J Food Sci Technol (March 2017) 54(3):802–809
DOI 10.1007/s13197-017-2526-7

ORIGINAL ARTICLE

An insight on the relationship between food compressibility and microbial inactivation during high pressure processing

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Revised: 16 January 2017 / Accepted: 31 January 2017 / Published online: 13 February 2017

Abstract This paper investigates the effect of high pressure liquid food compressibility on *S. cerevisae* inactivation. Honey with various adjusted sugar with different values of compressibility was selected as a model food. *S. cerevisiae* cells in different honey concentrations (0–80°Brix), 600 MPa (at ambient temperature) showed an increasing resistance to inactivation with increasing °Brix. *D*-values of *S. cerevisiae* at 200, 400 and 600 MPa, for 20 min/80°Brix were 136.99 ± 7.97, 29.24 ± 6.44 and 23.47 ± 0.86 min, respectively. These *D*-values resulted the Z_p-value of 526 ± 39 MPa. A significant correlation ($p < 0.05$) of cell reduction, °Brix and compressibility was found. Cell reduction in high pressure-treated samples varied linearly with °Brix suggesting that the baroprotective effect of the food was not solely due to sugar content, but also due to its compressibility. This research could have significant implications on the success of HPP (high pressure processing) preservation of foods containing high sugar content.

Keywords Compressibility · Sugar content · Microbial inactivation · *Saccharomyces cerevisiae* · High pressure processing · Honey

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Introduction

High pressure processing (HPP) has been widely used in food industry, since it has shown to keep foods' original freshness, enhance its functionalities and improve its shelf life. The application of HPP in various food including high sugar content food (i.e. honey) showed an improvement in its nutritional values and quality (Akhmazillah et al. 2013; Fauzi et al. 2014; Grainger et al. 2014; Fauzi and Farid 2015).

The efficacy of HPP is known to depend on the composition of the food; mainly sugars, fats and salts (Molina-Höppner et al. 2004; Senhajit and Loncins 1977; Van Opstal et al. 2003). High solute concentration foods have a baroprotective effect on microorganism cells undergoing pressure treatment (Basak et al. 2002; Goh et al. 2007). This baroprotective effect could be attributed to a lower water activity (Palou et al. 1997). Moussa et al. (2006), Oxen and Knorr (1993), Palou et al. (1997) and Satomi et al. (1995) showed that pressure sensitive microorganisms such as *Escherichia coli*, *Rhodotorula rubra* and *Zygosaccharomyces bailii* were highly dependent on the water activity of the treated product. Furthermore, it has been reported that pressure resistance of yeasts and moulds increased as sugar concentration (i.e. sucrose, fructose and glucose) increased (Ogawa et al. 1990; Oxen and Knorr 1993; Palou et al. 1997).

The free volume between molecules contributes to a high compressibility of liquids at low pressure (Bridgman 1970; Isaacs 1981). An increase in solute concentration (i.e. sugar content, °Brix) decreases liquid product's compressibility (Min et al. 2010). Min et al. (2010) also showed that increasing pressure significantly reduced liquid compressibility due to the loss of secondary and tertiary structure of protein and pressure effect on the hydrogen

bonding and carbohydrate composition (Shimada et al. 1993).

Although there are a number of studies concerning baroprotective effect on the increased solute concentration towards osmophilic microorganisms (yeasts cells) inactivation during HPP (Basak et al. 2002; Campos and Cristianini 2007; Goh et al. 2007; Parish 1998), yet it is not clear if cells protection is either solely due to the baroprotective effect of the solute concentration or to solute effect on compressibility or both. The study about the compressibility of foods under pressure carried out by Min et al. (2010) led to the hypothesis that compressibility might contribute to HPP microbial inactivation in high viscous foods.

This paper, therefore, aims to investigate if compressibility of honey, as a model food at different sugar content, has a major influence on the inactivation of *S. cerevisiae* (as the testing microorganism) after HPP. The main objective of this work is to establish the effect of compressibility and sugar concentration (°Brix) on the inactivation of *S. cerevisiae.* Thermal treatment at 55 °C for 10 min which is conventionally applied in honey pasteurisation in industry was carried out for comparison purpose.

Materials and method

S. cerevisiae strain and growth medium

S. cerevisiae ATCC 2601 (American Type Culture Collection, USA) was obtained freeze-dried from Fort Richard Laboratories (New Zealand). To rehydrate and revive the freeze dried culture, 1 mL of sterilized water was added into the freeze dried pellet. The entire content was transferred to a test tube containing about 5 mL of sterile water. Then, the culture was incubated at 30 °C for about 3 h before being plated.

For each experiment, *S. cerevisiae* cells were pre-cultured on yeast extract peptone dextrose (YPD) agar plates (BD Difco, Australia), at 30 °C, for 2 days. Following this process, a yeast colony was transferred into 20 mL YPD broth (BD Difco, Australia) in 50 mL Erlenmeyer flask, using a sterilized inoculation loop. The flask was continuously agitated (150 rpm) at 30 °C (Excella E24 Incubator Shaker Series, USA), for overnight. Inocula were prepared from the overnight culture by re-suspending 5 mL of the stock solution into 100 mL fresh YPD broth and incubating the sample (150 rpm, 30 °C), until the stationary phase was reached (24 h). The cell growth was checked by measuring the culture absorbance ($A_{660nm} = 1.0$) in order to ensure a similar inoculum level in all experiments ($\sim 10^8$ CFU/mL). The cells were washed three times by centrifugation at 10 °C, for 5 min, at $4000 \times g$, and sediment cells were aseptically re-suspended in sterilized distilled water before storage at 4 °C until use (<24 h).

Honey inoculation and packaging

Unprocessed Manuka honey used (pH 4.3, 80°Brix) was kindly donated by Comvita® (New Zealand). Honey and transparent plastic film pouches used to pack the honey (Cas-Pak plastic vacuum pouch, New Zealand) were sterilized at 121 °C for 15 min. Then, 0.2 mL of *S. cerevisiae* cell suspension, with initial count of ca. 10^8 CFU/mL, was added aseptically to 2 g of sterilized honey packed in 5 cm × 5 cm sterilized pouches (Cas-Pak, New Zealand). This food grade retort pouches composed of polyester coated with silicon oxide and laminated to cast polypropylene (PETSIOX(12)//ON(15)//RCPP(70)). This pouches were 1 mm thick, with low oxygen transmission rate (<2 cc/m^2/day) and could withstand temperatures up to 130 °C, being suitable for thermal and high pressure processing. The sample pouches were then vacuum-sealed (Vacuum sealer C200, MULTIVAC, Germany).

Preparation of honey samples with different sugar concentrations

Honey samples with different sugar concentrations (20, 30, 40, 50, 60 and 70°Brix) were prepared aseptically using sterilized distilled water. Honey with initial of 80°Brix was used to prepare these different sugar concentrations. The degree of soluble solids (°Brix) for each dilution and the undiluted honey sample (ca. 80°Brix) were measured using a RX-5000a digital refractometer (Atago®, UK), at 20 °C. Sterilized distilled water serves as control (0°Brix).

HPP equipment and operation conditions

The HPP unit used (QFP 2L-700 Laboratory Food Processing System, Avure Technologies, USA) consists of a 2-L cylindrical-shaped pressure treatment chamber (inner height = 254 mm, inner diameter = 101.6 mm) with a thermocouple, a water circulation system, a cooling system, a pumping system and a control system that is operated by a software supplied by the manufacturer. Distilled water was used as pressurization liquid. Temperature inside the pressure chamber during treatment was measured using thermocouples located at the mid and top of the vessel and immersed in the pressure medium. The cycle report (containing data for compression time, decompression time and average temperature during HPP) was directly obtained from the computed data logger.

Duplicate of 2 g vacuum-packed honey samples were subjected to different conditions of HPP (at 200, 400 and

600 MPa, from 2 to 30 min of treatment time). The treatment time was the holding pressure time and did not include the pressure come up and decompression times. The come-up time was recorded to be approximately 1 min which has negligible effect on microorganism inactivation and decompression time of less than 30 s. The adiabatic heating at 200, 400 and 600 MPa gave average processing temperatures during the holding pressure phase of 26.80 ± 2.10, 30.18 ± 1.14 and 32.60 ± 2.10 °C, respectively. The maximum temperature attained did not exceed 35 °C and is well within the growth range for the *S. cerevisiae* (Goh et al. 2007). After each treatment, samples were immediately cooled in ice water. All honey samples were taken from the same honey batch and every single treatment was carried out in triplicate.

Thermal processing of honey

Thermal processing was performed at 55 ± 0.10 °C, for 10 min, using a thermostatic water bath (W28, Grant Instruments, England). Process conditions were selected based on the conditions applied industrially.

Vacuum-sealed samples were fully submerged into the water bath. After each treatment, all samples were immediately placed in ice-cooled water before analysis (<1 h). The large surface area of the pouch bag relative to its volume enhanced heat transfer and minimized temperature difference within the sample, where the come up time was less than 50 s (Akhmazillah et al. 2013). All honey samples were taken from the same honey batch and the treatments were carried out in triplicate.

S. cerevisiae cells enumeration

Concentration of *S. cerevisiae* cell in honey samples were established before and after each HPP and thermal treatment. The number of surviving cells was determined after a proper dilution of the untreated/treated samples by the viable plate count method. Two gram of each sample was mixed and homogenized with 18 mL of sterilized 0.1% peptone water (Merck Milipore, UK) to prepare the initial dilution. Serial dilutions from 10^{-1} to $10^{-7}$of sample were carried out. Then, 0.1 mL of each dilution was aseptically inoculated onto YPD agar plates by the spread-plating technique. The plating was done in duplicate for each series of dilution. The inoculated plates were then incubated at 30 °C for 48 h. Microbial growth was examined and colonies were counted using a colony counter equipment (Colony Counter 570, Suntex Instrument Co., Taiwan). Only plates having 30–300 colonies were used for analysis. The number of viable cells were measured as Colony Forming Unit per mL (CFU/mL) using the Eq. (1).

$$CFU/mL = (no.\,of\,colonies \times dilution\,factor)/ volume\,of\,culture\,plate \quad (1)$$

CFU/mL is converted to log value by calculating Log (CFU/mL). Results were then expressed as log reduction using Eq. (2).

$$Logreduction = log_{10}\left(\frac{N}{N_o}\right) \quad (2)$$

where N = the number of viable *S. cerevisiae* cell after treatment; N_o = the number of viable *S. cerevisiae* cell before treatment.

Compressibility measurement

Compressibility-pressure data for various sugar concentrations was obtained from the literature. Compressibility of honey solution at different °Brix and pressure levels was determined by re-plotting the graphs obtained from Min et al. (2010). The compressibility versus different sucrose concentration (0, 2.5, 10, 50 and 80%) at different pressure is plotted in Fig. 1 and will be discussed in the Results and Discussion section.

Statistical analysis

One-way analysis of variance (ANOVA) was run to investigate any significant differences among treatments (Statistica version 8, USA). When significant differences were detected ($p < 0.05$), Tukey Honest Significant Difference (HSD) test was carried out to separate the average values for cells log reductions. The Pearson correlation coefficient was calculated using Statistica version 11, Statsoft® software (Statsoft®, USA). Other analyses were also performed using Microsoft Excel® 2010 (Microsoft©, USA).

Results and discussion

Compressibility and °Brix during HPP

°Brix refers to the sugar content, where 1°Brix *represents* 1 g of sucrose in 100 g of solution. To understand the relation between compressibility and °Brix, as well as their effect on microbial inactivation by HPP, previous measurements from Min et al. (2010) were re-plotted in the form of compressibility-°Brix diagram as shown in Fig. 1. In this study, we assumed that the compressibility is approximately the same for glucose and fructose. As stated by Min et al. (2010), an increase in sucrose concentration (°Brix) significantly ($p < 0.05$) decreased sucrose

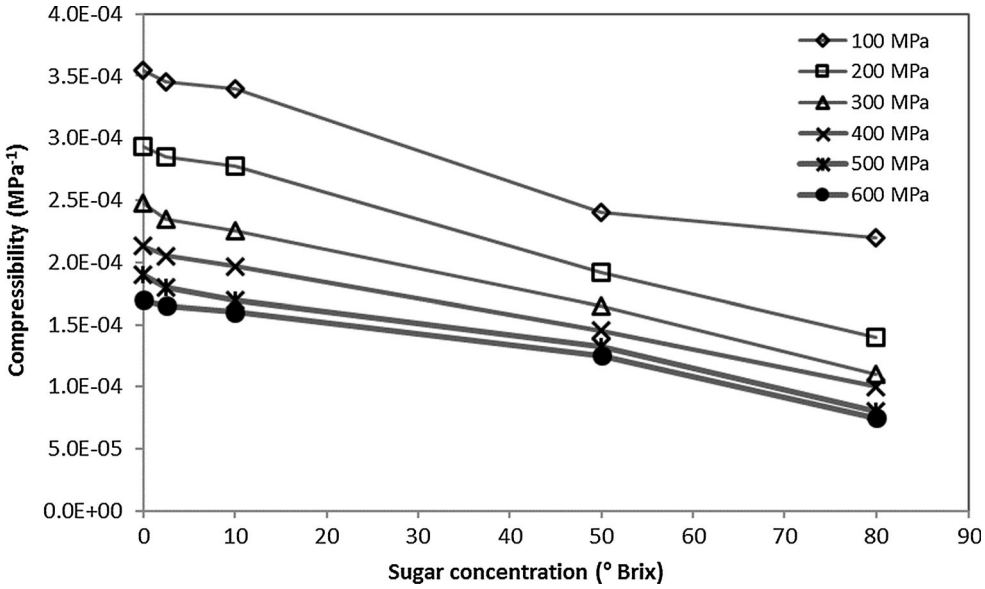

Fig. 1 Compressibility of honey solution at different pressure levels as a function of sugar concentration (°Brix). Data were taken and reassembled from Min et al. (2010)

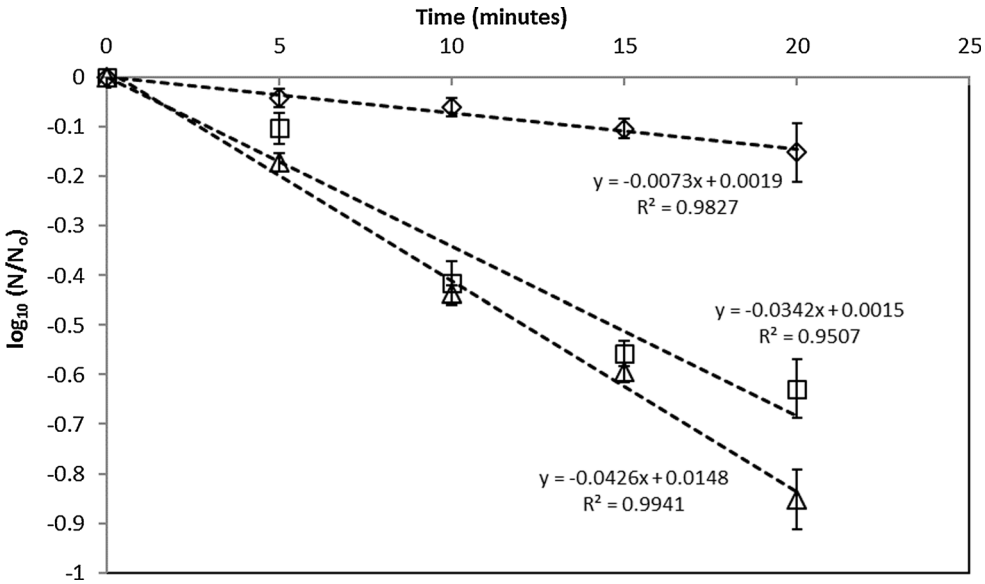

Fig. 2 *S. cerevisiae* cell survivors in honey (80°Brix) subjected to HPP (ambient temperature) at (i) ◇ = 200; □ = 400 and Δ = 600 MPa. The *error bars* are standard deviation (n = 3)

solution's compressibility, regardless of pressure, in the studied range (100–700 MPa). A very good correlation between compressibility and °Brix at the tested pressures was found with a correlation coefficient (r) better than 0.98.

Figure 1 also shows that increasing pressure significantly ($p < 0.05$) decreased compressibility for each °Brix tested. Bridgman (1970) and Isaacs (1981) stated that the relatively high compressibility of liquids at low pressure results from considerable free volume between molecules. Theoretically, as pressure increases, free volume decreases and compressibility is governed by the molecule's inherent compressibility as well as attractive and repulsive interactions between molecules (Min et al. 2010). Therefore, the lowest compressibility result was obtained when submitting 80°Brix honey to 600 MPa.

Ogawa et al. (1990) and Oxen and Knorr (1993) reported a higher sensitivity of microorganisms to pressure inactivation in the presence of lower concentration of solutes. Our preliminary work demonstrates that 80°Brix honey had a baroprotective effect at 200, 400 and 600 MPa (at ambient temperature), showing less than 1-log reduction of *S. cerevisiae*, even when it was submitted to 600 MPa for 20 min (Fig. 2). This is also in agreement with previous studies conducted with fruit juices and sucrose syrup (Basak et al. 2002; Campos and Cristianini 2007; Goh et al. 2007; Parish 1998).

A higher °Brix means lower water activity in honey. The survival of cells at lower water activity might be due to cell shrinkage which causes thickening of the cell membrane, reducing the membrane permeability and protecting cells

Table 1 °Brix effect of solute concentration on *S. cerevisiae* cells in various types of medium during HPP

°Brix	Media/food matrices	Process condition			Log reduction ($-\log_{10}N/N_o$)	*D*-value (min)	Z_p-value (MPa)	References
		Pressure (MPa)	Temperature (°C)	Time (min)				
10.5	Orange juice	100	84 (the maximum temperature reached)		0.60 ± 0.20			Campos and Cristianini (2007)
		200			4.90 ± 1.00			
		300			>5.60 ± 0.03			
10.7	Orange juice	350		0.02–5		0.63 ± 0.27	106 ± 6	Parish (1998)
		400				0.12 ± 0.03		
		450				0.07 ± 0.02		
		500				0.02 ± 0.002		
11.4	Single strength juice	100	20	30–120		82.2	135	Basak et al. (2002)
		150		20–80		38.2		
		200		10–40		26		
		250		5–20		5.4		
42	Concentrated orange juice	200	20	15–60		119	287	
		300				96.8		
		350				45.8		
		400				23.5		
50	Sucrose syrup in citrate–phosphate buffer	600	Ambient temperature (18–20)	0.25–2	1 (15 s)			Goh et al. (2007)
55					3 (120 s)			
60					<1 (120 s)			
80	Manuka honey	200	Ambient temperature (25–35)	5, 10, 15, 20	0.15 (20 min)	136.99 ± 7.97	526 ± 39	Current work
		400			0.63 (20 min)	29.24 ± 6.44		
		600			0.85 (20 min)	23.47 ± 0.86		

The errors bars are mean ± standard deviation with n = 3

* *D*-value = reciprocal of the slope log survivors versus time regression line; Z_p-values = reciprocal of the slope of log *D* versus pressure line

from destruction by HPP and temperature (Palou et al. 1998). This could explain the results obtained, keeping in mind that the presence of sucrose may impose only a transitory osmotic stress because cells are able to equilibrate extra- and intracellular concentrations of sucrose and lactose (Gibson 1973).

From thermodynamics point of view, the mechanical energy transferred to the cell during HPP can be observed by the change in volume of the system. The amount of mechanical energy transferred to the cell is strongly related to the compressibility which depends on water quantity in the cytoplasm. Water compression is involved in the antimicrobial effect of HPP (Moussa et al. 2006). An increase in water content in cytoplasm (higher a_w) results in an increase in compressibility and thus, in the mechanical energy transferred to the cell system. So, more microorganisms will be inactivated. On the other hand, lower water content in cytoplasm reduces the compressibility and thus reduces the mechanical energy transferred to the cell system, leading to less inactivation of microorganism.

Goh et al. (2007), Iwahashi et al. (1997) and Leslie et al. (1995) stated that sugars protect against changes in the physical state of membrane lipids and in the structure of sensitive proteins during physical stresses. Likewise, sucrose lowers the transmission temperature of the membrane by replacing the water between the lipid head groups, which then prevent phase transition and inhibit the fusion between the liposome due to glass formation. Furthermore, proteins are stabilized in their native state and, therefore, the integrity of the membranes is preserved during HPP (Crowe et al. 1997).

Table 1 summarizes the effect of HPP on the inactivation of *S. cerevisiae* in juices and also in buffer, as compared to honey (investigated in this study). *D*-value is the time required to inactivate 90% of *S. cerevisiae*, (at a given pressure in this case), whereas Z_p-value refers to the pressure required for 1-log reduction in the *D-value.* In comparison with other liquid food products, honey samples showed the largest *D*- and Z_p-values, indicating that higher °Brix caused cells to have higher resistance to HPP. This was claimed to be due to the protective nature of sugar (Goh et al. 2007). However, it is not clear if the low inactivation in *S. cerevisiae* is solely due to the protective nature of sugar or if the decrease in honey compressibility has also some contribution. This cannot be confirmed

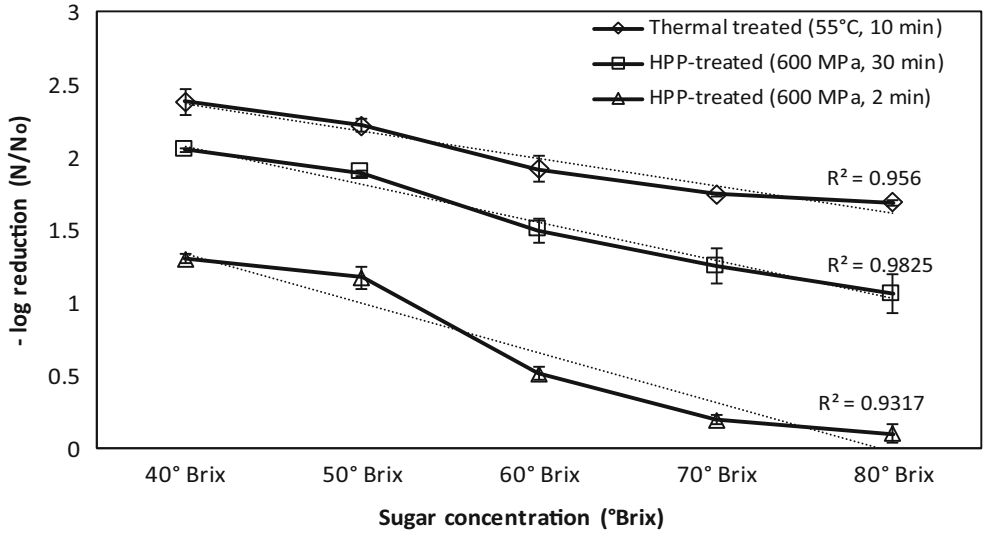

Fig. 3 Comparison of log reduction of *S. cerevisae* after HPP treatment (600 MPa for 2 and 30 min) and thermal treatment (55 °C for 10 min) for different sugar concentrations. *Error bars* represents standard deviation with n = 3

unless sugar solutions with different °Brix were submitted to HPP, which has been carried out in this paper. Results are shown in the following section.

Combined effects of compressibility and sugar protective nature on the inactivation of *S. cerevisiae*

To investigate the osmoprotection phenomenon of sugar concentration on the destruction of *S. cerevisiae* cells in HPP-treated honey, different concentrations of honey solution up to 80°Brix were treated by subjecting cells suspensions at 600 MPa for 2 and 30 min. As expected, at °Brix (0, 10, 20 and 30°Brix), the inactivation of the cells was greater when microbial concentration showed below the detection limit (data not shown). Lower °Brix presented higher water activity which decreased microbial resistance. Results demonstrate that a higher number of surviving cells was observed when cells were pressure-treated with increasing sugar concentrations of honey from 40 to 80°Brix (Fig. 3).

S. cerevisiae cells showed a gradual increase in the resistance to HPP with increasing sugar concentration. With an increase in sugar concentration to 60°Brix and above, less than 1-log reduction (for 2 min treatment) and less than 1.5-log reduction (for 30 min treatment) were observed. As expected, longer treatment time of 30 min in HPP-treated samples showed more cell reduction as compared to 2 min treatment at all sugar concentrations. The log reduction of *S. cerevisiae* cells in thermal-treated honey at 55 °C (for 10 min treatment) is slightly higher than the log reduction associated to HPP-treated honey, although longer treatment time (30 min) was applied, reflecting the baroprotective effect of honey concentration during HPP.

Table 2 The changes in log reduction (Δ log reduction) of *S. cerevisiae* subjected to HPP and thermal treatment with respect to different °Brix

°Brix	Processes		
	600 MPa, 2 min	600 MPa, 30 min	55 °C, 10 min
40 → 50	0.128 ± 0.01^{a}	0.161 ± 0.01^{a}	0.161 ± 0.01^{a}
40 → 60	0.787 ± 0.03^{a}	0.554 ± 0.04^{b}	0.465 ± 0.01^{b}
40 → 70	1.108 ± 0.02^{a}	0.803 ± 0.03^{b}	0.637 ± 0.01^{c}
40 → 80	1.151 ± 0.05^{a}	0.991 ± 0.01^{b}	0.695 ± 0.01^{c}

Mean values (mean ± standard deviation) within the same row with different letters are significantly different according to Turkey's HSD Test (Statistica version 11, Satsoft®) with n = 3

Hashizume et al. (1995) reported that sugars, particularly sucrose do protect against pressure inactivation of yeasts when pressurized at ambient temperature; however, it was not clear if the effect is solely due to sugar concentration or due to the decrease in the compressibility as sugar concentration increase.

Change (Δ) in log reduction provides a measurement of cells sensitivity to a change in the °Brix, as affected by processes. It is calculated as the difference between log reduction at 40°Brix and log reduction at particular °Brix (log reduction $_{40°Brix}$ − log reduction $_{50/60/70/80°Brix}$). A larger value in Δ log reduction indicates a higher microbial sensitivity to sugar concentration changes. Table 2 shows Δ log reduction of *S. cerevisiae* cells with respect to different sugar concentrations in honey (°Brix) for both HPP and thermal treatment.

In general, *S. cerevisiae* cells in HPP-treated samples (600 MPa/ambient temperature/2 and 30 min) are more

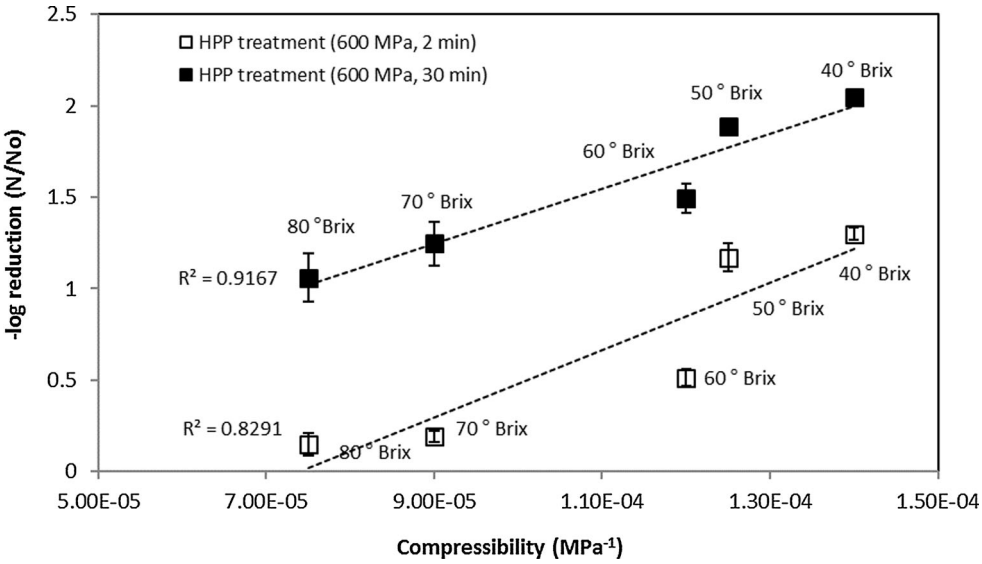

Fig. 4 Effect of compressibility and °Brix on the inactivation of *S. cerevisiae* subjected to HPP at 600 MPa for 2 and 30 min

Table 3 Correlation matrices for °Brix, compressibility and *S. cerevisiae* cells reduction subjected to HPP (600 MPa, ambient temperature), obtained using Pearson correlation from Statistica version 11, Statsoft®

	Honey concentration (°Brix)	Compressibility (MPa^{-1})	Cell reduction ($-\log_{10} N/N_o$)
Honey concentration (°Brix)			
2 min	1.000000	*0.956315	*0.957717
30 min	1.000000	*0.977376	*0.957438
Compressibility (MPa^{-1})			
2 min	*0.956315	1.000000	*0.910550
30 min	*0.977376	1.000000	*0.957453
Cell reduction ($-\log N/N_o$)			
2 min	*0.957717	*0.910550	1.00000
30 min	*0.957438	*0.957453	1.00000

* Correlation is significant at the 0.05 level

sensitive to sugar concentration changes than in thermally-treated cells (55 °C, 10 min), presenting higher Δ log reduction values (within the range of 0.13 ± 0.01 to 1.15 ± 0.05). Cell reduction due to °Brix change was significantly different ($p < 0.05$) between treatments at higher honey concentration of 70 and 80°Brix. The highest change in *S. cerevisiae* cells (at 40 → 80°Brix) was more pronounced for HPP-treated sample (600 MPa, 2 min; $\Delta = 1.151$ log reduction). A Δ log reduction for thermally-treated sample showed the lowest value (0.695). These results indicate that the inactivation of *S. cerevisiae* is not solely dependent on the baroprotective effect of honey, but also the compressibility effect when honey samples were subjected to HPP. Further study on the latter hypothesis is required to confirm the results by manipulating the °Brix solution with different compressibility.

The effect of compressibility on *S. cerevisiae* cells reduction at different °Brix subjected to HPP (at 600 MPa for 2 and 30 min) is shown in Fig. 4. A shorter pressure holding time (2 min) coupled with rapid decompression will lead to a fast adiabatic expansion of water that could result in rupturing the cell wall more effectively (Balasubramanian and Balasubramaniam 2003). The graph shows a gradual increase in the inactivation of the cells with increasing compressibility, showing a higher reduction cells when they were in honey with lower sucrose concentration (i.e. lower °Brix).

Table 3 shows the relationship between honey concentration and compressibility on the reduction of *S. cerevisiae* cells as affected by HPP. As tabulated, a very strong correlation ($r > 0.90$) was found between honey concentration, compressibility and cell reduction. The r value between cell reduction and honey concentration for 2 and 30 min was registered to about 0.95 ($p < 0.05$), whereas, r value between cell reduction and compressibility was found between 0.91 to 0.96 ($p < 0.05$). Cell reduction varied linearly with compressibility and honey concentration, indicating that these two factors play a significant role in HPP-treated honey. A strong and significant correlation ($p < 0.05$) between compressibility, honey concentration and cell reduction provides evidence that microbial inactivation during HPP was not solely influenced by °Brix but also due to compressibility.

Conclusion

The effect of pressure and °Brix on the inactivation of an osmophilic microorganism (*S. cerevisiae*) in model food (honey) was studied. Experimental work confirmed that, at a higher °Brix (80°Brix), the resistance of *S. cerevisiae* to HPP (600 MPa,T < 35 °C) was increased. This study proves that cell reduction is affected not only by the baroprotective nature of sugar but also due to compressibility effect when samples were subjected to HPP. The results reported from this study have practical implications in establishing efficient process design for commercial manufacturing of food preparation containing high concentration of sugars.

Acknowledgements The first author would like to thank the SLAB scholarship to The Ministry of Higher Education of Malaysia and Universiti Tun Hussein Onn Malaysia. The supply of honey samples from Comvita® (New Zealand) is appreciated. Special thanks to all technicians from Department of Chemical and Materials Engineering, The University of Auckland for their technical support.

Compliance with ethical standards

Conflict of interest The authors report no conflict of interest.

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