

## Phenolic Content of Honey Reduces *in Vitro* Starch Digestibility

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### Abstract

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The starch digestibility of foods containing honey, as a function of honey's phenolic content was evaluated. We have evaluated the *in vitro* digestibility of starch from potato when added to honey. Natural honey diminished the starch digestibility compared to simulated honey (0% phenolics). A negative correlation between starch digestibility and total phenolic content was found (samples grouped by the honey botanical origin): for rapidly available glucose  $R^2$  was 0.76, whereas for unavailable glucose  $R^2$  was 0.89. Our results suggest a possible beneficial capacity of phenolic contents present in honey to diminish starch digestibility. Hereafter this aspect requires more attention in order to confirm this property and elucidate details about it.

**Keywords:** functional food; natural resources; phytochemicals

It has been shown that honey has a variety of positive nutritional and health effects, including antimicrobial, antiviral, antiparasitory, anti-inflammatory, antioxidant, antimutagenic, and antitumor ones, if consumed at higher doses of 50 g to 80 g per intake. These properties can be related with the presence of several bioactive compounds such as flavonoids and carotenoids, which affect both total antioxidant activity and colour of honey (BOGDANOV *et al.* 2008). On the other hand, there are also increasing concerns regarding the adverse effects of excessive consumption of free sugars. However, the capacity of phenolic compounds that are present in honey to diminish starch digestion at the gastrointestinal level has not been explored so far.

In this context, it is known that the glycaemic index (GI) of honey varies from 32 to 85 (depending on the botanical source), which is less than in other sugars, mainly in relation to its fructose content (AGRAWAL *et al.* 2007; BOGDANOV *et al.* 2008; DEIBERT *et al.* 2010). Even more interesting is the fact that the glycaemic response of honey is lower

than that of simulated honey (a solution possessing similar fractions of glucose and fructose) (AHMAD *et al.* 2008). The mechanisms related with such situation remain unclear, but data suggests an effect of minor components like phenolics.

The aim of the present research was to evaluate the capacity of some Chilean honeys to reduce *in vitro* starch digestibility with regard to their phenolic content, thus starting the study of an unexplored property of honey components: their capacity to reduce the activity of enzymes related with starch digestion. Although it is not common as a bakery product, potato was used as a model food system since it is reproducible and easy to use, and because its starch digestion kinetics has been previously described.

### MATERIAL AND METHODS

**Honey samples.** In the present study, ten unprocessed natural honeys collected from several areas in

Table 1. Geographical origin of honey

Sample key	Geographical origin (Chilean region – specific sector)
M-228-11	La Araucanía – Carahue
M-145-11	Los Lagos – Palena
M-458-11	Los Ríos – San José de la Maruiquina
M-147-10	Los Lagos – Palena
M-252-11	Los Ríos – Valdivia
M-93-11	Los Ríos – Punucapa
M-317-11	Los Lagos – Cochamó
M-66-11	Metropolitana
M-81-12	Los Lagos – Palena
M-98-11	Libertador B. O'Higgins – San Fernando

Sample key – Internal code of the Honey Bank of Universidad Austral de Chile

Chile were used. Table 1 shows the geographical origin of each sample. Since phenolics are relatively stable compounds, resistant to heat, oxygen, and moderate degrees of acidity, honey samples were stored prior to analyses in a dark place at room temperature.

**Pollen analyses.** All honey samples were subjected to pollen analyses with the aim of identifying the honey type according to the qualitative microscopic analyses and frequency of the classes of pollen grains in indissoluble matter as described by the Official Chilean Norm (Honey by honeybees – Botanical origin name throughout melissopalinalogical test; NCh2981.Of:2005). The different pollen morphologies were compared with those of the pollen collection of Chilean honey plants, property of Austral University of Chile.

**Physicochemical analyses.** In order to determine the quality of honeys, several analyses were performed: moisture (norm NCh3026.n:2006); ash content (NCh3102:2007); electrical conductivity (NCh3064:2007); sucrose, glucose, and fructose (by gas chromatography) (BOGDANOV *et al.* 1997); hydroxymethylfurfural (HMF) (NCh3046.n:2006); and colour according to the Pfund classifier (15937-2:1995).

**Estimation of total phenolics.** The Folin-Ciocalteu method (with modifications) was used to determine the total phenolic content (SINGLETON *et al.* 1999). Gallic acid monohydrate CAS N° 0595-86-8 (J.T. Baker, Center Valley, USA) (0–500 mg/l) was used as a standard to produce the calibration curve. The mean of three readings was used. Total phenolic content was expressed in mg of gallic acid equivalents (GAE)/1000 g of honey.

**In vitro digestion of starch.** In order to evaluate the effect of honey phenolics on starch digestibility, samples made with potato and honey were analysed through an *in vitro* digestion procedure.

**Sample preparation.** Potatoes (*Solanum tuberosum* L.) obtained from a local market were washed, peeled, cut into small pieces (~1 cm<sup>3</sup>), and finally cooked by immersion in a boiling water bath (10 min). After cooking, potatoes were removed from the water bath and kept at room temperature for 30 min; they were mashed until a homogeneous consistence was achieved (all particles having less than 2 mm in size), mixed manually with each kind of honey at a 4 : 1 (w/w potato/honey) ratio until no honey lumps were noted (~1 min), and kept at room temperature for 1 hour. Additionally, simulated honey was also used (D-glucose 33.5 g, D-fructose 40.5 g, sucrose 1.5 g, and maltose 7.5 g dissolved in 17 ml of sterile deionised water). Simulated honey is a solution that represents the proportion of the 4 predominant sugars in natural honey and therefore was used as a honey-like product without phenolic compounds (control sample) (AHMAD *et al.* 2008).

**In vitro digestion procedure.** An *in vitro* enzymatic starch degradation assay that mimics human digestion was performed according to a variation of the method of ENGLYST *et al.* (1999). The enzymes used were pepsin No. P7000, pancreatin No. 7545, and amyloglucosidase No. A7095 (all Sigma-Aldrich; St. Louis, USA). Three replicates were performed for each sample. Rapidly Available Glucose (RAG), Slowly Available Glucose (SAG), and Unavailable glucose (UG) were calculated as follows:

$$\text{RAG (g/100 g)} = (G_{20} - G_h) / (TG - G_h) \times 100 \quad (1)$$

$$\text{SAG (g/100 g)} = (G_{120} - G_{20}) / (TG - G_h) \times 100 \quad (2)$$

$$\text{UG (g/100 g)} = (TG - G_{120}) / (TG - G_h) \times 100 \quad (3)$$

where:  $G_{20}$  and  $G_{120}$  – glucose released after 20 and 120 min of intestinal digestion, respectively; TG – glucose released by the complete breakdown of starch;  $G_h$  – glucose from honey for each sample

The glucose concentrations in the  $G_{20}$ ,  $G_{120}$ , and TG portions were measured using the gas chromatography (GC) method described by BOGDANOV *et al.* (1997).

**Statistical analysis.** The statistical design included one independent variable (honey type) and the dependent variables were measured in triplicate. A regression analysis was performed to relate *in vitro* digestion of starch with the phenolic content of honey. The analysis was performed using Statgraphics Plus for Windows 4.0 (StatPoint Inc., Herndon, USA).

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Table 2. Pollen analyses of used honeys

Botanic origin of grains	M-228-11	M-145-11	M-458-11	M-147-10	M-252-11	M-93-11	M-317-11	M-66-11	M-81-12	M-98-11
Ulmo		36.7	80.5	77.4	75.0	79.4			67.3	
Tiaca		55.0		20.0	4.1					
Huarapo	30.9									
Hierba azul	17.1		3.8							
Alfalfa chilota	12.9		4.3		7.0	4.2				16.4
Maitén									6.9	
Clover							70			
Quillay								75		45
Litre										14.6
Others	39.1	8.3	11.4	2.6	13.9	16.4	30	25	25.8	24
Honey type	multi floral	mono-floral of Tiaca	mono-floral of Ulmo	mono-floral of Ulmo	mono-floral of Ulmo	mono-floral of Ulmo	mono-floral of Clover	mono-floral of Quillay	mono-floral of Ulmo	mono-floral of Quillay

Results expressed as number of pollen grains/100 grains; values are averages of at least 3 independent replicates (microscopic analyses); the method has a variability of 5%

## RESULTS AND DISCUSSION

**Pollen analyses.** Pollen analyses were used to identify honey samples. Five samples were typified as monofloral honey of Ulmo (*Eucryphia cordifolia*), one was monofloral honey of Tiaca (*Caldcluvia paniculada*), one was monofloral honey of clover (*Trifolium* sp.), two were monofloral honeys of Quillay (*Quillaja saponaria*), and one sample was typified as multifloral honey (Table 2). According to the Chilean regulations, it is required that at least 45% of pollen grains in indissoluble matter has to be from a specific botanic source if honey is to be declared as monofloral honey (NCh2981.Of:2005).

**Physicochemical analyses.** In general, all honeys showed expected values and meet the Chilean regula-

tions, so they are suitable for consumption (Table 3). Only one sample showed a value for electrical conductivity (related to the content of minerals) higher than 0.8 mS/cm (M-66-11) (Codex Standard for Honey, Codex Stan 12). Additionally, only the sample M-145-11 showed ash content higher than 0.8% (Ministry of Health, Republic of Chile, RSA 2010).

Regarding the honey colour, results were as follows: M-317-11 white; M-147-10, M-145-11, M-228-11, M-252-11, M-458-11, M-81-12 extra light amber; M-66-11, M-93-11 light amber; M-98-11 amber. In general, honey can vary from nearly colourless to dark brown (Codex Stan 12). It has been shown that the colour intensity of honey is related to pigments such as carotenoids and flavonoids; therefore, the colour of honey would be related to its antioxidant

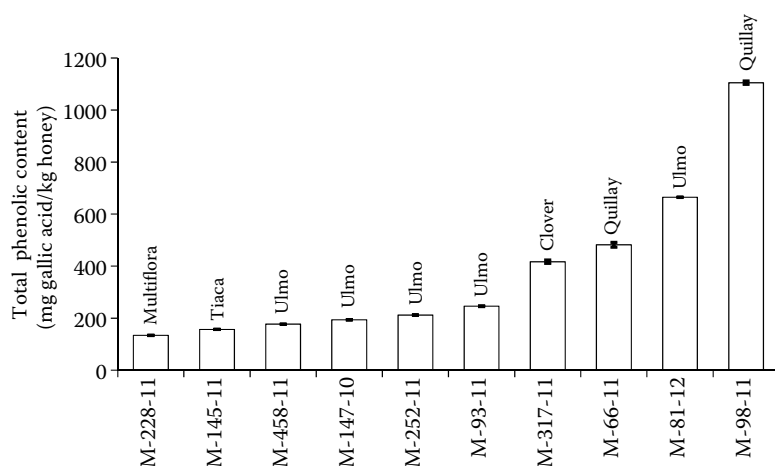


Figure 1. Total phenolic content of honeys (average  $\pm$  SD), showing botanical origin

Table 3. Physicochemical analyses of honey samples

	M-228-11	M-145-11	M-458-11	M-147-10	M-252-11	M-93-11	M-317-11	M-66-11	M-81-12	M-98-11
Moisture (g/100 g)	16.90 ± 0.07	16.2 ± 0.0	19.4 ± 0.07	16 ± 0	18 ± 0	15.6 ± 0.0	16.6 ± 0.0	17.3 ± 0.0	18.20 ± 0.07	16.8 ± 0.07
Ash (g/100 g)	0.18 ± 0.01	0.94 ± 0.02	0.42 ± 0.01	0.66 ± 0.00	0.76 ± 0.03	0.28 ± 0.00	0.17 ± 0.01	0.63 ± 0.00	0.25 ± 0.02	0.27 ± 0.01
Electrical conductivity (mS/cm)	0.52 ± 0.00	0.66 ± 0.01	0.61 ± 0.00	0.600 ± 0.006	0.58 ± 0.01	0.67 ± 0.01	0.30 ± 0.01	1.36 ± 0.01	0.56 ± 0.01	0.57 ± 0.01
Sucrose (g/100 g)	0 ± 0	0 ± 0	0.3 ± 0.0	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0	0 ± 0	0.3 ± 0.0	0.3 ± 0.0
Glucose (g/100 g)	43.0 ± 0.1	43.1 ± 0.0	26.0 ± 0.1	37.7 ± 0.2	33.9 ± 0.1	38.6 ± 0.1	38.2 ± 0.3	34.0 ± 0.3	35.8 ± 0.3	35.7 ± 0.2
Fructose (g/100 g)	32.0 ± 0.3	34.9 ± 0.2	45.6 ± 0.2	41.5 ± 0.3	40.4 ± 0.2	38.2 ± 0.1	37.0 ± 0.5	39.2	37.3 ± 0.2	38.5 ± 0.2
Σ glucose + fructose	75	78	71.6	79.2	74.3	76.8	75.2	73.2	73.1	74.2
HMF (g/100 g)	1.40 ± 0.42	18.00 ± 0.06	1.57 ± 0.32	16.00 ± 0.76	3.6 ± 0.0	4.0 ± 1.9	0.75 ± 0.00	1.5 ± 0.0	4.00 ± 0.12	1.98 ± 0.01
Colour (mm Pfund)	48.0 ± 1.4 extra light amber	45.0 ± 1.4 extra light amber	47.0 ± 1.4 extra light amber	40.0 ± 1.3 extra light amber	48.0 ± 1.4 extra light amber	57.0 ± 4.2 light amber	24 ± 0 white	66.00 ± 0.71 light amber	44.00 ± 0.71, extra light amber	73.8 ± 0.0 amber

activity (ESTEVINHO *et al.* 2008; SAXENA *et al.* 2010). Recently, a good correlation was observed between all parameters related to colour and total antioxidant activity (ESCRICHE *et al.* 2014).

**Phenolic contents.** For our study the Folin-Ciocalteu assay, a standardised method for measurement of antioxidant capacity of food products and dietary supplements, was used. This method is an electron transfer based assay and gives reducing capacity, and although such a reaction is not specific for phenolic compounds, an extraction procedure can eliminate a high proportion of potentially interfering compounds, so the results have normally been expressed as phenolic contents (PRIOR *et al.* 2005; AINSWORTH & GILLESPIE 2007).

For samples used in the study, total phenolic content (mg of GAE/1000 g of honey) varied from 134 mg to 1105 mg (Figure 1) using the standard curve of gallic acid ( $R^2 = 0.999$ ); this means an almost tenfold difference between samples presenting the highest and the lowest phenolic content. Results confirm a high variability among honeys in relation to their phenolic content (GHELDOLF & ENGESETH 2002; MUÑOZ *et al.* 2007). In our research, this high variation among samples seems to be related only in part to botanical origin. It has been observed that flavonoids and other phenolic compounds in honey, pollen, and propolis show a high variability with both botanical and geographical origin, as well as with the climate conditions (KENJERIC *et al.* 2007; BOGDANOV *et al.* 2008). Additionally, it has been observed that the antioxidant capacity of honey, which is derived from phenolics (but also from other compounds), can be as high that it is considered to use it like a replacement of other sweeteners having a minimal antioxidant activity. For instance, PHILLIPS *et al.* (2009) reported that a common honey available in the United States showed an intermediate antioxidant capacity, together with maple syrup and brown sugar (0.2–0.7 mmol FRAP/100 g), and concluded that honey and other alternative sweeteners offer the potential benefit of antioxidant activity compared to refined sugar (< 0.01 mmol FRAP/100 g) if consumed regularly. Finally, it has been reported that the natural antioxidants (especially flavonoids, which are present in honey) exhibit a wide range of biological effects, including antibacterial, anti-inflammatory, antiallergic, antithrombotic, and vasodilatory actions (COOK & SAMMON 1996).

**In vitro digestion of starch.** The starch digestibility of cooked mashed potato in the presence of diverse honey types is shown in Figure 2 (RAG, SAG, and UG).

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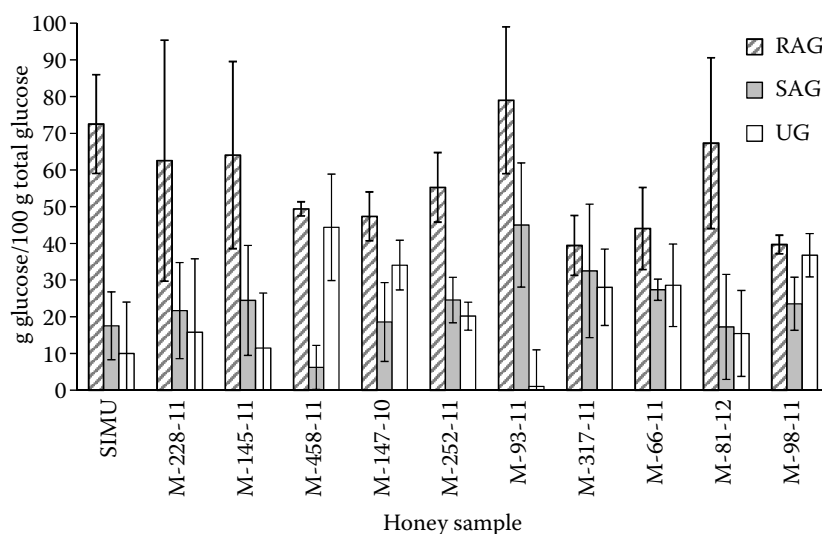


Figure 2. Glucose fractions from starch in potato samples having different types of honey (average  $\pm$  SD). Note that glucose from honey is not considered

Considering that several honeys have the same botanical origin, six groups were formed according to pollen analysis, and regression analysis was performed (Figure 3). For both RAG and UG, the  $P$ -value was lower than 0.05, showing a statistically significant relationship between total phenolic content and these fractions (95% confidence level). Additionally, for RAG the correlation coefficient was  $-0.87$ , showing a moderately strong relationship between the variables, whereas for UG this value was  $0.94$ , meaning that it is a relatively strong relationship between the variables. Clover and Quillay honey were the most efficient to reduce starch digestibility ( $\sim 40$  RAG,  $\sim 30$  SAG, and  $\sim 30$  UG) as well as being the samples presenting the highest phenolic content; whereas simulated honey (0 mg GAE/1000 g honey) generated food containing the highest starch digestibility ( $\sim 70$  RAG,  $\sim 20$  SAG, and  $\sim 10$  UG). These outcomes suggest that starch digestibility diminishes due to the presence of phenolic compounds in the honey.

Although the effect of honey phenolics on starch digestion has not been previously researched, the capacity of polyphenols to inhibit digestive enzymes involved in starch breakdown has already been examined for other foodstuffs. For example, a strong effect of the phenol content of berries on amylase activity was observed by GRUSSU *et al.* (2011); the authors reported that a change of phenol content for both yellow and red raspberries from 0  $\mu\text{g}$  to 50  $\mu\text{g}$  GAE was capable of diminishing the amylase activity from 100% to 0%, following a clear negative trend. Additionally, MCDUGALL *et al.* (2005) observed that the inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase is different depending on the polyphenol extract source; their results showed that strawberry and raspberry extracts were more effective  $\alpha$ -amylase inhibitors than blueberry, blackcurrant, or red cabbage. This is apparently related to the content of soluble tannins; whereas  $\alpha$ -glucosidase was more readily inhibited by blueberry and blackcurrant extracts, apparently re-

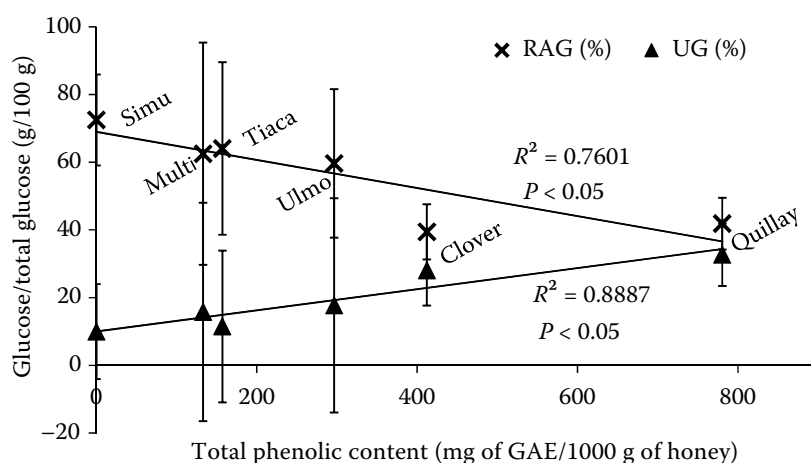


Figure 3. Relationship between glucose fractions (starch digestibility) and total phenolic content

Each point includes samples of the same botanical origin;  $P$ -value  $< 0.05$  indicates a statistically significant relationship between variables at the 95% confidence level; the relation between SAG and phenolic content was not statistically significant (results are not shown)



lated to the anthocyanin content. In a recent research, SOONG *et al.* (2014) examined the starch digestibility of muffins baked with rice, wheat, maize, oat, and barley flour. Outcomes showed that total phenolic content was inversely related to the RDS of muffins ( $y = -0.0547x + 478.98$ ;  $R = 0.9398$ ), most probably due to the inhibitory effect of phenolics (inhibition of digestive enzymes and interacting with starch). These results are concordant with our study.

Finally, it should be noted that although honey has a relatively low GI, there is a discrepancy regarding the nutritional effect of diets too rich in honey, due to their content of fructose. Evidence suggests that *de novo* hepatic lipogenesis is increased with a high consumption of this monosaccharide (FAO 1998; OUYANG *et al.* 2008). In murine models, it has also been observed that the lipid and liver metabolism changes indicate that even moderate fructose consumption might contribute to the onset or development of the metabolic syndrome, independently of significant effects on body weight (FIGLEWICZ *et al.* 2009). Nevertheless, there is also some evidence that the consumption of fructose with fruits or honey does not produce the same adverse metabolic effects as added fructose. This may be due to the presence of natural antioxidants and/or dietary fibres with fruits and honey (TAPPY *et al.* 2010). So, considering the current knowledge regarding the carbohydrate metabolism, outcomes of our research are valuable only to explore the potentiality of some honey constituents as health promoters, but not to give nutritional recommendations in the line of increasing honey consumption.

## CONCLUSIONS

We have evaluated the *in vitro* starch digestibility of potato when added to natural honey, finding a negative correlation between starch digestibility and total phenolic content. The botanical origin of honey appears to be a key factor in this sense. So, our results suggest a possible beneficial capacity of phenolic contents present in honey to diminish starch digestibility, although it is insufficient to support the idea of increasing honey consumption. Potato was used as a reproducible food model but the main conclusions of our study could be applied to other starchy foods. Additional research (including *in vivo* studies) is necessary to confirm these preliminary observations and study in detail the honey phenolics regarding this line.

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