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Antioxidant capacity of some Tasmanian Honeys

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Introduction

All over the world, honey is not only valued as a flavourful sweetener, but is also considered a part of traditional folk medicine. During the past two decades, the use of honey as a therapeutic substance has been reevaluated in a more scientific setting. Studies have shown that honey has both antibacterial (1) and anti-inflammatory properties (2), is useful in stimulation of wound and burn healing (3) and treatment of gastric ulcers and gastritis (4).

Additionally, honey has been found to have significant antioxidant activity by many workers (5-7) Honeys exhibits a wide range of antioxidant activity depending upon the floral source. In general, honey has a rich polyphenolic profile (8,9) and many of the honey flavonoids and phenolic acids are known to have antioxidant activity.

The aim of this work was to start building a platform of data to define the antioxidant properties of Tasmanian honey. With sufficient data, comparisons with other honeys from around the world will be possible. It has been frequently stated that only through a combination of antioxidant tests, can a rigorous characterization of the antioxidant activity of honey be achieved. The use of at least 3 or 4 different methods is mandatory to define the antioxidant profile. The reason for this is that no single test devised to date, can measure the antioxidant effect of all of the antioxidant components present. The following table shows some typical data taken from Beretta et al. (7). Although there is a strong correlation between all three assays used, certain honeys such as Africa 3 and Honeydew show high values in one assay and low values in another.

Sample	**FRAP value μM Fe(II)**	**Antiradical power DPPH, IC50**	**ORAC TE μmol/g**
Strawberry tree	1501	1.63	21.07
Africa 1	808	3.61	11.07
Buckwheat	801	4.00	11.60
Honeydew	772	8.48	6.30
Africa 2	448	5.13	18.23
Chestnut	389	7.93	8.90
Africa 3	381	3.47	18.00
Multi-flora	362	5.32	8.22
Dandelion 2	224	24.39	7.59
Chicory	210	5.81	6.72
Dandelion 1	212	47.62	2.00
Sulla	155	16.90	5.66
Acacia	80	45.45	2.12
Clover	73	25.00	2.15
Sugar analogue	0	0	1.40

The importance of this work resides in the need for accurate measurement of honey's antioxidant activity which is an essential tool for the understanding and demonstration of its antioxidant-linked therapeutic efficacy.

ORAC Methodology

It was decided to use the oxygen radical absorption capacity (ORAC) assay for this study. This assay is considered to be the most biologically relevant assay of antioxidant activity except for the cell-based assays. The method used, follows closely the work of Cao et al. (10) and employed the modifications made by Prior et al. (11) using fluorescein as the fluorescent probe.

Briefly, free radicals are thermally produced by AAPH and the fluorescent marker fluorescein is oxidized, losing its fluorescence. All reagents were prepared in phosphate buffer (75mM, pH 7.4) and Trolox (1μM to 10 μM, final concentration) was used as a standard. Each well of the microplate contained in the final volume of 200μL assay solution; fluorescein (250nM), 0.5 or 1mg/mL of honey, and AAPH 30mM (all final concentrations). After addition of the AAPH, the plate was shaken, and then fluorescence was measured every minute for 90 minutes with emission and excitation wavelengths of 535 and 485 nm respectively. All fluorescence measurements were made at 37^{o}C and the ORAC values were calculated from the areas under the fluorescence decay curves and expressed as μmol Trolox equivalents per gram of honey (TE μmol/g) . A honey analogue was prepared for comparison, consisting of 40% fructose, 30% glucose and 10% maltose in water.

Honey samples

Fourteen samples of honey from 6 producers were received,

Blue Hills Honey (BHH)
Miellerie Honey (M)
R.Stephens Honey (R.S.)
North Huon Apiaries (NHA)
Day Break Apiaries (DBA)
Tasmanian Honey Company (THC)

Results

The following table shows the ORAC values obtained from 12 replicate determinations. The coefficient of variance averaged 4.9% within a single determination and was less than 15% from day to day.

Sample	***Source***	***Producer***	***ORAC* TE μmol/g**	***Rank***
Leatherwood	Tarkine	BHH	7.25	2
	South West	M	3.44	13
	Mt. Arrowsmith	R.S.	4.14	12
	South	NHA	2.96	14
Manuka	North West	BHH	6.81	3
	North East	THC	6.33	4
	West coast	DBA	4.76	10
Kunzea		THC	4.91	9
Xanthorrhoea australis		THC	5.76	6
Prickly Box		BHH	7.83	1
Blackberry		BHH	4.40	11
Tallow wood		BHH	5.98	5
Pedder wildflower		M	5.71	7
Meadow		BHH	5.24	8
Honey analogue			1.28	

Discussion

The ORAC results show that the honey samples ranged in antioxidant capacity by more than a factor of 2.5. The highest activity 7.83 TE μmol/g was observed for prickly box honey supplied by Blue Hills Honey. This level of antioxidants is well above average (see table above). Of the fourteen samples 8 were above 5 TE μmol/g which would be considered high.

Leatherwood honey was generally low in antioxidant activity except for the sample from the Tarkine which was only just lower than the top honey, prickly box. Although 3 out of 4 leatherwood samples showed disappointingly low ORAC values, the sample from the Tarkine was outstanding. It was 2-3 times higher than the others and very close to the top prickly box honey. The reason for this can only be guessed at without a detailed chemical study.

The manuka honeys fell into two groups. The N.W. and N.E. samples being considerably higher in antioxidants than the West coast and *Kunzea* (closely related to *Leptospermum*) samples. Although only a few samples have been tested, manuka honey appears to be high in antioxidants.

Xanthorrhoea australis (black boy) honey was quite active in the ORAC assay, although perhaps not quite as much as its dark colour might have indicated. Tallow wood, Meadow, and Pedder wildflower all had ORAC activity only just lower than the N.W. and N.E. manuka samples.

The N.W. and N.E. manuka honeys, together with one Leatherwood sample and the prickly box honey were highest in antioxidants of the honeys tested, having ORAC values above 6 TE μmol/g. It remains to be seen if they also show high activity in other antioxidant tests.

Recommendations

This study has shown that many Tasmanian honeys have high levels of antioxidants as measured by the ORAC assay. It must be emphasised that these results must be viewed very cautiously being based upon so few samples and employing only one antioxidant assay. For example, if the Tarkine leatherwood sample had not been included in the study, leatherwood honey would remain of little interest for its antioxidant value. The following 3 recommendations are worthy of consideration;

1. Widening of the range of samples to see what further diversity there is in leatherwood honey and others.
2. Most importantly perhaps, other assays need to be carried out to complete evaluation of the samples already in hand. As previous studies have shown, at least 3 different assays need to be carried out to gain a reasonable measure of antioxidant capacity.
3. Given the very interesting outcome from the present investigation, it would seem that a study focussing on leatherwood is warranted.

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