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ORIGINAL PAPER

High-Pressure Processing of Manuka Honey: Improvement of Antioxidant Activity, Preservation of Colour and Flow Behaviour

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Abstract Manuka honey in New Zealand is known for its superior antimicrobial and antioxidant properties. However, these valuable properties are known to be compromised when raw honey goes through conventional thermal processing, thus reducing its final quality. As such, this present work is undertaken to assess the effect of high-pressure processing on quality of honey, namely, the antioxidant activity, colour and viscosity. The honey was subjected to different pressures (200–600 MPa) at ambient temperatures (25 to 33 °C) and combined with moderate temperatures (53 to 74 °C) for holding times (10 to 30 min). Thermal processing (49 to 70 °C) was also carried out for comparison purpose. In the absence of heat, the antioxidant activity of high-pressure processing (HPP)-treated samples (600 MPa, 10 min) was found to increase by about 30 % with no colour changes detected. The shear-thinning behaviour of the honey was also retained after HPP at ambient temperature, whereas for combined HPP–thermal treatment, no added benefit in antioxidant activity was observed particularly at higher temperature. Colour was significantly degraded when processed for ≥15 min at 70 °C and the flow behaviour was brought about from shear thinning to Newtonian. Thus, it can be concluded that the quality of honey can be enhanced by using high-pressure processing at ambient temperature.

Keywords High-pressure processing · Honey · Antioxidant activity · Colour · Viscosity

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Introduction

In commercial processing plant, honey is usually heated to 60 °C or above for inhibiting microorganisms, facilitating packing and delaying crystallization (Bath and Singh 1999; Tosi et al. 2004). However, the quality of honey is known to be compromised when it goes through thermal processing due to the unstable and thermolabile honey components which originated from the nectar and bees themselves (Nagai et al. 2001). Thus, the possibilities of thermal processing to improve the nutritional value look rather limited when honey is exposed to higher temperature. To maintain honey quality as high as possible, there is a need to develop novel processing technique such as non-thermal processing.

Manuka honey in New Zealand is known for its superior health benefits. Antioxidant activity, colour and viscosity are important quality characteristics and major factors affecting consumer acceptance. Honey is regarded as natural nutraceuticals due to its high antioxidant content with added value and has been shown to reduce the risk of heart disease, cancer, cataracts and inflammatory processes (Bogdanov et al. 2008). Flavonoids (Socha et al. 2011), amino acids (Bogdanov et al. 2008) and phenolic acids (Aljadi and Kamaruddin 2004) are the main antioxidant compounds in honey. However, most of these substances are unstable over time and thermolabile. Heating has been reported to decrease the antioxidative activities of honey due to decomposition of vitamins and also destruction of the integrity of the enzymes, particularly at higher temperature (Nagai et al. 2001).

The maintenance of naturally coloured pigments in foods is a major challenge. Colour relates directly to consumer perception of appearance and therefore has to be within an expected range for consumer acceptance. It is known that honey exists in a wide range of colours, varying from pale yellow to dark red. The floral origin and processing/storage temperature and time can affect honey colour (Pereyra et al.

1999). Lynn et al. (1936) reported that the darkening of honey could be due to instability of fructose (caramelization reaction) and oxidation of polyphenols. Wong and Stanton (1989) and Ibarz et al. (2000) revealed that one of the effects of thermal processing is non-enzymatic browning reactions including Maillard reaction. Browning, which occurs by thermal processing, is not desirable for consumers (Turkmen et al. 2006).

Understanding the flow behaviour of honey is of prime importance in all stages of honey production, processing, storage and packaging which in turn affects its quality (Anupama et al. 2003). Some honeys tend to crystallize at low temperature. Crystallized honey has an opaque waxy appearance and less visual impact than liquid honey. These features are not accepted for many consumers who prefer liquid honey. In addition, alteration in the consistency of honey makes it hard to use, handle and process. Although thermal processing is a convenient way to change the consistency of honey and protect it from fermentation (since an increase of water activity during crystallization tends to ferment), high temperature can be detrimental to the quality of honey and its biological properties. Most type of honey show Newtonian behaviour (Bhandari et al. 1999; Al-Malah et al. 2001; Zaitoun et al. 2001; Abu-Jdayil et al. 2002); however, there are some reports in the literature showing a non-Newtonian behaviour of honey (Munroe 1943; White 1978). Thermal treatment will change the flow behaviour of honey.

Losing the antioxidant activity, darkening of colour and changing of rheological properties of honey may occur during processing and these have detrimental effects on its quality as well as masking its originality. Thus, to maintain and improve the quality and nutritional value of honey, high-pressure processing (HPP) will be investigated for the first time as alternative to the conventional thermal processing. Due to the almost instantaneous isostatic pressure transmission (Abdul Ghani and Farid 2007), HPP-treated food has been shown to keep its original freshness and to improve functionalities (Butz 2010). No information is available on the effect of high pressure on the antioxidant activity and quality (namely, colour and rheological properties) of honey.

This paper, therefore determines the effect of HPP and combined HPP–thermal processing on the antioxidant activity of manuka honey, which has not been investigated so far. The paper also reports on the resulting quality changes occurring in manuka honey expressed in terms of colour and viscosity.

Material and Methods

Honey Samples Preparation

The fresh and unprocessed manuka honey used in this study (pH of 4.3±0.2, 79±0.3 °Brix and water content of 16.46± 1.4 %) was kindly donated by Comvita® New Zealand. The jar of honey sample was collected directly from beekeepers and sourced from manuka tree (*Leptospermum scoparium*), a native of New Zealand.

Honey (5 g) was packed in 5 cm×5 cm transparent plastic film pouches (Cas-Pak plastic vacuum pouch, New Zealand) and thermosealed under vacuum after manually stirred. A very thin pouch of 3 mm is deliberately used so that honey temperature approaches the surrounding water temperature in short time. The plastic film is made of cast polypropylene for excellent transparency and heat sealing qualities and can withstand temperatures up to 125 °C.

Processing of Honey

High-Pressure Processing Equipment

The HPP unit used in this research was QFP 2L-700 Laboratory Food Processing System (Avure Technologies, Columbus, OH, USA). The equipment consists of a 2-litre cylindrical-shaped pressure treatment chamber (inner height=10 in., inner diameter=4 in.) with a thermocouple, water circulation, a pumping system and a control system operated through a computer with software supplied by the manufacturer. Distilled water was used as the medium in the chamber where the packed honey samples were placed. The equipment can operate at maximum pressure and temperature of 600 MPa and 90 °C, respectively. The treatment time was the holding pressure time and did not include the pressure come up and the decompression times. The temperature inside the pressure chamber during treatment was monitored using a thermocouple, which was immersed in the pressure medium (distilled water). After processing, the packed honey samples were immediately cooled in ice water before analyzed. All honey samples were taken from the same honey batch and every single treatment was done twice.

HPP of Honey at Ambient Temperature

Five grams of vacuum-packed manuka honey samples were subjected to HPP with pressures of 200, 400 and 600 MPa, at close to ambient temperature (25 to 35 °C) for 10 min. Pressure come-up times were approximately 1.5 min and the decompression time was <20 s. The adiabatic heating of 200, 400 and 600 MPa gave an average processing temperature of 26.80±0.95 °C, 28.71±0.90 °C and 30.18±2.14 °C, respectively, during the holding pressure phase. The samples were then taken for the analysis of antioxidant activity, colour, rheological behaviour and viscosity.

Combined HPP–Thermal Processing of Honey

Five grams of vacuum-packed manuka honey samples were submitted to high pressure of 600 MPa and processing time of 10–30 min after initial heating. The samples were enclosed in

the pressure chamber for each condition tested. Pressure come-up times were approximately 1.5 min to reach 600 MPa, and adiabatic heating was observed during pressurization phase. The pressure increase up to the desired took less than 2 min. Initial temperature settings of 50, 60 and 70 °C for 600 MPa resulted in the average processing temperature of 53.62±0.30 °C, 62.65±0.47 °C and 72.99±0.38 °C during holding pressure phase. The samples were then taken for the analysis of antioxidant activity, colour, rheological behaviour and viscosity.

Thermal Processing of Honey

The thermal processing in the absence of high pressure was performed at 50, 60 and 70 °C for 10 min using a thermostatic water bath, W28 (Grant Instruments, Cambridge, UK). For the treatment, the vacuum-sealed samples were fully submerged into the water bath. Setting temperatures of 50, 60 and 70 °C resulted in average temperatures of 51.74±0.03 °C, 61.90± 0.10 °C and 71.58±0.04 °C, respectively. The come-up time of the centre of the packed honey was less than 1.5 min. After each treatment, all samples were immediately placed in ice-cooled water before analysis. All honey samples were taken from the same honey batch and every single treatment was repeated for two times. After processing, the packed honey samples were cooled in ice water before analyzed. The samples were then taken for the analysis of antioxidant activity, colour, rheological behaviour and viscosity.

Quality Determination

Antioxidant Activity

Antioxidant activity in the sample was determined using the 2, 2, diphenyl-2-picryl-hydrazyl (DPPH) method (Brand-Williams et al. 1995; Turkmen et al. 2006; Rauter et al. 2012). One gram of each honey sample was dissolved in 5 mL of distilled water. The solution was then centrifuged for 10 min at 10 000×*g* and filtered through Whatman no. 1 before precisely diluted to 4 °Brix with distilled water using an Atago RX-5000a digital refractometer. A 0.5 mL of honey extract was mixed with an aliquot of 1.5 mL of 0.1 mM DPPH radical (Sigma) in methanol. The reaction mixture was vortex-mixed and left to stand at 25 °C in the dark for 60 min. Absorbance at 517 nm was measured using a spectrophotometer (Shimadzu UV–vis) using methanol as blank, whereas distilled water was used as a control. The experiment was carried out two times, each time with duplicate samples. Antioxidant activity (DPPH scavenging activity) was expressed as percentage inhibition of the DPPH radical and was determined by the following equation (Yen and Duh 1994):

$$\mathrm{AA}\ (\%) = \frac{\mathrm{Abs}_{\mathrm{control}} - \mathrm{Abs}_{\mathrm{sample}}}{\mathrm{Abs}_{\mathrm{control}}} \times 100 \quad (1)$$

where AA (%)s the antioxidant activity in percentage, $\mathrm{ABS}_{\mathrm{control}}$ is the absorbance reading of the control and $\mathrm{ABS}_{\mathrm{sample}}$ is the absorbance reading of the sample.

Colour

Colour characteristics were assessed by the CIE $L^*a^*b^*$ method where lightness, L^*, and two colour coordinates, a^* and b^*, were measured by means of the Minolta CR-400 chromameter. The calibration was done using calibration plate with white background and 2° angle observer by taking Y, x, y as standard values (Y=85.9, x=0.3188, y=0.33578). The honey samples were heated to 50 °C for 30 min in a water bath to dissolve sugar crystals and decrease their viscosity (Gonzales 1999). The sample was placed in a container with 2.5-cm diameter and covered with a transparent plastic plate. The sample thickness was 4 mm. L^*, a^* and b^* values were measured against a white background and were directly obtained from the equipment. The measurement of the L^*, a^* and b^* values for each duplicate samples were read five times at five different locations. The experiment was carried out two times. Total colour difference (TCD) is a parameter that quantifies the overall colour difference of a processed sample (L^*, a^*, b^*) when compared to unprocessed sample (L_0^*, a_0^*, b_0^*) and calculated as (Silva and Silva 1999)

$$\mathrm{TCD} = \sqrt{\left(L^* - L_0^*\right)^2 + \left(a^* - a_0^*\right)^2 + \left(b^* - b_0^*\right)^2}. \quad (2)$$

Rheological Behaviour and Viscosity

Viscosity of unprocessed and processed honey samples were measured using a rheometer (AR-G2, TA Instrument, USA) which was connected to a computer with software (TA Instrument Advantage™ software). The measuring system consists of Smart Swap geometry with 40 mm, 2° steel cone. The method was adapted from Yanniotis et al. (2006) with some modification on shear rate range. Honey samples of 2 g were poured onto the sample plate. The rotational speed was increased to provide a shear rate in the range of 0.1 to 100 s^{-1}. Shear stress and viscosity were measured and recorded at different shear rates. The experiment was conducted at room temperature (25 °C) and carried out two times, each time with duplicate samples.

Statistical Analysis

Results were presented as mean±standard deviation of quadruplicate samples. One-way analysis of variance (ANOVA)

was used to compare the means. Differences were considered significant at $p<0.05$. The separation of treatment means was carried out with Tukey's honestly significant difference (HSD) test. All statistical analyses were performed with Statistica, version 11 (Statsoft®) and Microsoft Excell® 2010.

Results and Discussion

Effect of Processes on Honey Antioxidant Activity

The antioxidant activity of unprocessed manuka honey obtained from this study was 52.36±0.03 %, which exhibited the highest antioxidant activity in comparison with commercial Polish honey, 18.21–46.40 % (Socha et al. 2011), Malaysian floral honey, 22.4–41.3 % (Aljadi and Kamaruddin 2004; Mahaneem et al. 2010; Hussein et al. 2011) and Romanian honey, 35.8–49.19 % (Al et al. 2009). The results from this study emphasized the relevance of manuka honey as a healthy food supplement and a source of natural antioxidants due to its higher antioxidant activity and total phenolic content. The total phenolic content in unprocessed manuka honey was registered as 63.85±0.90 mg GAE/100 g (Akhmazillah et al. 2013) which was higher than other types of honey, 4.46–20.42 mg GAE/100 g (Ferreira and Aires 2009; Socha et al. 2011).

The antioxidant activity of manuka honey as affected by HPP, combined HPP–thermal and thermal processes are presented in Fig. 1. From the graph, all processed samples showed a significant increase in antioxidant activity as compared to unprocessed ($p<0.05$), except for combined HPP–thermally processed (600 MPa, 70 °C) and thermally processed samples (60 °C). Generally, the antioxidant activity for HPP-treated samples (at ambient temperature) showed the highest increment as compared to other treatments. This result is in agreement with measurements of the total phenolic content where HPP treatment at ambient temperature gave a significant increase (Akhmazillah et al. 2013). A strong correlation between the antioxidant activity and total phenolic content ($r=0.889$) was found in this study (Fig. 2). The same findings were also reported for Malaysian floral honeys (Aljadi and Kamaruddin 2004; Mahaneem et al. 2010). Since phenolic compounds play a major role in increasing antioxidant activity in honey, high pressure is expected to create significant increase in antioxidant activity.

Results demonstrated that although HPP-treated samples (at ambient temperature) lead to a significant increase to about 30 % in antioxidant activity, no added benefit was observed when combining HPP with thermal processing. This phenomenon could be explained by the ability of the hydroxyl molecules (OH), the functional group of phenolic compounds, to react with water molecules. The solubility which is due to hydrogen bonding was enhanced by the application of high pressure, whereas the hydration of charged group is loosened at high temperature (Mozhaev et al. 1996).

Previous studies revealed that high-pressure and combined HPP–thermal processing showed a different effect on antioxidant activity for different food products such as vegetables (McInerney et al. 2007), persimmon (de Begoa et al. 2000), orange juice and tomato puree (Fernández García et al. 2000; Garcia et al. 2001), legume seeds (Doblado et al. 2007), aloe vera gel (Vega-Gálvez et al. 2012), carrot juice (Van Loey Indrawati and Hendrickx 2004) and apple juice (Fernández García et al. 2000). The increase of antioxidant activity and phenolic content in these food matrices is mostly attributed to the disintegration of cell membrane. The cell membranes and organelles are disrupted and enzymes are released from vacuoles which then affect the phenolic content and antioxidant activity in the samples (Prasad et al. 2009; Keenan et al. 2010). However, since there is no intact cell in honey, the possible reason might be due to the presence of pollen. As enzymes and protein in honey conceivably arise from pollen, this can contribute to the phenolic content increment when HPP is applied.

The present study found that HPP treatment (600 MPa/ambient temperature/10 min) is beneficial not only in retaining antioxidant activities in honey but also in improving them by increasing significantly ($p<0.05$) the value to about 30 % from the unprocessed sample.

Effect of Processes on Colour of Honey

The colour parameters (L^*, a^* and b^*) and TCD of manuka honey as affected by conventional thermal processing, HPP and combined HPP–thermal are presented in Table 1. The result showed that there is no significant difference ($p<0.05$) in L^*, a^* and b^* values for all treatments as compared to unprocessed sample.

With respect to TCD, the result from this study shows that HPP-treated samples at ambient temperature had the lowest effect on TCD (range between 0.41 and 0.83) compared to others. While the colour change of combined HPP–thermal (600 MPa/70 °C) showed significant difference ($p<0.05$) where the TCD value was more intense (>6) as the processing time was longer (15 and 30 min). The difference in colour could be due to the presence of heat sensitive compounds particularly at higher temperature of 70 °C. These compounds involve the degradation of thermolabile pigments which in turn result in the formation of dark compounds that reduces luminosity (Barreiro et al. 1997). Besides, it also might be due to caramelization because of high sugar content present in honey. Monosaccharides particularly fructose will go initial enolization and progress to subsequent complex reactions, like dehydration, dicarboxylic cleaving and aldol condensation (Kroh 1994).

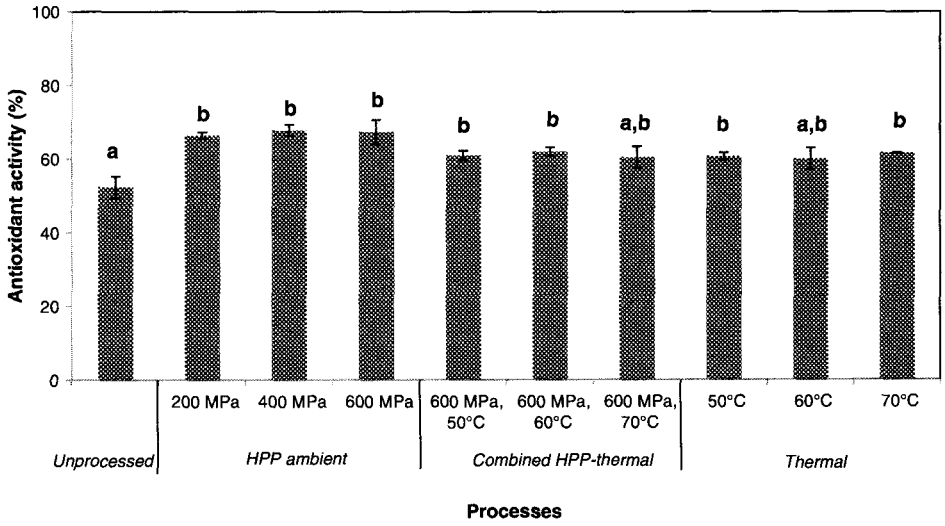

Fig. 1 Antioxidant activity (%) of manuka honey for different processes. All the processes had the duration of 10 min; *a* and *b* values are means±standard deviation. Different letters are significantly different according to Tukey's HSD test (Statistica version 11, Statsoft®) with $n=4$

The results from this study suggest that HPP at ambient temperature preserves the original colour of honey. However, for combined HPP–thermal process and in order to minimize colour change, it is recommended to treat at temperatures below 70 °C with shorter time of less than 15 min.

Effect of Processes on Honey Flow Behaviour

The unprocessed manuka honey investigated in this study displayed a non-Newtonian behaviour. Figure 3a shows the graph of shear stress (Pa) against shear rate (s^{-1}) for unprocessed honey, indicating a shear-thinning or pseudoplastic behaviour. This was confirmed by the value of flow behaviour index "n" which is less than 1, showing that the viscosity of unprocessed honey decreases with increasing shear rate. The non-Newtonian behaviour has been attributed to the presence of colloids or high molecular weight compounds such as proteins or polysaccharides (Mossel et al. 2000; Juszczak and Fortuna 2006). Although this result differs from some published studies where honey is reported as Newtonian fluid (Pan and Ji 1998; Bhandari et al. 1999; Mossel et al. 2000; Zaitoun et al. 2001; Abu-Jdayil et al. 2002; Lazaridou et al. 2004; Juszczak and Fortuna 2006; Yanniotis et al. 2006), there are some reports in the literature showing similar behaviour to the unprocessed manuka honey investigated in this study such as heather honey (*Calluna vulgaris*), buckwheat honey (*Fagopyrum esculentum*), white clover honey (*Trifolium repens*) and Indian Karvi honey (*Carvia callosa*). Nigerian honey (*Opuntia engelmannii*) and several eucalyptus honeys (e.g. *Eucalyptus ficifolia*) were also reported to have non-Newtonian behaviour but with dilatancy type. The HPP-treated samples (200, 400 and 600 MPa) at ambient temperature for 10 min displayed shear-thinning or pseudoplastic behaviour, similar to unprocessed honey (Fig. 3a). On the contrary, HPP–thermally and thermally treated samples displayed a Newtonian behaviour ($n=1$), where the shear stress directly proportional to the shear rate as shown in Fig. 3b.

The viscosity curve of the pressure-treated samples (200, 400 and 600 MPa at ambient temperature) as a function of shear rate is shown in Fig. 4a. The viscosity decreased with an

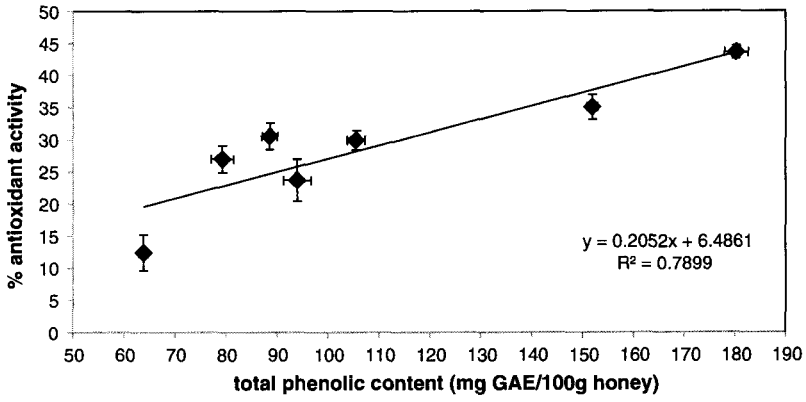

Fig. 2 Correlation between antioxidant activity of HPP-treated manuka honey (600 MPa at ambient temperature) and its total phenolic content. The *error bars* are means±standard deviation with $n=4$ for both antioxidant activity and total phenolic content

Table 1 The values of L^*, a^*, b^* and total colour difference (TCD) for HPP, combined HPP–thermally and thermally treated manuka honey

Treatment	L^*	a^*	b^*	TCD
Unprocessed	58.41±0.36a	3.61±0.1a	35.14±0.16a	0a
HPP at ambient temperature				
200 MPa, 10 min	57.97±2.74a	3.47±0.37a	34.89±1.83a	0.53±0.12a,b
400 MPa, 10 min	58±4.83a	3.64±0.19a	35±3.5a	0.43±0.30a,b
600 MPa, 10 min	57.88±0.12a	3.55±0.04a	34.65±0.09a	0.72±0.11a,b
Combined HPP–thermal				
600 MPa, 50 °C,10 min	55.13±2.31a	4.25±0.24a	33.3±1.58a	3.81±0.68a,b
600 MPa, 60 °C, 10 min	56.06±0.53a	3.76±0.24a	33.59±0.32a	2.82±0.58a,b
600 MPa, 70 °C, 10 min	60.18±3.7a	3.29±0.54a	36.26±2.45a	2.12±0.12a,b
600 MPa, 70 °C, 15 min	63.45±0.27a	3.35±0.09a	38.85±0.06a	6.26±0.02b
600 MPa, 70 °C, 30 min	63.7±0.29a	3.28±0.34a	38.68±0.46a	6.38±0.01b
Thermal process at ambient pressure (0.1 MPa)				
50 °C, 10 min	59.6±2.66a	3.48±0.29a	36.18±1.83a	1.59±0.20a,b
60 °C, 10 min	59.9±4.42a	3.18±0.41a	35.84±3.2a	1.70±0.12a,b
70 °C, 10 min	62.35±0.27a	3.28±0.05a	38.11±0.04a	4.95±0.21a,b

Mean values (means±standard deviation) within the same column with different letters are significantly different according to Tukey's HSD test (Statistica version 11, Statsoft®) with $n=4$

increase of the shear rate. The difference in viscosity of the samples was largest at a lower shear rate and becomes flatter at a higher shear rate. Meanwhile, Fig. 4b shows the viscosity of heated honey with and without HPP as a function of shear rate. The apparent viscosity for non-Newtonian honey samples and viscosity for Newtonian honey samples were presented in Tables 2 and 3, respectively. The important finding is that at low shear rate (usually when the honey is consumed), the viscosity is not affected by pressure treatment particularly at pressure<400 MPa. However, it was reduced by more than a factor of 2 through the thermal treatment.

Previous studies have reported that viscosity was correlated with high pressure for many fruits and vegetables products such as apple puree (Landl et al. 2010), tomato puree (Krebbers et al. 2003; Sánchez-Moreno et al. 2006), mango pulp (Ahmed et al. 2005), navel orange juice (Polydera et al. 2005), fruit yogurt (Walker et al. 2006), spinach and cauliflower (Prestamo and Arroyo 1998), chopped tomatoes

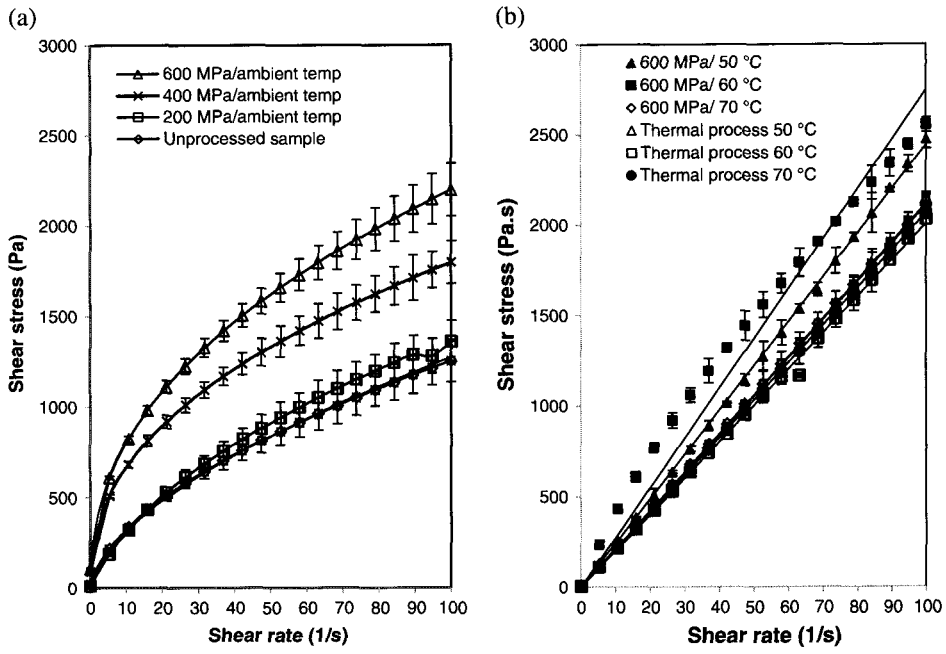

Fig. 3 Flow curves of **a** unprocessed sample and HPP-treated samples at ambient temperature and **b** combined HPP-thermally and thermally treated manuka honey. The *error bars* are means±standard deviation with $n=4$

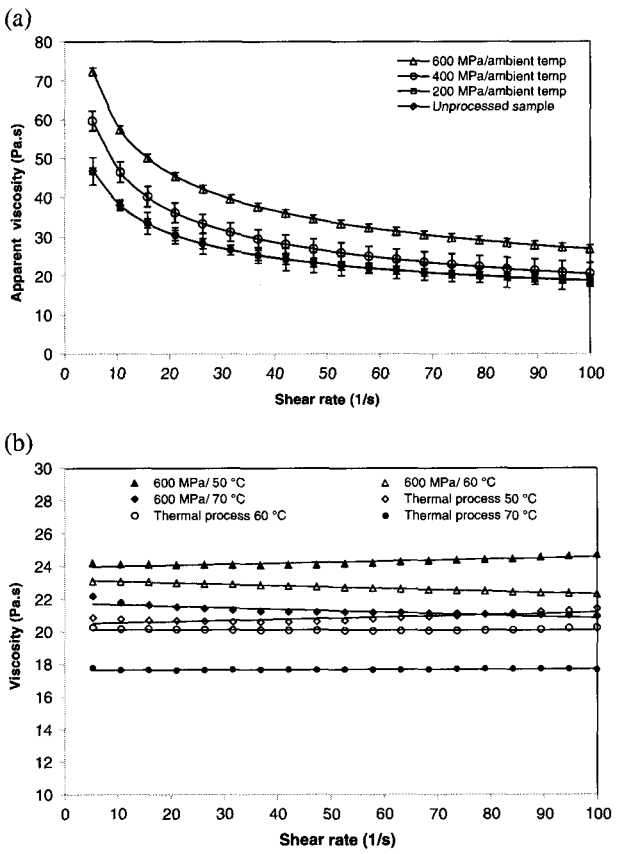

Fig. 4 The viscosity curves as a function of shear rate. **a** Unprocessed and HPP-processed manuka honey at ambient temperature. The *error bars* are means±standard deviation with $n=4$. **b** Combined HPP-thermal and thermal processing. The range of standard deviation ($n=4$) for the treatments were found as follows: *closed triangle*= 2.58–2.65; *open triangle*=0.12–93; *closed diamond*=2.86–3.34; *open diamond*=1.88–2.01; *open circle*=0.83–1.07; *closed circle*=1.02–1.18

(Rovere et al. 1997), guava puree (Yen and Lin 1996) and tomato juices (Porretta et al. 1995). Sánchez-Moreno et al. (2006) and Oey et al. (2008) revealed that the increase in viscosity was attributed to an increase in linearity of the cell walls and volumes of particles due to the permeabilization of

Table 2 Apparent viscosity of unprocessed and HPP-treated manuka honey at shear rate 5.39 s^{-1}

	Apparent viscosity (Pa s)
Unprocessed	46.86±1.42a
HPP (ambient temperature)	
HPP, 200 MPa	46.73±2.23a
HPP, 400 MPa	59.71±2.56a,b
HPP, 600 MPa	72.30±3.20b

Mean values (means±standard deviation) within the same column with different letters are significantly different according to Tukey's HSD test (Statistica version 11, Statsoft®) with $n=4$

Table 3 Viscosity of combined HPP–thermally treated and thermally treated manuka honey

	Viscosity (Pa s)
Combined HPP–thermal	
HPP (600 MPa), 50 °C	24.29±0.21a
HPP (600 MPa), 60 °C	22.82±0.26a
HPP (600 MPa), 70 °C	21.31±0.32a,b
Thermal (ambient pressure, 0.1 MPa)	
Thermal, 50 °C	20.68±0.26a,b
Thermal, 60 °C	20.16±0.07b,c
Thermal, 70 °C	17.64±0.06c

Mean values (means±standard deviation) within the same column with different letters are significantly different according to Tukey's HSD test (Statistica version 11, Statsoft®) with $n=4$

the cell walls. However, in the case of honey, the increase of viscosity as affected by HPP (at ambient temperature) might be due to the high molecular weight sugars (oligosaccharides) and also residual bees wax present in honey (Assil et al. 1991). HPP may affect oligosaccharides and may also induce a phase shift of the wax under pressure and hence can cause the change in viscosity. Further work is needed to verify this hypothesis.

This study has shown that in the absence of heat, high pressure can retain the shear-thinning behaviour of manuka honey, whereas combined HPP–thermal and thermal process brought about the manuka honey from shear-thinning behaviour to a Newtonian behaviour.

Conclusion

The determination of antioxidant activity, colour and rheological properties of manuka honey as affected by HPP is reported here for the first time. HPP at ambient temperature shows a great potential in increasing antioxidant activity which, in turn, improves the natural nutritional value of honey. The original colour of honey was also preserved at this condition, whereas the combined HPP–thermal process at higher temperature and longer time alters the original colour of the honey. Rheological measurement indicated that combining HPP with thermal brought about manuka honey from shear-thinning behaviour to a Newtonian behaviour, unlike HPP treatment alone in which the viscosity at lower shear rate was not affected by HPP treatment, while thermal treatment has reduced it to less than half. Based on the work presented in this paper, showing significant improvement in the nutritional value of honey, we expect that the HPP treatment could lead to a higher-value product.

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References

Abdul Ghani, A. G., & Farid, M. M. (2007). Numerical simulation of solid–liquid food mixture in a high pressure processing unit using computational fluid dynamics. *Journal of Food Engineering, 80*(4), 1031–1042.

Abu-Jdayil, B., Al-Majeed Ghazawi, A., Al-Malah, K. I. M., & Zaitoun, S. (2002). Heat effect on rheology of light- and dark-colored honey. *Journal of Food Engineering, 51*(1), 33–38.

Ahmed, J., Ramaswamy, H. S., & Hiremath, N. (2005). The effect of high pressure treatment on rheological characteristics and colour of mango pulp. *International Journal of Food Science & Technology, 40*(8), 885–895.

Akhmazillah, M. F. N., Farid, M. M., & Silva, F. V. M. (2013). High Pressure Processing (HPP) of honey for the improvement of nutritional value. *Innovative Food Science and Emerging Technologies*. doi:10.1016/j.ifset.2013.06.012.

Al, M. L., Daniel, D., Moise, A., Bobis, O., Laslo, L., & Bogdanov, S. (2009). Physico-chemical and bioactive properties of different floral origin honeys from Romania. *Food Chemistry, 112*(4), 863–867.

Aljadi, A. M., & Kamaruddin, M. Y. (2004). Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chemistry, 85*(4), 513–518.

Al-Malah, K. I. M., Abu-Jdayil, B., Zaitoun, S., & Al-Majeed Ghazwi, A. (2001). Application of WLF and Arrhenius kinetics to rheology of selected dark-colored honey. *Journal of Food Process Engineering, 24*, 341–357.

Anupama, D., Bhat, K. K., & Sapna, V. K. (2003). Sensory and physico-chemical properties of commercial samples of honey. *Food Research International, 36*(2), 183–191.

Assil, H. I., Sterling, R., & Sporns, P. (1991). Crystal control in processed liquid honey. *Journal of Food Science, 56*(4), 1034–1037.

Barreiro, J. A., Milano, M., & Sandoval, A. J. (1997). Kinetics of colour change of double concentrated tomato paste during thermal treatment. *Journal of Food Engineering, 33*, 359–371.

Bath, P. K., & Singh, N. (1999). A comparison between *Helianthus annuus* and *Eucalyptus lanceolatus* honey. *Food Chemistry, 67*, 389–397.

Bhandari, B., D'Arcy, B., & Chow, S. (1999). Rheology of selected Australian honeys. *Journal of Food Engineering, 41*(1), 65–68.

Bogdanov, S., Jurendic, T., Sieber, R., & Gallmann, P. (2008). Honey for nutrition and health: a review. *Journal of the American College of Nutrition, 27*(6), 667–689.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und Technologie, 28*, 25–30.

Butz, P. (2010). *High pressure: minimal processing. Encyclopedia of agricultural, food, and biological engineering* (pp. 819–822). New York: CRC Press.

de Begoa, A., Gonzalez, E., & Pilar Cano, M. (2000). Effect of high-pressure treatment on the carotenoid composition and the radical scavenging activity of persimmon fruit purees. *Journal of Agricultural and Food Chemistry, 48*(8), 3542–3548.

Doblado, R., Frías, J., & Vidal-Valverde, C. (2007). Changes in vitamin C content and antioxidant capacity of raw and germinated cowpea (*Vigna sinensis* var. *carilla*) seeds induced by high pressure treatment. *Food Chemistry, 101*(3), 918–923.

Fernández García, A., Butz, P., & Tauscher, B. (2000). Does the antioxidant potential of high pressure treated apple juice change during storage? *High Pressure Research, 19*(1–6), 153–160.

Ferreira, I. C. F. R., & Aires, E. (2009). Antioxidant activity of Portuguese honey samples: different contributions of the entire honey and phenolic extract. *Food Chemistry, 114*(4), 1438–1443.

Garcia, A. F., Butz, P., & Tauscher, B. (2001). Effects of high-pressure processing on carotenoid extractability, antioxidant activity, glucose diffusion, and water binding of tomato puree (*Lycopersicon esculentum*). *Journal of Food Science, 66*(7), 1033–1038.

Gonzales, A. P. (1999). Color changes during storage of honeys in relation to their composition and initial color. *Food Research International, 32*(3), 185–191.

Hussein, S. Z., Yusoff, K. M., Makpol, S., & Yusof, Y. A. M. (2011). Antioxidant capacities and total phenolic contents increase with gamma irradiation in two types of Malaysian honey. *Molecules, 16*, 6378–6395.

Ibarz, A., Pagán, J., & Garza, S. (2000). Kinetic models of non- enzymatic browning in apple puree. *Journal of the Science of Food and Agriculture, 80*, 1162–1168.

Juszczak, L., & Fortuna, T. (2006). Rheology of selected Polish honeys. *Journal of Food Engineering, 75*(1), 43–49.

Keenan, D. F., Brunton, N. P., et al. (2010). Effect of thermal and high hydrostatic pressure processing on antioxidant activity and colour of fruit smoothies. *Innovative Food Science & Emerging Technologies, 11*(4), 551–556.

Krebbers, B., Matser, A. M., Hoogerwerf, S. W., Moezelaar, R., Tomassen, M. M. M., & Van den Berg, R. W. (2003). Combined high-pressure and thermal treatments for processing of tomato puree: evaluation of microbial inactivation and quality parameters. *Innovative Food Science & Emerging Technologies, 4*(4), 377–385.

Kroh, L. W. (1994). Caramelization in food and beverages. *Food Chemistry, 51*, 373.

Landl, A., Abadias, M., Sarraga, C., & Picouet, P. A. (2010). Effect of high pressure processing on the quality of acidified Granny Smith apple purée product. *Innovative Food Science & Emerging Technologies, 11*(4), 557–564.

Lazaridou, A., Biliaderis, C. G., Bacandritsos, N., & Sabatini, A. G. (2004). Composition, thermal and rheological behaviour of selected Greek honeys. *Journal of Food Engineering, 64*(1), 9–21.

Lynn, E. G., Englis, D. T., & Milum, V. G. (1936). Effect of processing and storage on composition and color of honey. *Food Research, 1*, 2–27.

Mahaneem, M., Sirajudeen, K. N. S., Swamy, M., Yaacob, N. S., & Sulaiman, S. A. (2010). Studies on the antioxidant properties of Tualang honey of Malaysia. *Journal of Traditional, Complementary and Alternative Medicines, 7*(1), 59–63.

McInerney, J. K., Seccafien, C. A., Stewart, C. M., & Bird, A. R. (2007). Effects of high pressure processing on antioxidant activity, and total carotenoid content and availability, in vegetables. *Innovative Food Science & Emerging Technologies, 8*(4), 543–548.

Mossel, B., Bhandari, B., D'Arcy, B., & Caffin, N. (2000). Use of an Arrhenius model to predict rheological behaviour in some Australian honeys. *LWT - Food Science and Technology, 33*(8), 545–552.

Mozhaev, V. V., Lange, R., Kudryashova, E. V., & Balny, C. (1996). Application of high hydrostatic pressure for increasing activity and stability of enzymes. *Biotechnology and Bioengineering, 52*(2), 320–331.

Munroe, J. A. (1943). The viscosity and thixotropy of honey. *Journal of Entomology, 36*, 769–777.

Nagai, T., Sakai, M., Inoue, R., Inoue, H., & Suzuki, N. (2001). Antioxidative activities of some commercially honeys, royal jelly, and propolis. *Food Chemistry, 75*, 237–240.

Oey, I., Lille, M., Van Loey, A., & Hendrickx, M. (2008). Effect of high-pressure processing on colour, texture and flavour of fruit- and

vegetable-based food products: a review. *Trends in Food Science & Technology, 19*(6), 320–328.

Pan, J., & Ji, C. (1998). General rheological model for natural honeys in China. *Journal of Food Engineering, 36*(2), 165–168.

Pereyra, A. G., Burin, L., & Buera, M. P. (1999). Color changes during storage of honeys in relation to their composition and initial color. *Food Research International, 32*, 185–191.

Polydera, A. C., Stoforos, N. G., & Taoukis, P. S. (2005). Quality degradation kinetics of pasteurised and high pressure processed fresh navel orange juice: nutritional parameters and shelf life. *Innovative Food Science & Emerging Technologies, 6*(1), 1–9.

Porretta, S., Birzi, A., Ghizzoni, C., & Vicini, E. (1995). Effects of ultrahigh hydrostatic pressure treatments on the quality of tomato juice. *Food Chemistry, 52*(1), 35–41.

Prasad, K. N., Yang, E., Yi, C., Zhao, M., & Jiang, Y. (2009). Effects of high pressure extraction on the extraction yield, total phenolic content and antioxidant activity of longan fruit pericarp. *Innovative Food Science and Emerging Technologies, 10*(2), 155–159.

Prestamo, G., & Arroyo, G. (1998). High hydrostatic pressure effects on vegetable structure. *Journal of Food Science, 63*(5), 878–881.

Rauter, A. P., Dias, C., Martins, A., Branco, I., Neng, N. R., Nogueira, J. M., et al. (2012). Non-toxic *Salvia sclareoides* Brot. extracts as a source of functional food ingredients: phenolic profile, antioxidant activity and prion binding properties. *Food Chemistry, 132*(4), 1930–1935.

Rovere, P., Sandei, L., Colombi, A., Munari, M., Ghiretti, G., Carpi, G., et al. (1997). Effects of high-pressure treatment on chopped tomatoes. *Industrial Conserve, 72*(1), 3–12.

Sánchez-Moreno, C., Plaza, L., De Ancos, B., & Cano, M. P. (2006). Impact of high-pressure and traditional thermal processing of tomato puree on carotenoids, vitamin C and antioxidant activity. *Journal of the Science of Food and Agriculture, 86*(2), 171–179.

Silva, F. M., & Silva, C. L. M. (1999). Colour changes in thermally processed cupuacu (*Theobroma grandiflorum*) puree: critical times and kinetics modelling. *International Journal of Food Science and Technology, 34*(1), 87–94.

Socha, R., Juszczak, L., Pietrzyk, S., Galkowska, D., Fortuna, T., & Witczak, T. (2011). Phenolic profile and antioxidant properties of Polish honeys. *International Journal of Food Science & Technology, 46*(3), 528–534.

Tosi, E. A., Re, E., & Bulacio, L. (2004). Effect of honey high-temperature short-time heating on parameters related to quality, crystallisation phenomena and fungal inhibition. *LWT- Food Science and Technology, 37*(6), 669–678.

Turkmen, N., Sari, F., Poyrazoglu, E. S., & Velioglu, Y. S. (2006). Effects of prolonged heating on antioxidant activity and colour of honey. *Food Chemistry, 95*(4), 653–657.

Van Loey Indrawati, A., & Hendrickx, M. (2004). Pressure and temperature stability of water-soluble antioxidants in orange and carrot juice: a kinetic study. *European Food Research and Technology, 219*(2), 161–166.

Vega-Gálvez, A., Giovagnoli, C., Perez-Won, M., Reyes, J. E., Vergara, J., Miranda, M., et al. (2012). Application of high hydrostatic pressure to aloe vera (*Aloe barbadensis* Miller) gel: microbial inactivation and evaluation of quality parameters. *Innovative Food Science & Emerging Technologies, 13*, 57–63.

Walker, M. K., Farkas, D. F., Loveridge, V., & Meunier-Goddik, L. (2006). Fruit yogurt processed with high pressure. *International Journal of Food Science & Technology, 41*(4), 464–467.

White, J. W. (1978). Honey. *Advances in Food Research, 24*, 288–354.

Wong, M., & Stanton, D. W. (1989). Nonenzymic browning in kiwifruit juice concentrate systems during storage. *Journal of Food Science, 54*, 669–673.

Yanniotis, S., Skaltsi, S., & Karaburnioti, S. (2006). Effect of moisture content on the viscosity of honey at different temperatures. *Journal of Food Engineering, 72*(4), 372–377.

Yen, G. C., & Duh, P. D. (1994). Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species. *Journal of Agricultural and Food Chemistry, 42*, 629–632.

Yen, G. C., & Lin, H. T. (1996). Comparison of high pressure treatment and thermal pasteurization effects on the quality and shelf life of guava puree. *International Journal of Food Science and Technology, 31*, 205–213.

Zaitoun, S., Al-Majeed Ghazwi, A., Al-Malah, K. I. M., & Abu-Jdayil, B. (2001). Rheological properties of selected light colored Jordanian honey. *International Journal of Food Properties, 4*(1), 139–148.