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Some Evidences on Effect of Intake Aguamiel (Agave sap)

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Abstract

Honeywater or aguamiel (Agave sap) has been consumed by Mexican population since pre-columbian times. Although, it has been claimed by folk belief that aguamiel possesses some medicinal properties, scientific studies on its effect on human health have not been well documented. The behavior of blood components in nine volunteers (two young males, three adult females and four adult males) after aguamiel consumption (250 mL every three days during a period of 35 days) was analyzed. It was found that, serum red blood cell count, serum white blood cell count, platelet count, minerals (Zn, Mg and Fe) and iron-related proteins (ferritin and transferrin) levels were not negatively affected because of all of these blood indicators ranged within normal reference values. However, this study showed that aguamiel presented a specific functional effect since hypercholesterolemic adult males showed normal levels of serum total cholesterol after aguamiel consumption, whereas total cholesterol levels were kept in normal ranges after aguamiel consumption for normocholesterolemic subjects. Furthermore, aguamiel consumption did not cause hyperglycemia in any of the tested groups.

Keywords: Aguamiel, hematic biometry, cholesterol, human being

1. Introduction

Aguamiel is the sap extracted from 7-10 year old plants of maguey (*Agave spp.*) grown (wild or cultivated) in the arid and semi-arid regions of Mexico. The flower stalk of the plant (called quiote) is removed and after a period of several months, the sap (aguamiel) is collected from the core of the plant twice a day during a period of 3-6 months, accumulating a total of 600-900 liters all over the period (Granados, 1993). When fresh, the sap is consumed as a natural fortifying beverage and when naturally fermented, the sap becomes in an alcoholic drink, called Pulque. Aguamiel is characterized by a sweet, savory taste (Granados, 1993). It contains water, sucrose, glucose, fructose, gum, proteins, minerals, vitamins, and beneficial organisms such as *Kluyveromyces marcianus* var. *bulgaricus* (Estrada et al., 2001; Ortiz et al., 2008). It also contains pre-biotic inulin (11%) (Martínez, 1999; Cruz-Guerrero et al., 2006), and concentrations of iron (2.15 mg $100g^{-1}$) and zinc (1.41 mg $100g^{-1}$) higher than those reported in cow milk (Silos-Espino et al., 2007). In this respect, *Agave salmiana* honeywater can be used as a source of these minerals for humans in Mexico, where the iron deficiency is prevalent (Rosado et al., 1995). Aguamiel is a natural source of micronutrient whose benefits on health could be tentatively matched with micronutrient-fortified beverages containing iron, iodine, zinc, vitamin A, vitamin C, niacin, riboflavin, folate, vitamin B-12, vitamin B-6, and vitamin E that have been shown to reduce the risk of iron-deficiency anemia and increase the hemoglobin and ferritin concentration in pregnant women (Makola et al., 2003). There is little evidence about medicinal studies of agave sap on human beings. Colin (1928) reported that for gonorrhea, it is recommended to drink on a daily basis, half a liter of boiled aguamiel for about two weeks while Pardo (2007) has been claimed properties for cleaning the urinary system by drinking aguamiel. Recently, Tovar-Robles et al. (2011) analyzing the effect of agave sap on rabbits, found that the fresh aguamiel promoted weight increases of about 13% by the seventh week, haemoglobin counts increased 4.5% and 9% when rabbits were fed with fresh and boiled aguamiel (respectively), hematocrit counts increased from 2.6 to 5.3%, while mean corpuscular volume did not show change, mean corpuscular haemoglobin significantly increased up to 12%, which is higher than previously reported on rabbits. They observed that Fe (fixed to transferrin), transferrin and ferritin content increased slightly when fresh aguamiel was included in the diet, as compared to the control (without aguamiel). Thus, in the current study we examined, for the first time in humans, the effects of regular consumption of aguamiel by healthy subjects on their serum glucose, serum total cholesterol content, serum minerals, serum blood red cell count, serum blood white cell count, and cells of immune system.

2. Method

2.1 Aguamiel Extraction

Aguamiel samples were collected from *Agave salmiana* plants cultivated in the region of Calvillito, Aguascalientes and Salinas de Hidalgo, San Luis Potosi, Mexico. The aguamiel was boiled during 5 min to avoid fermentation and keep its microbiological stability. Then it was kept at 4 °C until its utilization.

2.2 Aguamiel Consumption

Nine healthy subjects were grouped according to sex and age as follows: Group I: two young men (between 22 and 23 years old) Group II: three women (between 48 and 60 years old) and Group III: four men (between 48 and 55 years old; Three of them hypercholesterolemic). The subjects ingested before lunch, a sample of 250 mL (a cup) of aguamiel every three days, during a period of 35 days. The subjects kept their individual food habits during the entire experimental period. Aguamiel was ingested before lunch in order to maximize its gut absorption. One cup was considered as a standard measure for a beverage commonly ingested alone or accompanying a meal. It was ingested every 3 days in order to avoid the possible weariness of a frequent drinking and a period of 35 days was considered long enough to observe any possible effect derived from aguamiel consumption.

2.3 Blood Analyses

In total, 5 mL of blood was extracted from the subjects, before and after the total aguamiel ingestion period. Three mL were used in the determination of glucose, total cholesterol, iron, Mg and Zn contents, and 2 mL were used in hemogram analyses (hematocrit, hemoglobin and erythrocyte index). In serum, the glucose, total cholesterol and iron content were done using the GOD-PAP photometric enzyme test, the CHOD-PAP photometric test and the ferene-based photometric tests, respectively. Magnesium content was done by the UV-VIS-xylidine blue assay. Zinc content was determined by the atomic absorption spectroscopy method using a Perkin-Elmer (3110 equipment, NJ, USA).

Transferrin was done by the IVD-turbidimetric assay and Ferritin content by the immune-enzymatic assay using a microplate Reader (Eldex 3.1, CA, USA). Leucocyte count was done by the Wright stain method using microscope at 100x (The Primo Star Zeiss, Göttingen, Germany). Hemogram analyses were done in an automated hematology analyzer (Horiba ABX Micros 60, Minnesota, USA).

2.4 Data Analysis

The data were organized in first and second sampling. First sampling was done before aguamiel intake, whereas the second sampling after the 35-day period (Before and After). For all blood analysis the individual data were compared to reference ranges from “Instituto Mexicano del Seguro Social” (Mexican Institute for Health Care). A normal condition for a given parameter from blood serum is present when the parameter value is within its reference range. When the level of a given parameter fall outside the reference range, this is considered as abnormal level. When data fall outside the reference range before aguamiel intake but fall within range after the intake, the effect is attributed to aguamiel consumption.

3. Results and Discussion

3.1 Initial Health Status of Subjects

The blood and general health status of the nine healthy subjects grouped according to sex and age was established using the following parameters: Red blood cell indices (hemoglobin, hematocrit, erythrocyte count, Mean Corspuscular Volume, Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin Concentration and Red blood cell Distribution Width), white blood cell indices (total leukocytes, total neutrophills, eosinophils, basophils, monocytes and limphocytes) and platelet count. Based on the results observed on the Complete Blood Cell Count, all the subjects were considered healthy, inclusive a subject from the group of adult females that exhibited levels of hemoglobin, hematocrite, MCV and MCH slightly lower than the respective reference range. On the other hand, serum iron, iron-related protein, serum glucose and total cholesterol measured before aguamiel intake showed normal levels for all the groups. However, three subjects from the group of adult males exhibited hypercholesterolemia and a female adult showed a level of 2.9 mg/dL of serum Mg, although slightly higher than normal range, it was regarded as hypermagnesemia.

3.2 Serum Glucose and Total Cholesterol

Values for glucose in serum between measurements before and after aguamiel global intake ranged from 81.7 to 98 mg/dL for young males (group I), from 76.9 to 22.6 mg/dL for adult females (group II) and from 84.9 to 117.9 mg/dL for adult males (Table 1). In general, the normal range for most people (fasting adults) is about 70 to 115 mg/dL. A subject with a consistent range above 126 mg/dL is generally regarded to have hyperglycemia, whereas a consistent range below 70 mg/dL is considered hypoglycemic. Aguamiel showed to be safe in terms of the minor glucose changes derived from its consumptions as beverage. Sugars in aguamiel that have been reported are glucose, polyfructans, arabinose, xylose, and galactose, whereas the fructose/glucose ratio has been reported as 3:16 (Ortiz-Basurto et al., 2008). Values for total cholesterol in serum between measurements before and after aguamiel global intake ranged from 154.6 to 185.9 mg/dL in young males, from 134 to 224.9 mg/dL in adult females and from 141.6 to 263 mg/dL in adult males.

Table 1. Serum glucose and total cholesterol in men and women before and after consumption of aguamiel for a 35-day period (aguamiel consumption: 250 mL every third day)

Group	*Subject*	*Glucose (mg/dl)*		*Cholesterol*	*(mg/dl)*
		Before	After	Before	After
I	Young Male	93.9	98.0	172.8	185.9
	Young Male	81.7	97.0	154.6	181.0
II	Adult Female	84.4	85.8	134.0	118.0
	Adult Female	86.0	76.9	171.1	198.1
	Adult Female	95.1	122.6	207.0	224.9
III	Adult Male	112.9	103.2	205.0	180.7
	Adult Male	117.9	107.8	263.0	186.7
	Adult Male	90.8	84.9	141.6	174.5
	Adult Male	87.2	96.2	252.0	207.8

Normal Reference Ranges: Serum glucose levels: 60 to 109 mg/dL; Total cholesterol levels: 0 - 200 mg/dL.

Young males showed optimal levels (less than 200 mg/dL of total cholesterol), one adult female showed values between 207 and 224.9 mg/dL corresponding to the borderline high levels of the reference range (200 to 239 mg/dL), whereas two adult males showed high levels of total cholesterol (greater than 240 mg/dL) and one adult male showed borderline high levels (200 to 239 m/dL) before de aguamiel global intake and diminished significantly to optimal levels (186.7 mg/dL) in a first case, to borderline high levels in a second case (207.8 mg/dL) and to optimal levels in the third case (less than 200 mg/dL), all of them after the global intake of aguamiel.

These results suggest that aguamiel provoked a drop of total cholesterol in the hypercholesterolemic adult males. In humans, decreases between 6–35 % of serum total cholesterol by fruit pectin consumption have been observed (Stasse-Wolthuis et al., 1980; Hillman et al., 1985). The total cholesterol-lowering effect by fruit pectin (fiber) is well known. Aguamiel contains only 0.57 % of crude fiber (Palmer & Dixon, 1966; Judd & Truswell, 1982). Hence, its hypocholesterolemic effect must be the result of a mechanism that does not involve its fiber content. Other plant component that has been also suggested to help lower serum cholesterol levels is phytosterol as seen in flax seeds and peanuts (Ostlund et al., 2003). Phytosterol at a dose of 2–3 g/d has shown a significant cholesterol-lowering effect in humans according to Plat and Mensink (2005). The presence of phytosterol in aguamiel has not been reported. However, a cholesterol-lowering effect has been observed in mice fed with a diet supplemented with 10% of agave fructans (inulin). Mice showed a reduction of 20 % of serum cholesterol levels (Urías-Silvas et al., 2008). It has been mentioned that aguamiel contains 11% of inulin (Martínez, 1999; Cruz-Guerrero et al., 2006) and inulin might explain the lowering effect in humans. However, Cholesterol-lowering effect of aguamiel in humans remains to be elucidated.

3.3 Behavior of Zinc, Iron, Magnesium and Iron-Related Proteins Content

Blood levels of Zinc, Iron and Magnesium varied within their respective reference ranges for all the groups of subjects (Table 2). This is important because in the one hand the clinical features of severe zinc deficiency in humans are growth retardation, delayed sexual and bone maturation, skin lesions, diarrhea, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioral changes (Hambidge, 1987). On the other hand, iron deficiency can affect negatively physical working capacity in a population if a long-standing iron deficiency is kept. However, an improvement in working capacity is shown after iron administration (Scrimshaw, 1984). Furthermore, iron deficiency is probably the most frequent nutritional deficiency disorder in the world (DeMaeyer et al., 1985) and in the case of Mexico, iron deficiency is prevalent according with Rosado et al. (1995), and the bio-availability of iron can be poor (Rosado *et al*., l992). Aguamiel also possesses important contents of Zn (14.1 mg/L) and Iron (21.5 mg/L). Levels of zinc and iron in aguamiel are sufficient to satisfy daily human requirements, provided people consume approx. 850 ml of aguamiel (Silos-Espino *et al*., 2007). Although Iron from vegetables, fruits, and grains, is hard for the body to absorb, this seems not to be the case for aguamiel because of according to our results, serum iron levels were kept under normal reference ranges for males (50 to 160 μg/dL) and females (45 to 150 μg/dL).

From this, it can be suggested that aguamiel does not contain substances that bind to iron and Zn that could negatively affect their bioavailability. Ferritin and Transferrin were also varying within the normal reference ranges supporting the normal serum iron level behavior. Magnesium is necessary for nearly all biochemical processes in the body. It helps maintain normal muscle and nerve function, keeps the bones strong, controls the heartbeat, and helps regulate blood pressure. Magnesium also controls blood sugar levels and helps support the body defense (immune) system. Serum magnesium levels were kept at normal levels for all the subjects of this study after aguamiel consumption, including the adult female that showed a slight hypermagnesemia (2.9 mg/dL) before aguamiel consumption.

Table 2. Serum minerals and iron related proteins in men and women before and after consumption of aguamiel for a 35-day period (aguamiel consumption: 250 ml every third day)

Group	Iron (μg/dL)		Ferritin (ng/mL)		Transferrin (mg/dL)		Zn (μg/dL)		Mg (mg/dL)	
	B	A	B	A	B	A	B	A	B	A
I	133.8	75.7	48.2	47.2	280.1	274.5	86.7	62.7	2.5	2.7
	124	99.4	49.6	49	312.2	365.1	51.2	78.9	2.5	2.5
II	56.8	61.8	0.4	0.1	333.8	349.7	64.1	89.2	2.2	2.5
	82.8	76.4	26.4	9.7	368.7	278	60.1	69.3	2.9	2.1
	61.9	72	40.9	37.8	241.8	118	80.6	82.6	2.6	3.1
III	96.2	121.3	264.9	121.6	315.1	337.8	72.9	72.3	2.8	2.0
	99.4	24.3	378.7	143	289.2	290.8	77.9	53.8	2.5	1.8
	137.3	73.3	63.8	37.2	344.3	240.5	77.6	75.4	2.2	2.1
	75.8	107.6	72.5	61.8	292.5	123.2	79.5	58.1	2.1	2.0

B: Before; A: After

3.4 Complete Blood Cell Count and Immune Response

Red blood cell, white blood cell indices and platelet count were kept at normal levels after the global period of aguamiel consumption with the exception of one adult female who kept levels slightly lower than normal values for hemoglobin, hematocrit, MCV and MCH (Tables 3 and 4). Based on the effect of aguamiel consumption for prolonged periods as in this study (250 mL every 3 days for a 35-day period) on the red blood cell and white blood cell indices and platelet count, it is clear that aguamiel is an innocuous beverage for population of young males, and adult males and females.

Table 3. Red blood cell counts in men and women before and after consumption of aguamiel for a 35-day period (aguamiel consumption: 250 ml every third day)

Group	Hmg g/dL		Hmt %		Eryth x 10^6/μL		MCV (fl)		MCH (pg)		MCHb (g/dL)		RDW (± %)	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A
I	14.8	14.3	42.5	42.5	5.3	5.3	80.2	80.2	27.9	27.0	34.8	33.6	12.5	13.3
	15.6	14.4	43.7	41.5	4.9	4.7	89.2	83.0	31.8	30.6	35.7	34.7	12.7	14.4
II	11.5	10.7	33.9	30.8	4.5	4.3	75.3	71.6	25.6	24.9	33.9	34.7	13.9	14.6
	13.0	13.5	37.9	39.2	4.5	4.7	84.2	82.7	28.9	28.5	34.3	34.4	13.0	14.2
	13.9	12.7	40.4	38.4	5.2	4.9	77.7	78.4	26.7	25.9	34.4	33.1	13.4	12.7
III	17.5	18.1	51.0	50.4	5.7	5.8	89.5	86.9	30.7	31.2	34.3	35.9	13.7	13.3
	16.4	16.6	47.6	47.6	5.3	5.4	89.8	88.0	30.9	30.7	34.5	34.9	14.7	14.4
	16.0	15.9	46.3	46.4	5.7	5.8	81.2	80.4	28.1	27.6	34.6	34.3	13.9	13.7
	16.8	15.3	48.5	44.3	5.6	5.2	86.6	85.4	30.0	28.0	34.6	32.0	13.6	13.9
RL	13.0-16.0		39.0-48.0		4.3-5.3		83.0-100.0		28.0-32.0		32.0-34.5		12.8 ± 1.2	

Hmg: Hemoglobin, Hmt: Hematocryte, Eryth: Erythrocyte, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, RDW: Red Blood Cell Distribution Width, RL: Reference Levels, B: before, A: After.

Table 4. White blood cell and platelet count in men and women before and after consumption of aguamiel for a 35-day period (aguamiel consumption: 250 mL every third day)

Group	T-Leuco /μL		T-Neutroph /μL		Eosinoph /μL		Basoph /μL		Monoc /μL		Limphoc /μL		PL-Count ($x10^5$) /μL	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A
I	6500	6900	3705	3726	0	0	130	69	585	276	2080	2829	2.64	2.34
	5500	5100	3025	2907	165	102	0	51	165	153	2145	1887	3.13	3.20
II	7600	5500	5092	3135	0	0	0	0	228	275	2280	2090	2.91	3.32
	4800	4200	264	2394	96	84	0	42	96	168	1344	1512	2.57	2.52
	5500	4800	3190	3024	0	0	20	48	385	192	1870	1536	2.12	2.06
III	8700	10800	5220	8208	87	216	0	0	435	432	2958	1944	2.41	2.50
	6600	7800	4818	4524	0	234	0	0	66	234	1716	2808	2.73	2.45
	8500	10300	6290	6489	0	412	0	103	510	309	1700	2897	3.01	2.91
	6800	5300	4828	3180	68	159	68	53	272	265	1564	1643	2.73	2.34
RL	4500-11000		1800-7700		20-450		20-100		0-800		1000 - 4800		1.5 – 4.5	

T-Leuko: Total Leukocytes, T-Neutroph, Eosinoph: Eosinophils, Basoph: Basophils, Monoc: Monocytes, Limphoc: Limphocytes, PL-Count: Platelet Count, RL: Reference Levels, B: Before, A: After.

Furthermore, it seems to possess hypocholesterolemic properties when drunk by hypercholesterolemic adults, showing a regulatory/homeostatic effect on total cholesterol levels since the subjects with normal levels of total cholesterol were kept within the reference range. This effect could be regarded as a functional/nutraceutical property of aguamiel in favor to human health avoiding potential cardiovascular diseases. The most of the measured clinical parameters were not significantly affected by the consumption of aguamiel since they were varying within the normal reference ranges during the 35-day period of aguamiel consumption.

4. Conclusions

Aguamiel possesses beneficial effects on human health, as demonstrated by reduced serum total cholesterol levels observed after aguamiel consumption by hypercholesterolemic subjects and that aguamiel consumption did not affect negatively to the levels of serum glucose. This is the first report about functional/nutraceutical aguamiel properties. However, this is a first a study about aguamiel functional properties involving human volunteers and there is a need for further studies investigating in a more profound approach about the cholesterol-lowering effect of aguamiel and the possible mechanism implied.

References

Colin, G. (1928). A contribution to the chemistry, pharmacology and therapeutics of Agave salmiana. *Journal of the American Pharmaceutical Association, 17*, 1182-1189. http://dx.doi.org/10.1002/jps.3080171204.

Cruz-Guerrero, A. E., Olvera, J. L., García-Garibay, M., & Gómez-Ruíz, L. (2006). Inulinase hyperproducing strains of Kluyveromyces sp. isolated from aguamiel (Agave sap) and pulque. *World Journal of Microbiology & Biotechnology, 22*, 115-117. http://dx.doi.org/10.1007/s11274-005-9005-4

DeMaeyer, E., Adiels-Tegman, M., & Raystone, E. (1985). The prevalence of anemia in the world. *World Health Stat Q, 38*, 302-316.

Estrada-Godina, A. R., Cruz-Guerrero, A. E., Lappe, P., Ulloa, M., García-Garibay, M., & Gomez-Ruiz, L. (2001). Isolation and identification of killer yeast from Agave sap (aguamiel) and pulque. *World Journal of Microbiology & Biotechnology, 17*, 557-560. http://dx.doi.org/10.1023/A:1012210106203

Granados, S. D. (1993). *Los Agaves en México* (p. 252). Universidad Autónoma Chapingo. Estado de México.

Hambidge, K. M. (1987). Zinc. In W. Mertz (ed.), *Trace elements in human and animal nutrition* (Vol. 1., 5th Ed., pp. 1-137). Orlando, Florida: Academic Press, Inc.

Hillman, L. C., Peters, S. G., Fisher, C. A., & Pomare, E. W. (1985). The effects of the fibre components pectin, cellulose and lignin on serum cholesterol levels. *American Journal of Clinical Nutrition, 42*, 207-213.

Judd, P. A., & Truswell, A. S. (1982). *Comparison of the effects of high- and low-methoxylpectins on blood and faecal lipids in man*. *British Journal of Nutrition, 48*, 451-458. http://dx.doi.org/10.1079/BJN19820130

Makola, D., Ash, D. M., Tatala, S. R., Latham, M. C., Ndossi, G., & Mehansho, H. (2003). A micronutrient-fortified beverage prevents iron deficiency, reduces anemia and improves the hemoglobin concentration of pregnant Tanzanian women. *Journal American Society for Nutritional Sciences, 133*, 1339-1346.

Martínez, M. (1999). *Determinación, cuantificación e hidrólisis de inulina en el aguamiel de* Agave *pulquero*.Tesis de Licenciatura, UNAM, Facultad de Química, México, D.F., México. (In Spanish).

Ortiz-Basurto, R. I., Pourcelly, G., Doco, T., Williams, P., Domier, M., & Belleville, M. P. (2008). Analysis of the main components of the aguamiel produced by the maguey pulquero (Agave mapisaga) through out the harvest period. *Journal of Agricultural and Food Chemistry, 56*, 3682-3687. http://dx.doi.org/0.1021/jf072767h

Ostlund, R. E., Racette, S. B., & Stenson, W. F. (2003). Inhibition of cholesterol absorption by phytosterol-replete wheat germ compared with phytosteroldepleted wheat germ. *American Journal of Clinical Nutrition, 77*, 1385-1589.

Palmer, G. H., & Dixon, D. G. (1966). Effect of pectin dose on serum cholesterol levels. *American Journal of Clinical Nutrition, 18*, 437-442.

Pardo, O. (2007). *The agave (*Agave americana *L.) in prehispanic cultures*: bibliographical revision. Chlorischilencis. Revista chilena de flora y vegetación. Año 10. No 1. Retrieved Sep. 2010, from www.chlorischile.cl/pardoagavez/Agaveamericana.htm

Plat, J., & Mensink, R. P. (2005*)*. Plant sterols and sterol esters in the control of blood cholesterol levels: mechanism and safety aspects. *American Journal of Cardiology, 96*(1A), 15D-22D. http://dx.doi.org/10.1016/j.amjcard.2005.03.015

Rosado, J., Lopez, P., Morales, M., Muñoz, E., & Allen, L. H. (1992). Bioavailability of energy, nitrogen, fat, zinc, iron and calcium from rural and urban Mexican diets. *British Journal of Nutrition, 68*, 45-58. http://dx.doi.org/ 10.1079/BJN19920065

Rosado, J. L., Bourges, H., & Saint-Martin, B. (1995*).* Deficiencia de vitaminas y minerales en México. Una revisión crítica: II minerales. *Salud Pública de México, 37*, 130-139 (In Spanish).

Scrimshaw, N. S. (1984). Functional consequences of iron deficiency in Human populations. *Journal of Nutritional Science and Vitaminology, 30*, 47-63. http://dx.doi.org/10.3177/jnsv.30.47

Silos-Espino, H., González-Cortez, N., Carrillo-López, A., Guevara-Lara, F., & Valverde-González, M. E. (2007). Chemical composition and in vitro propagation of Agave salmiana 'Gentry. *The Journal of Horticultural Science and Biotechnology, 82*, 355-359.

Stasse-Wolthuis, M., Albers, H. F., Van–Jeveren, J. G., Wil, D. J., Hautvast, J. G., Hermus, R. J., … Eastwood, M. A. (1980). Influence of dietary fiber from vegetables and fruits, bran or citrus pectin on serum lipids, fecal lipids, and colonic function. *American Journal of Clinical Nutrition,33*, 1745-1756.

Tovar-Robles, C. L., Perales, P. C., Nava, C. A., Valera, M. L. L., Gómez, L. J. F., Guevara, G. L., … Silos, E. H. (2011). Effect of aguamiel (Agave sap) on hematic biometry in rabbits and it antioxidant activity determination. *Italian Journal of Animal Science, 10*(21), 106-110. http://dx.doi.org/10.4081/ijas.2011.e21

Urías-Silvas, J. E., Cani, P. D., Delmée, E., Neyrinck, A., Lopez, M. G., & Delzenne, N. M. (2008). Physiological effects of dietary fructans extracted from Agave tequilana Gto. and Dasylirion spp. *British Journal of Nutrition, 99*, 254-261. http://dx.doi.org/10.1017/S0007114507795338