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Article

Polyphenol Antioxidants in Savory and Other Snacks

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Abstract: Whole grain consumption in the US has increased due to the introduction of whole grains initially in breakfast cereals and then in snacks. Most people consider fiber as the health benefit of eating whole grains since it inhibits the absorption of fats and sugar in the GI tract. Polyphenols, the major antioxidants in the US diet, are primarily covalently bound to the fiber matrix of grains that are broken during digestion. Human biomarkers have been found after cereal grain consumption, thus proving bioavailability of polyphenols. Savory snacks (n=32) studied included potato chips, tortilla chips, pretzels and crackers. The snacks contained as the first ingredient enriched wheat, whole wheat, corn, potatoes, and beans. We have used both a Folin and ferric reducing antioxidant power (FRAP) colorimetric method with catechin as the standard for the commercial snack assays. Total polyphenols were analyzed after basic hydrolysis. Maximum bio-accessible polyphenols were measured after sample hydrolysis simulating in vivo digestion. FRAP measurements were done on the same samples. FRAP total antioxidant capacity values were significantly higher than Folin total polyphenols but followed the same trends and were highly correlated. Digestion significantly decreased both polyphenols and antioxidant capacity. Whole grain snacks, defined as having > 50% whole grains, had significantly more polyphenols (7 times) than enriched grain snacks. Savory snack Folin polyphenols after in vitro digestion averaged 64% of the total polyphenol value. This indicates polyphenol bio-accessibility might be favorable after snack consumption. Using published data and this work, the order of total polyphenols in all snacks is nuts >> dried fruits > popcorn > pretzels > tortilla chips > crackers > potato chips. Potato chips are the most consumed snack but are lowest in polyphenol antioxidants

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1. Introduction

Snacks are defined as small portions of food eaten between meals and they are usually processed. The first-generation snacks include popcorn, peanuts, nuts and dehydrated fruits. Second-generation snacks are produced by direct extrusion (corn chips, puffed corn) or by cutting/sheeting/forming such as potato chips, tortilla chips and pretzels. The processing of whole grains removes fiber, polyphenols as well as vitamins and minerals. The resulting flour has a much longer shelf life. Snacking by adults from 1977-1978 to 2007-2008 has increased significantly ($p < 0.01$) [1]. The mean frequency of snacking is also up from 1.0 to 2.2 snacks/day and the % of adult snacking on a given day is up from 59 to 90%. US consumers are 37% more likely to eat snacks when feeling sad or depressed during the pandemic than they did in 2019 [2]. Savory or salty snacks are also comforting to consumers when anxious or bored. The percentage of total calories from snacks doubled in the last ten years from 12 to 24% and amounts to 500 calories/day. Currently only 4% of Americans said they have eaten no snacks the previous day [2].

Since savory snacks are plant-derived they contain polyphenols. Polyphenols are synthesized by the plant as a means of warding off insects due to their bitter taste. They also protect the plant from free radical damage due to UV light from the sun. Polyphenols are the major antioxidants in plant foods but in whole grains are covalently bound by the fiber. In order to be absorbed into the

body grain polyphenols must be hydrolyzed during the digestive process. Free polyphenols are then taken up and metabolized primarily in the liver. Systemic polyphenols and their metabolites make their way to cells, tissues and organs and exert health effects throughout the body [3]. Polyphenols and phenols (found to a lesser extent) in the human diet are well known to be healthy as a result of 50 years of epidemiological studies and from recent very large intervention studies investigating foods in the Mediterranean diet [4]. Polyphenols have been found to be significantly better antioxidants against in vitro LDL oxidation than vitamins A, C and E [5]. LDL oxidation in vivo is the initiation step of atherosclerosis commonly known as hardening of the arteries. Intake of polyphenols is hypothesized to inhibit in vivo LDL oxidation and thus decrease the risk of heart disease.

The health benefits of whole grains are a subject that has been part of many studies over the last 20 years. Consumption of whole grains has been linked to several health benefits including reducing the risk of chronic diseases such as type 2 diabetes, coronary heart disease, and hypertension [6]. These health benefits were previously believed to be linked to the fiber content in grains. However recent research now indicates that these health effects are due to the presence of phenolic acids, primarily ferulic acid, in the grains. In other words the whole grain content of food is more relevant to health than the fiber content shown on the label [7]. The fiber-bound polyphenols are released during digestion as phenolic biomarkers which have been found after human consumption [8]. Grains also contain other classes of polyphenols including anthocyanins, flavanols, flavonols, flavones, flavonones and chalcones. A review discussed the antioxidant properties of polyphenols both in vitro and in vivo [9].

Up to now there are no publications on polyphenols in US snacks for use in consumer decisions. Thirty-two commercial savory snacks were analyzed for total polyphenol antioxidants after alkaline hydrolysis, and after in vitro digestion by a standardized single reagent specific Folin-Ciocalteu method that does not respond to reducing sugars or proteins [10]. The FRAP method [11] previously used for popcorn was used for analysis of antioxidant capacity on the same snack samples.

2. Materials and Methods

We obtained snacks from local grocery stores. Samples were ground to a fine powder under liquid nitrogen. Lipids were extracted twice with hexane. One hundred mg (flour) or 1 g of snack sample, done in duplicate or triplicate, was extracted and hydrolyzed with 10 ml of with 1M NaOH in methanol under nitrogen overnight with shaking. The basic solution was neutralized with HCl and an aliquot used to measure polyphenols by the single reagent Folin and the FRAP colorimetric method to give the total phenols as catechin equivalents (TP) and total antioxidant capacity as TAC, respectively. Three samples each of commercial whole grain wheat flour and whole grain corn flour were also analyzed by the Folin method.

The in vitro digestion method used was a modification of the the procedure previously described by Liu [12]. In brief, 0.5 g of ground snack sample in duplicate was mixed with 18 mL of saline (140 mM NaCl, 5 mM KCl and 150 μ M butylhydroxytoluene) and acidified to pH 2 with 0.1 M HCl. Then the sample was mixed with 0.5 mL of pepsin solution (0.2 g of pepsin in 5 mL of 0.1 M HCl) and incubated in a shaking water bath at 37 °C for 1 h. After simulated gastric digestion, the pH of the digestate was increased to 6.9 with 0.1 M NaHCO₃. Further intestinal-simulated digestion was performed with the addition of 2.5 mL of pancreatin-bile solution (0.240 g of bile extract and 0.040 g of pancreatin in 20.0 mL of 0.1 M NaHCO₃) and incubated in a shaking water bath at 37° C for 2 h. The total digestate was then acidified to pH 2 and adjusted to a total volume of 37 mL with 2.4 M HCl. Solids were filtered using gravity filtration and the digest solution was stored at -80°C until further analysis. Folin polyphenol and FRAP antioxidant capacity (AC) analysis of these digested samples determined the maximum bio-accessible polyphenols (MBP) and maximum bio-accessible antioxidant capacity (MBAC).

Statistical comparison of two groups was done with two sample student t test when there was a normal sample distribution or a Wilcoxon Ranked Sum test when not normally distributed. Correlations were done with the Spearman test. Significance was defined as $p < 0.05$.

3. Results

In Figure 1 are the results for the TP and MBP found in the 32 snacks. They are listed from left to right in descending order of TP. The range of the TP was 0.270 to 16.25 mg/g and the average was 3.16 ± 3.29 mg/g. The average % standard deviation was 4.1% for individual TP analysis. After digestion the average decreased significantly to 1.93 ± 1.76 , a decrease of 37% which was highly significant ($p < 0.001$).

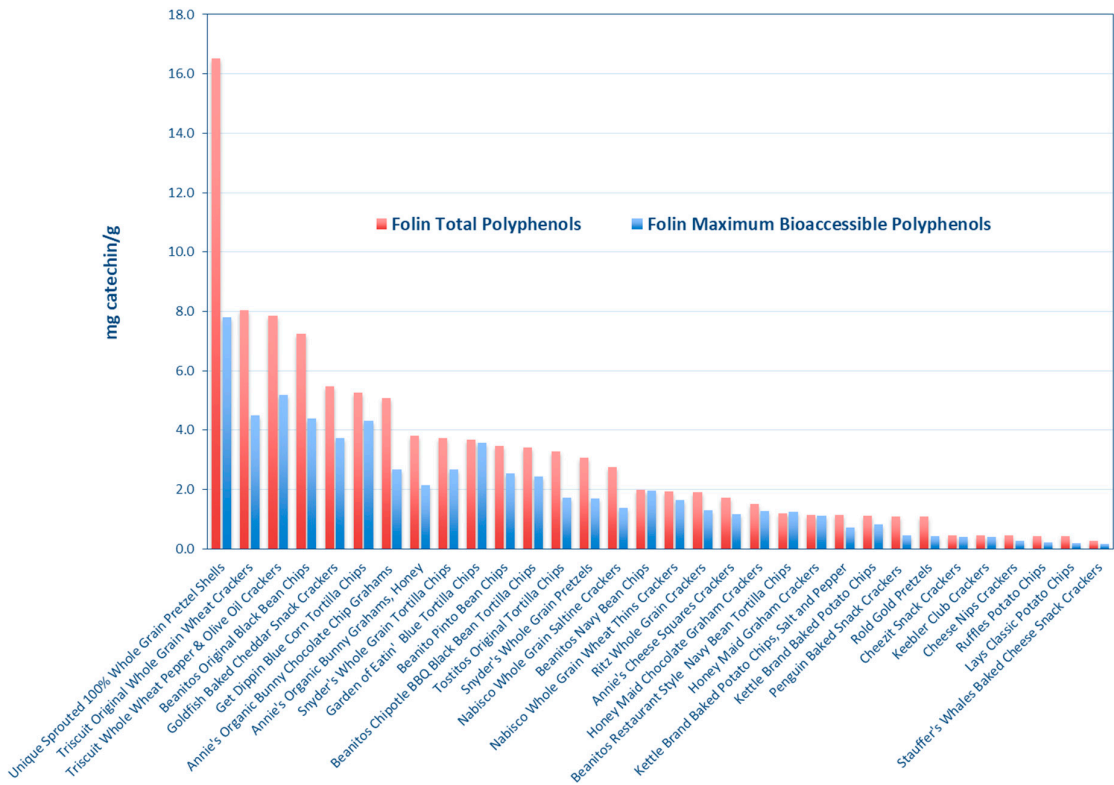


Figure 1. Total Polyphenols and Maximum Bio-accessible Polyphenols in Snacks. (mg catechin/g).

3.1. Flours

Our analysis of TP in two enriched flours found that enriched flour was 1.8 ± 0.2 mg/g and in three whole wheat flours was 8.2 ± 1.0 mg/g. Whole corn flours averaged 9.9 ± 1.0 mg/g. These latter two values represent the theoretical upper limit of wheat and corn snacks' TP.

3.2. Wheat Snack Crackers TP

There are two types of wheat crackers. There are those made with enriched wheat flour and those with whole grain wheat flour. There were nine enriched grain crackers analyzed for TP which averaged 1.57 mg/g. Compared with four whole grain crackers (two Triscuit and two Annie's Grahams) which averaged 6.2 mg/g, the whole grain was significantly higher ($p < 0.05$). There were five crackers which had whole wheat flour as a second ingredient (two Honey Maid Grahams, Annie's Cheese Squares, Ritz Crackers and Wheat Thins). They averaged 1.63 mg/g which is equivalent to that of enriched wheat crackers. There was one aberrantly high enriched wheat cracker Goldfish Baked Cheddar which was 5.48 mg/g. However, it contained a spice mix, and spices are well documented to be very high in TP.

3.4. Potato Snacks TP

3.5. Bean Snacks TP

3.6. Pretzel Snacks TP

mg catechin/g

FRAP Total Antioxidant Capacity

FRAP Maximum Bioaccessible Antioxidant Capacity

Product	FRAP Total Antioxidant Capacity (mg catechin/g)	FRAP Maximum Bioaccessible Antioxidant Capacity (mg catechin/g)
Unique Sprouted 100% Whole Grain Pretzel Shells	23.5	2.5
Tropicana Original Whole Grain Wheat Crackers	14.8	1.8
Goldfish Baked Cheddar Blue Corn Crackers	14.5	1.5
Get Tropicana Blue Corn Tortilla Chips	14.2	1.5
Annie's Organic Bunny Chocolate Chip Crackers	12.8	1.8
Snyder's Whole Grain Tortilla Chips	12.5	1.5
Annie's Organic Bunny Grahams	11.5	1.2
Garden of Eatin' Blue Tortilla Chips	5.8	1.2
Beanitos Chipotle BBQ Black Bean Tortilla Chips	5.5	1.2
Tostitos Original Tortilla Chips	5.5	1.2
Snyder's Whole Grain Tortilla Chips	5.5	1.2
Nabisco Whole Grain Saline Crackers	5.5	1.2
Nabisco Whole Grain Thin Crackers	5.2	1.2
Beanitos Navy Bean Crackers	5.2	1.2
Annie's Cheese Squares Crackers	3.5	1.2
Honey Maid Chocolate Graham Crackers	3.2	1.2
Honey Maid Graham Crackers	3.2	1.2
Newman's Own Potato Chips	2.5	1.2
Penguin Baked Snack Crackers	2.2	1.2
Rold Gold Pretzels	1.8	1.2
Keebler's Cheesecake Crackers	1.5	1.2
Stouffer's Baked Cheese Snack Crackers	1.2	1.2
Beanitos Navy Bean Crackers	1.2	1.2
Annie's Cheese Squares Crackers	1.2	1.2
Honey Maid Chocolate Graham Crackers	1.2	1.2
Honey Maid Graham Crackers	1.2	1.2
Newman's Own Potato Chips	1.2	1.2
Penguin Baked Snack Crackers	1.2	1.2
Rold Gold Pretzels	1.2	1.2
Keebler's Cheesecake Crackers	1.2	1.2
Stouffer's Baked Cheese Snack Crackers	1.2	1.2

Figure 2. Total Antioxidant Capacity and Maximum Bio-accessible Antioxidant Capacity in Snacks.

3.7. Snacks MBP

For MBP the range was 0.151 to 7.81 mg/g and the average was 2.04 ± 1.75 mg/g. The average standard deviation for MBP was 8.9%. TP was significantly higher than MBP ($p < 0.001$) but they were highly correlated (Pearson correlation coefficient $r^2 = 0.9216$). The in vitro digestion procedure decreased the polyphenols an average of 35.4%. This indicates that polyphenols after in vivo digestion might still have favorable bio-accessibility. Beanitos Puffs containing navy beans had only a decrease of 15% polyphenols during digestion and Beanitos Tortilla Chips with navy beans actually increased 17%.

There is an excellent paper on in vitro digestion of wheat and corn whole grains which measured polyphenols by Folin assay and HPLC [17]. Corn had a significantly higher TP than wheat as found for our whole grain flours TP. They also found after digestion a decrease in TP, as well as HPLC polyphenols, which was ascribed to the loss of monomeric polyphenols. The TP sample of corn and wheat had the highest cellular antioxidant assay in this model.

3.8. Snacks TAC and MBAC

On the previous page in Figure 2 are listed the results for TAC and MBAC in the snacks. For TAC the range was 0.294 to 23.2 mg/g and the average was 5.41 ± 5.62 mg/g. The average standard deviation for TAC was 6.84%. MBAC averaged 0.90 ± 0.69 mg/g and ranged from 0.01 to 2.62 mg/g. The average standard deviation for MBAC was 6.5%. TAC and MBAC were significantly different ($p < 0.001$) but highly correlated ($r^2 = 0.8780$). The in vitro digestion procedure decreased the antioxidant capacity an average of 83.4%.

It is readily apparent from comparing the two figures that although the TAC values are higher than the TP values, after digestion the MBAC value are significantly lower than the corresponding MBP values ($p < 0.01$). Apparently, antioxidant capacity is much more sensitive to digestion than polyphenols. Antioxidant capacity is reduced to negligible amounts for the enriched wheat flour chips, pretzels, and crackers after digestion.

The snack trends were the same for TAC and MBAC as for TP and MBP. TP and TAC values were highly correlated ($r^2 = 0.9312$, $p < 0.001$) as were MBP and MBAC ($r^2 = 0.9389$, $p < 0.001$) so trends in TAC among the snacks will not be outlined in this section. The order of the snacks was approximately the same before and after digestion for TP and AC.

4. Discussion

4.1. Processing

The effects of processing to manufacture snacks can be illustrated by several examples. Potatoes are fried twice to make potato chips at temperatures greater than 180°C . The % water goes from 80% in the potato to 3% in the chip. Comparing polyphenols: potatoes (dry weight) are 5.9 mg/g TP [14] and potato chips are 0.8 mg/g. TP in potatoes are thus reduced 86% by heating and dilution with oil and salt involved in the processing to produce potato chips. Preparing potato chips using colored potatoes which are high in anthocyanins produces snacks with 2-3 higher antioxidant activity [18]. Dry beans average 30 mg/g dry weight [16] and the Beanitos snacks averaged 3.5 mg/g, a decrease of 88% in TP due to cooking and processing.

Popcorn represents the savory snack with the least processing and is the only snack that is 100% whole grain. TP of microwaved popcorn with no added salt or oil showed no decline compared to the intact kernels in either TP or TAC [11]. It is important to consume the pericarp of the popcorn (the tough skin surrounding the kernel) as it contains almost all of the TP. Although some nuts are roasted rather than eaten raw, there is no change in TP in nuts due to roasting. Walnuts had the highest TP of the nine nuts studied (20.1 mg/g) [19]. Advice for the consumer is to eat nuts with skins if there is a choice (peanuts for example) as the TP is higher. Peanut butter (made with no skins) has ~60% of the TP of peanuts [19]. Dried fruits are also minimally processed as they are dried by the sun or in

ovens or specialized dehydrators. Six dried fruits averaged 15% higher in TP than that of the fresh fruit [20].

Consumption of heavy/ultra processed foods, like potato chips, crackers and pretzels increases the risk of chronic diseases such as cancer, high blood pressure and diabetes. The average American diet consists of 57.5% of calories from ultra processed foods. These types of foods have too much sugar, sodium and fat, lack nutritional value, are calorie dense and addicting and full of artificial ingredients. The longer the ingredient list on the label, the more processed is the food or snack [21].

4.2. *Snack Consumption in the USA and TP in Snacks*

A 2015 survey of 40,000 subjects determined the average % consumption of the seven varieties of snacks eaten in the USA [22]. Not included were dried fruits. A pie graph of the data is shown in the following Figure 3. Potato chips (average 0.78 mg/g TP) ranked 30th and 31st in TP among the 32 snacks but were the most consumed snacks. Second in snack consumption, corn-based tortilla chips, averaged 3.99 mg/g. Nuts were third in consumption (average 13.8 mg/g); crackers were fourth in consumption (average 2.86 mg/g); fifth was popcorn (5.93 mg/g); sixth, cheese puffs (not analyzed but very low in fiber); and pretzels seventh (6.89 mg/g). Cheese puffs are made with enriched wheat flour diluted with cheese and thus are expected to be quite low in TP. The 32 savory snacks of the 6 types we measured averaged 3.1 mg/g. Ranking TP in all snacks give the following order: **nuts >> dried fruits > popcorn > pretzels (including sprouted) > tortilla chips > crackers > potato chips**. Potato chips were the most consumed snack but had the least amount of healthy polyphenol ingredients.

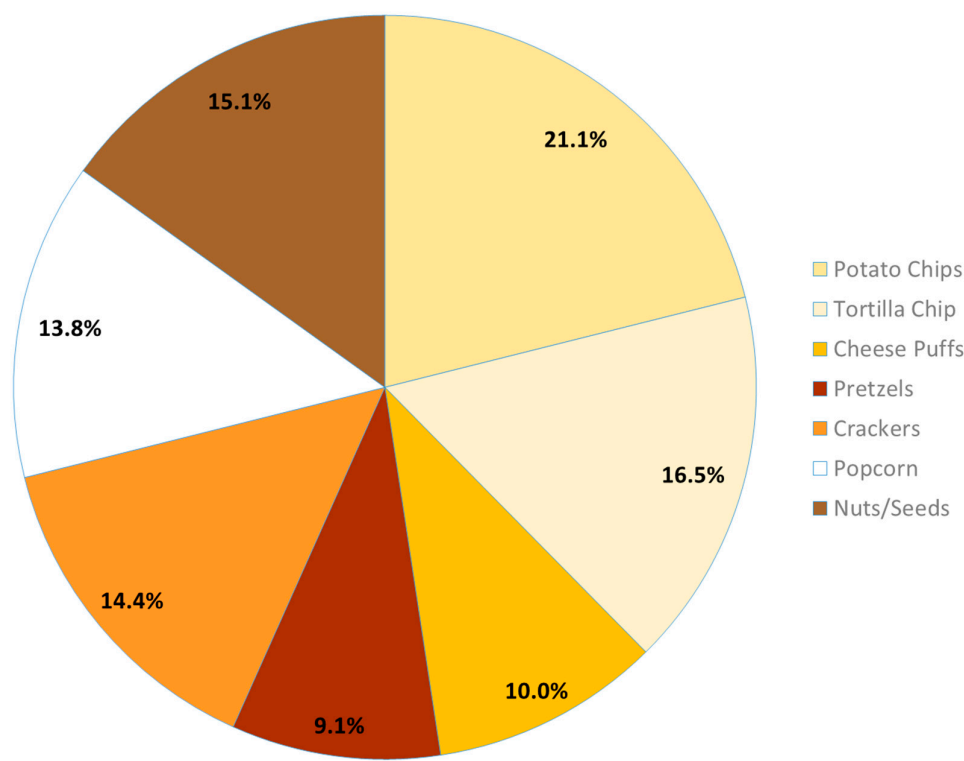


Figure 3. Percentage of Snack Consumers Consuming Each Snack.

4.3. *Sprouted Wheat*

Sprouted pretzels had the highest TP and AC of all the savory snacks. Sprouting of whole grain is germination/fermentation done in a controlled setting. This processing has been shown to change the appearance, flavor, and taste of the grain as well as improve the nutritional value of the product.

Sprouting also liberates polyphenols bound to the polysaccharide fiber matrix of the grain [23,24]. The increased TP in sprouted pretzels is shown by the extraordinarily high value compared to whole grain pretzels (15 times higher). Sprouting also releases simple sugars from the starches, making addition of sugar unnecessary. Addition of sugar is needed in enriched grain or whole grain pretzels to cut the bitterness of the wheat.

4.4. Recommendations

The global snack market is valued at \$440 billion (US share \$110 billion) and is expected to grow annually at a rate of 6.2% [25]. The dietary guidelines for the USA recommend a maximum 2000 calories per day should come from grain consumption and that half of that consumption be whole grains. As of 2017 only 14% of the population follow that whole grain recommendation. Since 2017 wheat consumption has increased 23 % while corn consumption has increased 202% [26]. That is a healthy trend if it were whole corn since that is higher in TP than wheat. Based on our data whole grain snacks are healthier as they contain more polyphenols. We examined 17 of the savory snacks (without added polyphenols in the form of nuts, seeds or fruits) to see if there was a correlation of TP/serving with the amount of whole grain in a serving of the snack as found on the Oldways Whole Grain Stamp on the products' label. The positive correlation was highly significant, $p < 0.001$ with a correlation coefficient of 0.805. Using the label value of fiber content of all snacks the correlation with TP/serving is also significant ($p < 0.02$) but with a much lower correlation coefficient of 0.183. Thus, the consumer can get an idea of the amount of TP by looking at whole grain content or alternatively fiber content of a snack. Potato chips and enriched wheat flour crackers should be avoided. Tortilla chips made from corn and having more polyphenols are preferred over potato chips. Snacks containing non-grain high polyphenol ingredients like fruits, nuts and spices will diversify the flavor and greatly increase polyphenol intake of snacks. Since snacking is on the rise consumers should use science to help make informed decisions about purchase and consumption.

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Conflicts of Interest: The authors declare no conflict of interest.

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