimethoxystilbene, and (37) (*E*)-3,3'-dimethoxy-4,4'-dihydroxystilbene.



**Figure S2.** Cytotoxic effects of AGE and MGO on normal human colon CCD-18Co cells. Untreated cells were grown in 10% FBS-supplemented DMEM for 2 days. The viability of cells treated with MGO, AGE, or each co-treated with 50 µg/mL MSX were measured by the CCK-8/MTT assay and compared to the untreated cells. Relative cell viability was plotted as mean ± SE (n=3).



**Figure S3.** The effects of MSX on p38 phosphorylation. The normal human colon CCD-18Co cells were treated with 250  $\mu\text{g}/\text{mL}$  MSX for 15 mins or for 12 h. The cell lysates were analyzed for p-p38 and p38 by western blot analyses.

**Table S1.** The phenolic and sugar contents of MSX

Sample	Phenolic content (as % of MaPLES equivalents)	Sugar content (Sucrose, as %)
MSX	92.41	<0.1



**Table S2.** Transition midpoint temperatures (Tms) and enthalpy ( $\Delta H$ ) determined by Differential scanning calorimetry

samples	Tm1 (°C)	Enthalpy ( $\Delta H$ ) kcal/mol	Tm2 (°C)	Enthalpy ( $\Delta H$ ) kcal/mol
Natural BSA	65.2	150.6	74.1	56.2
Glycated BSA	67.6	233.7	78.8	488.8
MSX treated BSA	67.5	168.1	78.4	230.9