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# Impacts of Grape Pomace on the *In Vitro* Starch Digestion and Overall Digestibility of Extruded Food Products

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## Abstract

The objective of this study was to evaluate the effects of adding grape pomace (GP), a polyphenol and insoluble dietary fiber (IDF) rich residue of grapes from wine making, on the digestibility of starch and overall digestion of extruded corn-based product. Yellow corn-grits was mixed with different amount of GP (0, 5, 10 and 15%, w/w) and water (15, 17 and 19%, w/w). The mixtures were extruded at 160°C and 175 rpm. At each moisture level, the product with 0% GP served as control. The overall digestibility of extruded products was evaluated by total dietary fiber (TDF) of products without correction of non-digestible protein. The starch digestibility of extruded products was evaluated by simulated gastric fluid (SGF) digestion and *in vitro* amylase digestion methods in which the reducing sugar content of digesta was used as indicator of starch digestion. The study found that the TDF content of the extruded product increased with GP content almost linearly, and were affected by the moisture of formula; the reducing sugar content of amylase digested samples decreased with GP content in dose dependent manner; and for samples obtained from simulated gastric-intestine digestion, only those containing 5 and 10% GP showed significantly reduced reducing sugar content in the digesta ($P < 0.05$). The results indicate significantly reduced overall digestibility of extruded food product and starch digestibility in the presence of GP.

***Keywords:*** *Grape Pomace; Extrusion; Overall Digestibility; Starch Digestion; Reducing Sugar*

## Introduction

Extrusion cooking is a multi-step, multi-functional and short-time thermal/ mechanical process. This food processing technology has many beneficial effects including the ability to destroy antinutritional factors and microbes while increasing the digestibility of protein and starch which makes extrusion well-suited to produce nutritious foods for at-risk populations, particularly infants and children [1], but it also caused some undesirable effects such as reduced insoluble dietary fiber and increased starch digestibility [2]. Almost all breakfast cereals were made by extrusion process using starch rich materials such as wheat and corn flours. Consumption of rapidly digested starch rich food product can cause a rapid rise in blood sugar and insulin levels after meals that can lead to insulin insensitivity and Type 2 diabetes [3,4], and may be partially responsible for obesity because it was shown that pig fed with low moisture extruded corn-based feed gained more weight than those fed with non-extruded feed [5]. Therefore, packing sufficient amounts of fiber into breakfast cereal and slowdown the digestion of starch become necessary.

Grape pomace (GP) is rich in dietary fiber and polyphenols which could form complex with macronutrients and made them less digestible [6]. Other studies also reported that dietary polyphenols inhibited the activities of digestive enzymes [7]. Adding GP in the food formula also partially replaces some digestible components and the overall digestibility of food product is anticipated to be reduced because up to 70% of the component of GP is non-digestible. The objective of this study was to evaluate the influence of incorporating GP in corn-based product on dietary fiber content and starch digestibility of extruded foods.

## Materials and Methods

### Product formulation and extrusion

Ingredients for extrusion product include yellow corn-grits, corn oil, Muscadine Carlos grape pomace (GP). The reason of using Muscadine Carlos GP is that Carlos GP does not contain anthocyanins which impact product color too much. The moisture contents of all ingredients were measured. Yellow corn grits was mixed with 2.5, 5 and 10% of GP (dry base). At each GP content, water was added to total moisture of 13, 15 and 17%. The formulas were extruded at 160°C and 175 rpm using a twin screw extruder (Brabender MARK III, CTSE-V, C.W. Brabender® Instruments, Inc., South Hackensack, NJ). At each moisture level, the product without grape pomace was used

### Determination of total dietary fiber content of extruded product

The effects of grape pomace content on the overall digestibility of extruded product was evaluated by total dietary fiber (TDF) contents according to AOAC method 991.43 (1994) [8] without adjustment of non-digestible protein. The TDF assay kit was purchased from Sigma-Aldrich (St. Louis, MO).

### Determination of total polyphenols in the extruded products

Polyphenols in extruded product samples were extracted using 70% ethanol. Briefly, 20 ml of 70% ethanol was added to 3g of ground product sample in a 50 ml centrifuge tube. The samples were homogenized for three minutes, and then centrifuged for 20 minutes at 3000g using an Eppendorf 5810 R centrifuge (Westbury, NY, USA). The supernatants were collected and placed into clean tubes. The volume was measured using a graduated cylinder. Total polyphenol (TP) concentrations of the extracts were determined by Folin-Ciocalteu micro-method [9].

### Evaluation the effect of grape pomace content on the digestibility of starch in the extruded products

The effect of GP and extrusion condition on the digestibility of corn starch of extruded products was evaluated by *in vitro* amylase digestion, simulated gastric fluid (GSF)-intestine digestion method. At different digestion stages, starch was hydrolyzed by stomach acidic, amylase and amyloglucosidase. The acid digestion of starch usually produces dextrin which is a group of polysaccharides with different molecular weights. The dextrin is non-reducing sugar. The digestion of starch and dextrin by amylase and amyloglucosidase forms reducing sugar: maltose and glucose. Therefore, the reducing sugar content of digesta was used as indicator of starch digestion.

**Simulated *In vitro* amylase digestion:** The extruded products was ground into powder using a coffee grinder and a motor/pestle. Two grams of dry sample powder was suspended in 20 ml of phosphate buffer (pH 6.8, 10 mM) in a 50 ml flask, and 0.1 ml of heat stable α-amylase solution(Sigma-Aldrich, St. Louis) was added. The digestion was conducted in a water bather shaker at 37°C for 30 minutes. The enzyme hydrolysis reaction was terminated by adjusting pH to 4.5 - 4.7 using 6N HCl solution.

**Simulated gastric fluid (SGF) and intestine digestion:** Two grams of dry sample powder was suspended in 10 ml of deionized water in a 50 ml flask. A 5 ml of simulated gastric fluid (pH 1.5, containing pepsin) was added to the sample mixture. The SGF digestion was conducted in a water bath shaker at 30°C for 60 minutes. The SGF digested mixture was adjusted to pH 6.8 - 7.0 using 10N NaOH solution, then 5 ml of amyloglucosidase (intestine amylase) was added. The digestion was continued at 37°C for 60 minutes. During this digestion, the corn starch in the product should be first hydrolyzed into dextrin, than maltose and glucose.

After centrifugation, the volumes of supernatants were recorded, and the reducing sugar concentrations of the supernatants were determined by Dinitrosalicylic acid (DNS) methods [10] and expressed mg/g product.

## Results and Discussion

### Effect of grape pomace inclusion on dietary fiber content of extruded product

Figure 1 shows that the TDF content of the extruded product increased with increasing amount of GP almost linearly, and was affected by the moisture of formula ($R^2$ = 0.975 - 0.997) and for every 1% increase in GP, an average of 0.78% increase of TDF in the extruded product was observed. The TDF of extruded product was 2.76 - 3.09% without GP, 7.26 - 7.5% with 5% GP, 9.33 - 11.77% with 10% GP and 14.22 - 15.78% with 15%GP. This indicates that the overall digestibility of extruded food was reduced in the presence of GP. These data indicate that the overall digestibility of extruded food was reduced by inclusion of 2.5 - 10% of GP.

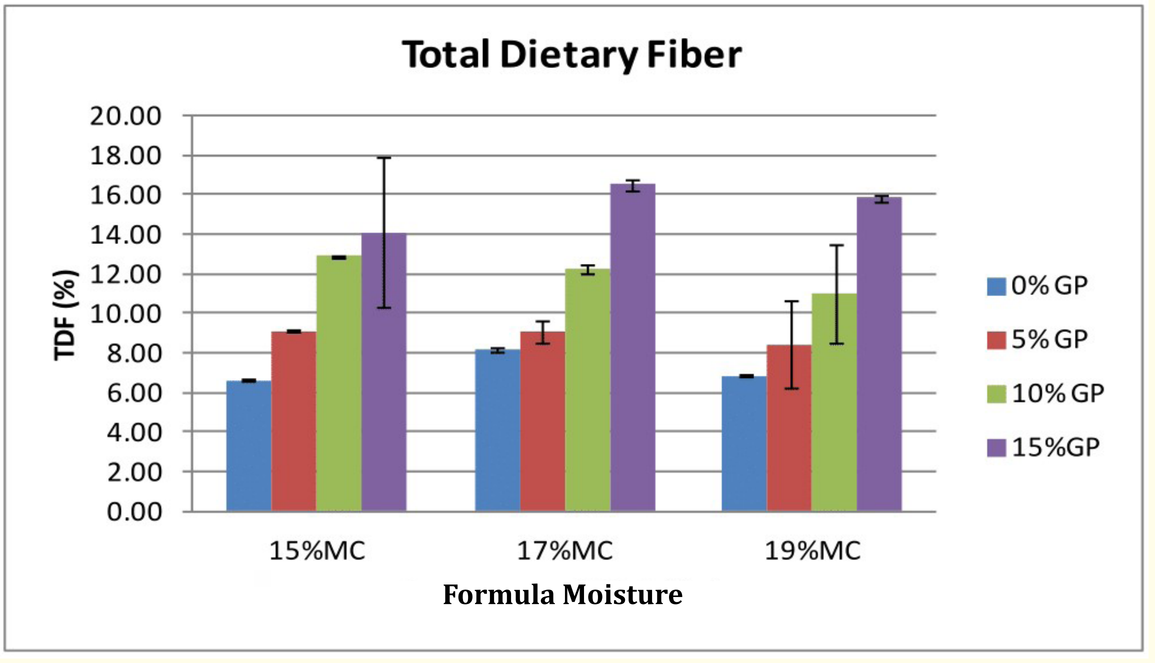


***Figure 1:*** *Effects of GP content on the overall digestion of extruded products at different extrusion moisture (MC) as indicated by TDF change.*

### Effects of grape pomace inclusion on total polyphenol content of extruded products

Figure 2 show that total polyphenol contents (TPC) of extruded products increased greatly with GP level in the formulation, but the impact of moisture on TPC was not significant until the moisture of formulation was 17%. The low extractable TPC in the high moisture product suggests that polyphenol was less stable at high moisture and high temperature (160ºC). Although many type of polyphenols are unstable to heat and high temperature, significant amount of polyphenol from GP survived the extrusion process. Therefore, GP can be a great source of polyphenol for extruded product.

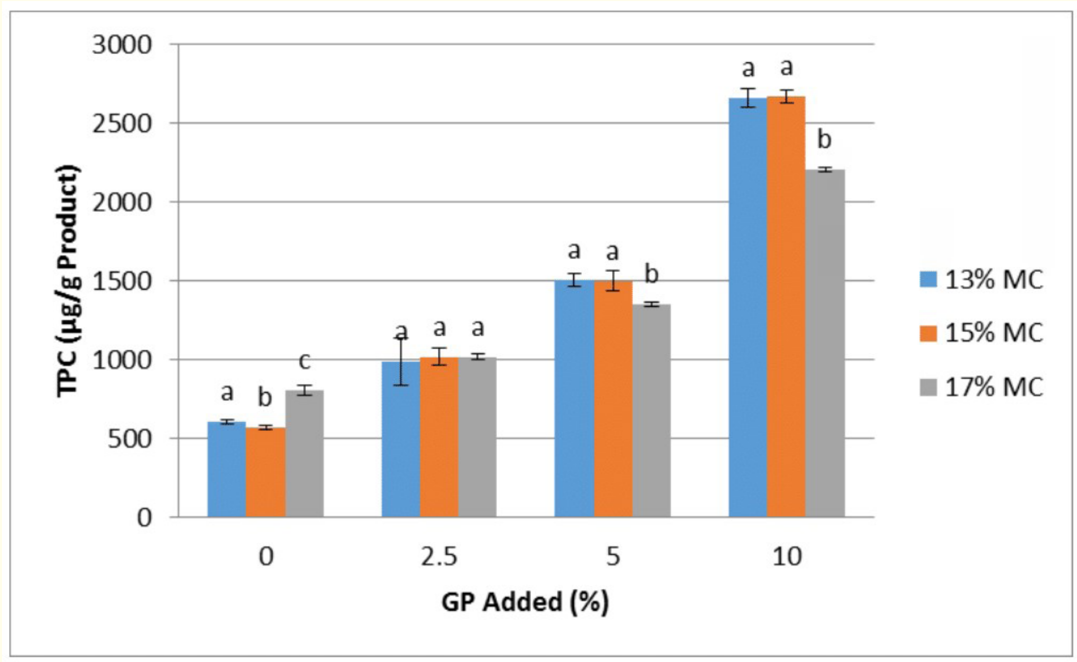


***Figure 2:*** *Polyphenol contents of extruded products containing different amount of GP. (At same GP level, data with sample label are not significantly different at 95% confidence interval).*

### Effects of grape pomace inclusion on amylase digestion of corn starch in the extruded products

The reducing sugar content of amylase digested samples decreased with GP content in dose dependent manner and was affected by the extrusion moisture (Figure 3). At 13% extrusion moisture, the reducing sugar concentration of amylase digested product was decreased by 2.41% to 19.35% when GP increased from 2.5% to 10%; at 15% moisture, the reduction of reducing sugar was 2.87 - 24.49%, at 17% moisture, 2.5 - 10% increase in GP resulted in 6.13 - 27.589% decrease in reducing sugar of amylase digested samples.

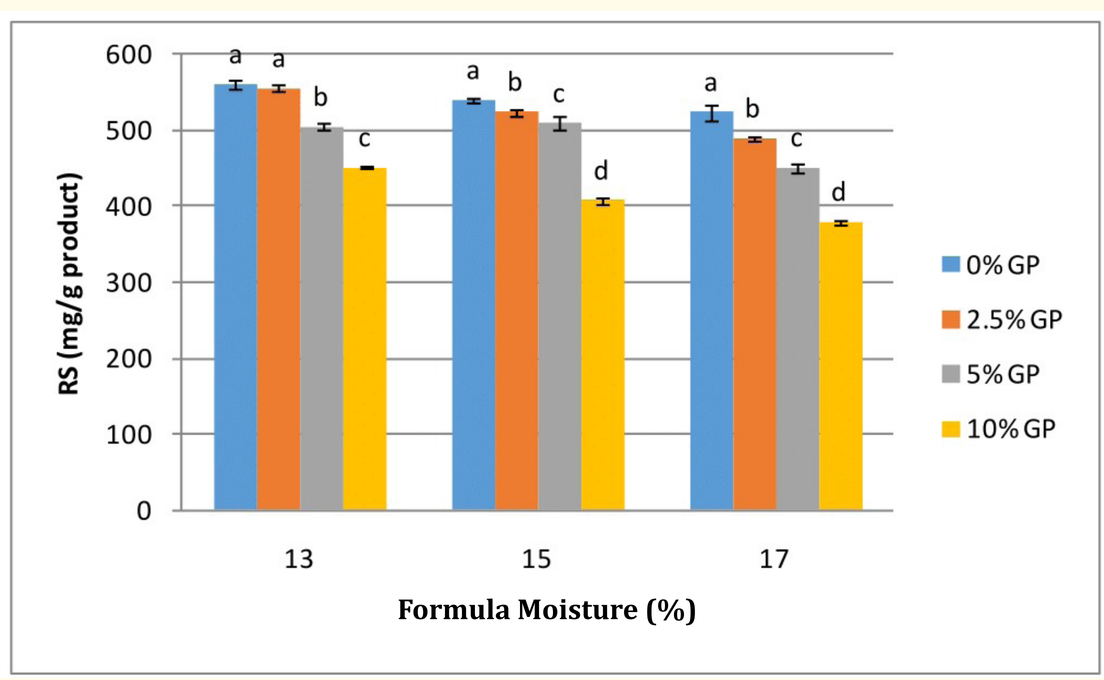


***Figure 3:*** *Reducing sugar (RS) concentration of amylase digested extruded product (digesta) as affected by grape pomace content and extrusion moisture (At same moisture level, data with sample label are not significantly different at 95% confidence interval).*

### Effect of grape pomace inclusion on starch digestion by simulated gastric fluid and amyloglucosidase

For samples obtained from simulated gastric-intestine digestion, the digested GP containing products showed obviously reduced RS contents (Figure 4). Among samples containing GP, the reducing sugar content of digesta decreasing with the increase of GP slightly but significantly ($P < 0.05$). At same GP level the reducing sugar content of the SGF-intestine digesta decreased with moisture of formula except extruded sample containing 2.5% GP. Compared to Figure 3, it can be seen that amylase digested corn starch in the extruded product more effectively than amyloglucosidase.

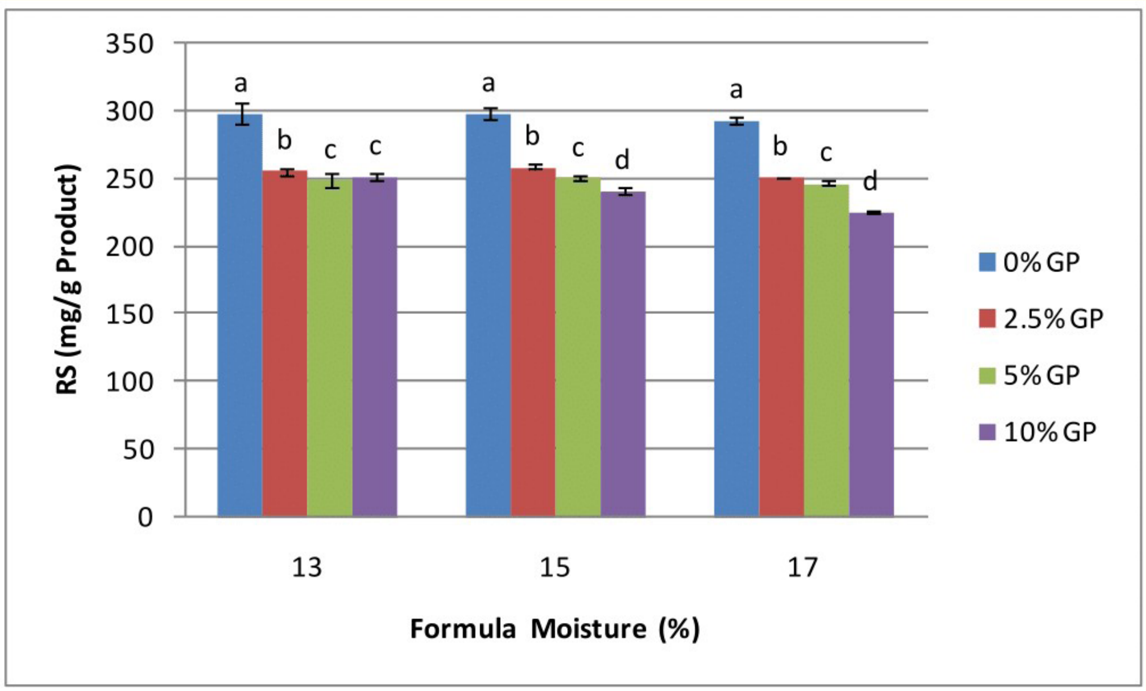


***Figure 4:*** *Reducing sugar (RS) concentration of simulated gastric fluid (SGF) digested extruded products as affected by of GP content and extrusion moisture (At same moisture level, data with sample label are not significantly different at 95% confidence interval).*

## Conclusion

The study suggests that GP could serve as an economic source of dietary fiber and polyphenols in extruded food products. Inclusion of GP in the formula of extrusion process reduced overall product digestibility and starch digestibility. This will contribute to reduced sugar absorption after intake of starch rich extruded products. Therefore, adding GP in the extruded product may be good for overweight population and people with Type-2 diabetes.

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## Author Disclosure Statement

No competing financial interests exist.

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