Title: Prebiotic Carbohydrates in Strawberry and Raspberry as a Food Source to Reduce Obesity

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Objectives: Determine types and concentrations of prebiotic carbohydrate in strawberry and raspberry as a possible dietary source to reduce obesity.

Justification: One-third of the Americans are obese (body mass index ≥ 30 kg m^{-2}). The 2011-2012 National Health and Nutrition Examination Survey found 35% of Americans and 17% of children and adolescents obese (CDC, 2014). The prevalence of obesity in South Carolina is similar to the national average at 31.6%, while North Carolina is slightly below the national average. Obesity increases the risk of cardiovascular disease, type 2 diabetes, osteoarthritis, and several cancers (NIH, 1998). In addition, health care costs have been skyrocketed, therefore new sustainable prevention methods are required to combat obesity related non communicable diseases in the USA (Volkow, 2011). Recent research suggests that the intestinal microbiome and a prebiotic-rich, low-calorie diet can play important roles in reducing obesity and related non-communicable diseases. According to these studies, prebiotic-rich diets stimulate the immune system, promote mineral micronutrient absorption (especially iron and selenium), decrease the risk of developing colon cancer, reduce excessive blood glucose and cholesterol levels, and improve insulin sensitivity. Therefore, whole foods high in prebiotics are seen as healthy food choices to reduce obesity and to improve the general health of Americans.

Naturally occurring prebiotic carbohydrates are categorized into two groups: dietary fiber and sugar alcohols. Major dietary sources of prebiotic carbohydrates are cereals, pulses, fruits, and vegetables. As a result of heavy food processing practices, cereal based food products have low levels of prebiotic carbohydrates than pulses, small fruits, and vegetables. Americans 20+ yrs of age consume less than half of recommended amounts of prebiotic carbohydrates (e.g., 7-10 g/d of prebiotic carbohydrates). Pulses especially lentils can provide 13 g of prebiotics per 100 g of serving, which level can be doubled after cooking/processing (Johnson et al., 2013, 2014). No prebiotic data are available for small fruits. Therefore, studies on small fruit prebiotics are likely to offer new opportunities to elucidate the true nutritional value of popular small fruits including strawberries and raspberries. Although prebiotic data for cereals and pulses are beginning to appear, data on small fruits including strawberries and raspberries are not yet available.

Strawberries and raspberries are a major part of the US fruit industry, worth more than $2.2 billion. Fresh market strawberry includes approximately 80 percent of total strawberry production (30.1 billion pounds) and processing strawberries includes 18 percent of total production (6 billion pounds). California is the major strawberry producing state, recently North Carolina emerged as fourth major strawberry producing state in the USA. The current drought conditions in California has affected on US strawberry production. Industry estimated that a range from a half-million to 1 million acres of agricultural land in California likely to be affected by the current drought (Agriculture Marketing Resource center, 2014). Therefore, more small fruit production and new marketing opportunities will arise for Carolina producers. Raspberries is the third most popular berry in the United States after strawberries and blueberries. Raspberry production in North Carolina is relatively small; however, this fruit could be promoted through increased consumer awareness of its nutritional quality. Therefore, studies on North Carolina and South Carolina grown small fruit prebiotics will provide important nutritional quality data, and new opportunities to promote these fruits for greater consumer health.

Methodologies:

Strawberry samples: Approximately 35 fresh South Carolina (SC) and North Carolina (NC) grown strawberry samples were collected in 2015 spring **(Table 1)**. Total of 21 strawberry samples representing three cultivars (Albion, Camarosa, and Chandler) were collected from SC. Total of 14 strawberry samples representing 14 genotypes were collected from NC state strawberry breeding program **(Table 1)**.

Raspberry samples*:* Total of 41 raspberry samples were collected in 2014 and 2015 from NC State University raspberry breeding program. Raspberry cultivars, growing year, and number of samples are presented in **Table 2**.

Chemicals: Carbohydrate standards, reagents, and high-purity solvents used for high performance liquid chromatographic (HPLC) analyses were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and VWR International (Radnor, PA, USA). These were used without any further purification. Water, distilled and deionized (ddH_2O) to a resistance of ≥18.2 MΩ (Milli-Q Water System, Millipore, Milford, MA), was used for sample extractions and preparation.

Analysis of prebiotic carbohydrates: Ground fresh strawberry samples were weighed (~500 mg) into 15 mL polystyrene conical tubes. Extraction was carried out using a previously described method (Muir et al., 2009). Samples were combined with 10 mL of ddH_2O, agitated to assure proper homogenization, and incubated in a water bath at 80° C for 1 hour. This was followed by centrifuging at 3000 x *g* for 15 minutes at 22° C using an Eppendorf 5810 R centrifuge (Hauppage, USA). After being centrifuged, 1 mL of the supernatant was diluted with 10 mL of ddH_2O and passed through a 13 mm x 0.45 µm nylon syringe filter (Chromatographic Specialties, Brockville, ON). Analysis of sugar alcohols, monosaccharide, disaccharide, and oligosaccharides was performed on a Dionex system (ICS-5000 Dionex, Sunnyvale, CA, USA) using a method previously described [2]. Carbohydrates were separated by a CarboPac PA-100 4 x 250 mm column in series with a CarboPac PA-100 4 x 50 mm guard column (Dionex, Sunnyvale, CA, USA). Flow rate was maintained at 1mL per minute during the mobile phase. Solvents used for elution were 100 mM sodium hydroxide/600 mM sodium acetate (solvent A), 200 mM sodium hydroxide (solvent B), and ddH$_2$O (solvent C). Solvents B and C were used at 50% for the initial 16 minutes. This was followed by a gradient change of 3% A, 48.5% B, and 48.5% C at the 16 minute mark. At 18 minutes, the gradient changed linearly to 16% A, 42% B, and 42% C. Finally, at the 19 minute mark the gradient returned to 50% B and 50% C, the original gradient. The total run time was 23 minutes. Detection was carried out using a pulsed amperometric detector (PAD, Dionex) in congruence with a working gold electrode (Thermo Fisher Scientific, Inc.) with a silver-silver chloride electrode at 2.0 μA. Reported carbohydrate concentrations were based on pure standards from Sigma-Aldrich, selected primarily on the basis of high relative abundance in strawberry and raspberry: sorbitol, mannitol, glucose, sucrose, raffinose, stachyose, verbascose, nystose, 1-kestose, and galactinol. The concentrations of the analyzed carbohydrates were detected within a linear range of 0.1 – 100 ppm. The minimal detectible limit was 0.1 ppm (signal to noise ration of ≥3).

Statistical Analysis: The experimental design was a complete randomized design with two separate small fruit types, states, and growing seasons as class variables. A mixed model analysis of variance for each experiment was performed using the PROC GLM procedure of SAS version 9.2 (SAS User's Guide: Statistics, 2009). Means were separated by Fisher's protected least significance difference (LSD) at $p < 0.05$.

Table 1: Growing state, cultivar, and number of strawberry samples collected in 2015.

State	Cultivar	Number of Samples
South Carolina	Albion	6
	Camarosa	9
	Chandler	6
Total		**21**
North Carolina	Chandler	1
	Festival	1
	Radiance	1
	Sweet Charlie	1
	Ann	1
	Camino Red	1
	Benecia	1
	Albion	1
	San Andreas	1
	Merced	1
	NC10-038	1
	NC10-777	1
	NC10-032	1
	NC09-11	1
Total		**35**

Table 2: Sample description of raspberry samples collected in 2014 and 2015.

Growing year	Cultivar	Number of samples
2014	NC 682	1
	NC 669	1
	NC 693	1
	NC 705	1
	NC 712	1
	Tula Magic	1
	Crimson Giant	1
	Crimson Night	1
	Himbo Top	1
	Nautahala	1
	Polka	1
Total		**11**
2015	Cascade Gold	2
	Crimson Giant	2
	Dormanred	2
	NC 713	2
	NC 344	2
	NC 548	2
	NC 612	2
	NC 666	2
	NC 712	2
	Latham	2
	Mac Black	2
	Nantahala	2
	Polka	2
	Tulamagic	2
	Tulameen	2
Total		**30**

Results:

***Strawberry*:** Mean prebiotic carbohydrate concentration in strawberries are listed in **Table 3**. 100g serving of fresh strawberry provides 12-87 mg of sugar alcohol, 26-3168 mg of simple sugars, 105-207 mg of raffinose oligosaccharides, 90-190 mg of kestose, and 50-1752 mg of hemicellulose **(Table 3)**. In addition, approximately 2g of unknown prebiotic carbohydrates were identified in these strawberries (data not shown; research underway to classify this unknown prebiotic carbohydrates). A single 100g serving of Carolina grown strawberries provides 4-8 g of dietary fiber, therefore, fresh strawberry is a significant source of dietary fiber **(Table 3)**. Prebiotic carbohydrate concentrations significantly vary with cultivar and the growing location. Camarosa and Chandler grown strawberry cultivars in SC showed significantly higher concentrations of sucrose, sorbitol, and arabinose compared to Albion **(Table 4)**. Strawberry cultivar, Merced grown in NC showed relatively higher concentrations of glucose, fructose, sucrose, xylose than the other cultivars **(Table 5)**. In summary, our preliminary first year data showed that strawberry is a significant source of prebiotic carbohydrates, however second year data is required to obtain significant cultivar differences, and provide recommendations for breeding and cultivar selections for high prebiotic carbohydrates.

Table 3: Mean prebiotic carbohydrates present in strawberry cultivars grown in SC.

Dietary Fiber Component*	Range	Mean†
Sugar alcohol (mg/100g)		
Sorbitol	12-87	53
Simple sugars (mg/100g)		
Glucose	1157-2180	1521
Fructose	1410-3168	2044
Sucrose	26-331	161
Raffinose family oligosaccharides (mg/100g)	105-207	151
Kestose (mg/100g)	90-190	142
Hemicellulose (mg/100g)		
Arabinose	50-181	84
Xylose	948-1752	1231
Total dietary fiber (g/100g)	*4-8*	5

* n=21, Data presented in fresh weight basis (90% moisture)

Table 4: Prebiotic carbohydrates concentrations of three strawberry cultivars grown in SC, USA.

Cultivar	Simple sugars			Sugar alcohol	Hemicellulose		RFO	Kestose
	glucose	fructose	sucrose	sorbitol	arabinose	xylose		
	mg/100g (fresh weight)							
Albion	1566 *a*	1982 *a*	42 *b*	39±4 *b*	77 *b*	1285 *a*	156 *a*	142 *a*
Camarosa	1432 *a*	1930 *a*	209 *a*	59±3 *a*	86 *a*	1144 *a*	152 *a*	140 *a*
Chandler	1607 *a*	2276 *a*	205 *a*	59±2 *a*	90 *a*	1308 *a*	146 *a*	143 *a*
Mean ± SE	1521±57	2011±87	160±12	53±4	85±7	1231±46	151±7	142±6

†Means within a column followed by different letters are significantly different at $p < 0.05$. SE, standard error (n=21). RFO, Raffinose family oligosaccharides include total of stachyose, raffinose, and verbascose.

Table 5: prebiotic carbohydrates of strawberry cultivars grown in NC, USA.

Cultivar*	Simple sugars			Sugar alcohol	Hemicellulose
	glucose	fructose	sucrose	sorbitol	xylose
	mg/100 g of fresh weight				
Chandler	1218	551	ND	78	583
Festival	1452	763	698	24	670
Radiance	1457	802	696	ND	714
Sweet Charlie	1351	763	737	111	681
Ann	1379	746	619	22	728
Camino Red	1043	557	443	10	491
Benecia	1250	673	687	49	575
Albion	1394	751	605	75	524
San Andreas	1324	735	640	ND	660
Merced	1703	922	1082	11	835
NC10-038	1459	728	ND	79	721
NC10-777	805	639	474	159	536
NC10-032	1392	820	820	65	697
NC09-11	1326	762	1076	21	631
Mean ± SE	1325±210	729±99	715±199	59±45	646±95

*SE, standard error (n=14; no biological replications). ND, not detected. Raffinose family oligosaccharides and kestose are not reported.

Raspberries: Mean prebiotic carbohydrate concentrations in raspberry cultivars are listed in Table 6. A single 100g serving of fresh raspberry provides 53-60 mg of sugar alcohol, 66-1991 mg of simple sugars, 28-32 mg of raffinose oligosaccharides, 28-32 mg of kestose, and 118-588 mg of hemicellulose **(Table 6)**. In addition, approximately 2-3g of unknown prebiotic different carbohydrates were identified in these raspberries (data not shown; research underway to classify this unknown prebiotic carbohydrates). A single 100g serving of these raspberries provides 3-4 g of dietary fiber **(Table 6)**. Prebiotic carbohydrate concentrations significantly vary with cultivar and the growing year. In 2014, Crimson Giant showed relatively high concentrations of simple sugars, moderate levels of sugar alcohols and hemicellulose **(Table 7)**. In 2015, raspberry cultivar, Dorman Red showed significantly higher concentrations of glucose, fructose, sorbitol, and xylose than the other cultivars **(Table 8)**. Raspberry cultivar, NC 612 showed higher concentrations of sucrose, than the other cultivars. Both Dorman Red and NC 612 cultivars had significantly higher concentrations of raffinose oligosaccharides and kestose. In summary, our preliminary first year data showed that raspberry also has significant amounts of prebiotic carbohydrates, however second year data is required to obtain significant cultivar differences and to provide recommendations for breeding and cultivar selection for high prebiotic carbohydrates.

Table 6: Mean prebiotic carbohydrates present in raspberry cultivars grown in NC.

Dietary Fiber Component*	**Mean (2014)**	**Mean (2015)**
Sugar alcohol (mg/100g)		
Sorbitol	60±22	53±2
Simple sugars (mg/100g)		
Glucose	1269±276	624±10
Fructose	1991±453	468±8
Sucrose	66±20	1835±67
Raffinose family oligosaccharides (mg/100g)	28±11	32±2
Kestose (mg/100g)	28±11	30±2
Hemicellulose (mg/100g)		
Arabinose	118±23	ND
Xylose	588±141	358±6
Total dietary fiber (g/100g)	4.1±1	3.3±1

* n=11 for 2014, and n=30 for 2015, Data presented in fresh weight basis (90% moisture), ND, not detected.

Table 7: Prebiotic carbohydrate concentrations of raspberry cultivars grown in NC, USA, 2014.

Cultivar*	Simple sugars			Sugar alcohol	Hemicellulose		RFO	Kestose
	glucose	sucrose	fructose	sorbitol	arabinose	xylose		
	mg/100 g of fresh weight							
NC 682	1651	90	2240	102	152	770	23	21
NC 669	948	38	1365	21	103	422	22	20
NC 693	1256	84	1939	68	108	551	21	20
NC 705	1078	56	1612	53	114	517	34	31
NC 712	1458	94	2336	67	138	736	32	29
Tula Magic	1203	41	2226	56	136	721	54	49
Crimson Giant	1676	83	2733	90	137	679	31	28
Crimson Night	851	65	1424	60	125	381	25	23
Himbo Top	1192	43	1839	46	73	418	26	24
Nautahala	1119	72	1660	52	112	462	12	11
Polka	1531	61	2524	48	97	592	24	22
Mean ± SD	1269±276	66±20	1991±453	60±22	118±23	588±141	28±11	25±10

*n=11, Data presented in fresh weight basis (90% moisture). SD, standard deviation (n=11; no biological replications). RFO, Raffinose family oligosaccharides include total of stachyose, raffinose, and verbascose.

Table 8: Prebiotic carbohydrate concentrations of raspberry cultivars grown in NC, USA, 2015.

Cultivar*	Simple sugars†			Sugar alcohol†	Hemicellulose†		RFO†	Kestose†
	glucose	sucrose	fructose	sorbitol	arabinose	xylose		
	mg/100 g of fresh weight							
Cascade Gold	619 *cde*	1993 *cd*	493 *dc*	25 *def*	ND	370 *cde*	32 *bc*	30 *bc*
Crimson Giant	736 *bc*	1732 *de*	631 *b*	58 *c*	ND	457 *ab*	23 *bc*	21 *bc*
Dorman Red	1208 *a*	41 *g*	756 *a*	209 *a*	ND	512 *a*	49 *ab*	45 *ab*
Latham	680 *bcd*	30 *g*	520 *c*	33 *de*	ND	413 *bc*	31 *bc*	29 *bc*
Mac Black	430 *fg*	389 *fg*	262 *f*	109 *b*	ND	199 *g*	11*c*	10 *c*
Nantahala	389 *g*	2142 *cd*	253 *f*	42 *cd*	ND	277 *f*	20 *bc*	18 *bc*
NC 344	518 *ef*	1950 *cd*	357 *e*	20 *efg*	ND	303 *ef*	39 *abc*	37 *abc*
NC 548	585 *de*	2649 *bc*	417 *de*	44 *cd*	ND	340 *def*	28 *bc*	25 *bc*
NC 612	549 *de*	3799 *a*	470 *cd*	17 *efg*	ND	438 *cb*	40 *abc*	37 *abc*
NC 666	778 *b*	955 *ef*	677 *ab*	2 *g*	ND	379 *cd*	69 *a*	64 *a*
NC 712	423 *fg*	2370 *cd*	443 *cde*	9 *fg*	ND	299 *ef*	41 *abc*	37 *abc*
NC 713	576 *de*	3307 *ba*	429 *cde*	10 *fg*	ND	377 *cd*	42 *abc*	38 *abc*
Polka	615 *cde*	1640 *de*	446 *cde*	42 *cd*	ND	323 *def*	15 *c*	14 *c*
Tulamagic	649 *cd*	2234 *cd*	475 *cd*	92 *b*	ND	317 *def*	28 *bc*	25 *bc*
Tulameen	567 *de*	2306 *cd*	405 *de*	92 *b*	ND	368 *cde*	18 *bc*	17 *bc*
Mean ± SD	624±10	1835±67	468±8	53±2	ND	358±6	32±2	30±2

*n=30, Data presented in fresh weight basis (90% moisture). SD, standard deviation (n=30). RFO, Raffinose family oligosaccharides include total of stachyose, raffinose, and verbascose. †Means within a column followed by different letters are significantly different at $p < 0.05$. SE, standard error (n=30). ND, not detected.

Conclusions: Both strawberry and raspberry are significant food sources could provide daily dietary fiber requirements for these two fruit consumers. However, to confirm this claim, it is required that this project to continue for year two to gather more data to understand the genetic and environment interactions variations. The results of this study will also enable breeding selections for higher prebiotic levels in these small fruits.

Impact Statement: Strawberry and raspberry are significant food sources could provide daily dietary fiber requirements for these two fruit consumers. *Eat more small fruits that provide dietary fiber!*

Citations for any publications:

1. Alex Abare submitted an abstract American Agronomy Society.
2. Peer review manuscript will be prepared after collecting second year data.
3. Dr. Fernandez will use our preliminary data to for future Specialty Crop Multi State grant.

References:

1. Agriculture Marketing Resource center, 2014; http://www.agmrc.org/commodities__products/fruits/strawberries/commodity-strawberry-profile/
2. CDC, 2014; http://www.cdc.gov/obesity/data/adult.html
3. Johnson, C.R., Thavarajah, D., Thavarajah, P., Payne, S., Moore, J., and Ohm, J. B. (2015). Processing, cooking, and cooling affect prebiotic concentrations in lentil (Lens culinaris Medikus). *J. Food Composition and Analysis*, *38*, 106-111.
4. Johnson, CR., Thavarajah, D., Combs, Jr, GF. and Thavarajah, P. (2013). Lentil (*Lens culinaris* L.): A prebiotic-rich whole food legume. *Food Res Inter,* 51(1) 107–113.
5. Muir, J.G, Rose, R., Rosella, O., Liels, K., Barrett, J.S., Shepherd, S.J., and Gibson, P.R. (2009). Measurement of Short-Chain Carbohydrates in Common Australian Vegetables and Fruits by High-Performance Liquid Chromatography (HPLC). *J Agric Food Chem.* 57: 554-565.
6. National Institute of Health (1998). Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults. T*he evidence report*, 6(2), 51S-209S.
7. SAS Institute. (2009). User's Guide: Statistics SAS Institute (Version 9.2). In. Cary, NC: SAS Institute.
8. Volkow, N., Wang, G., and Baler, D. (2011). Reward, dopamine and the control of food intake: implications for obesity. *Trends Cogn Sci*. 15(1):37-46.