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APPLICATION OF NON-THERMAL PROCESSING FOR PRESERVATION OF ORANGE JUICE

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ABSTRACT

The application of non-thermal processing for inactivation of yeast (*Saccharomyces cerevisiae*) in orange juice was investigated and compared with conventional thermal processing. The results showed that less than 1 log of yeast in orange juice could be inactivated during thermal processing at 50 ^{0}C and 15 min treated time. In contrast high pressure treatment at moderate temperature (50 °C) and 100 to 300 MPa resulted in total (up to 6 log) inactivation of yeast in orange juice. Supercritical CO_2/liquid CO_2 treatment at ≥6 MPa and 50 °C was sufficient to inactive yeast up to 6 log after 15 min treatment time. For high electric field pulses**,** the field strength used in this study (15 kV/cm) was high enough to reduce the yeast number up to 2 log after 60 pulses in orange juice. Inactivation of yeast in orange juice using ultrasound is possible. Ultrasound amplitude at ≥40% (≥80 W) combined with moderate temperature (50 ^{0}C) resulted in up to 6 log inactivation of yeast in orange juice after 15 min treatment.

KEYWORDS: non-thermal processing, orange juice, yeast inactivation, ultrasound

1. INTRODUCTION

The objective of food preservation technologies used by food industry is to control microorganisms. Heating is the method of bacterial destruction more frequently used in the food industry. Thermal processing is very effective technology for microbial inactivation, however, excessive heat treatment may cause undesirable effects on foods such as protein denaturation, non enzymatic browning, and loss of vitamins and volatile flavours. In order to reduce the negative effects of heat treatments in foods, alternative technologies capable of inactivating microorganisms at temperatures below those used during thermal processing are being demanded by the food industry.

Non-thermal food processing techniques are receiving considerable attention because of their potential for quality and safety improvement of food. Non-thermal processing method such as high electric field pulses (HELP), Supercritical carbon dioxide ($ScCO_2$), high hydrostatic pressure (HP) and ultrasound could be applied to reduce the number of microorganisms in foods and extend the storage time.

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Orange juice has become a strong candidate for the application of non thermal processes due to degradation of fresh flavour characteristics by the thermal processes currently in use for ready to drink products, such as pasteurisation.

High electric field pulses has the potential of inactivating microorganism at temperatures that avoid the harmful effect of heat in organoleptic properties and nutrient value of liquid foods. It has been demonstrated that HELP treatments inactive bacterial vegetative cells, moulds, and yeast [1, 2] but bacterial spores are resistant to HELP treatment. In general, it has been reported that yeasts are the most HELP sensitive microorganisms, and Gram-positive bacteria are more resistant than gram-negative bacteria [3].

The main process parameters that determine HELP treatments are electric field strength, shape and width of the pulse, treatment time, frequency, specific energy, and temperature. Among them, electric field strength, treatment time, and/ or pulse energy are the basic control parameters of HELP processes [4].

High hydrostatic pressure has been shown to be more effective against vegetative bacteria [5]. More recently, by combining higher pressure from 700 to 1000 MPa and higher temperatures from 70 to 90 $^{\circ}$C, HP has been successfully applied to the sterilization of low-acid foods. The lethal effect of high pressure on bacteria is due to a number of different processes taking place simultaneously. In particular, damage to the cell membrane and inactivation of key enzymes, including those involved in DNA replication and transcription are thought to play a key role in inactivation [5, 6].

Supercritical fluid technology can be applied in pharmaceutical and food industries. The antimicrobial effect of high-pressure CO_2 treatment has been widely demonstrated in latest years [7-10]. This technique represents a promising alternative for pasteurisation of foodstuffs with respect to their natural properties: at moderate temperature and pressure CO_2 treatment significantly inactivates bacterial vegetative cells, moulds and yeasts and, at suitable conditions, high-pressure CO_2 can inactive intracellular and pectolytic enzymes [11, 12].

The application of ultrasonic waves generating cavitation in suspension which contain microorganisms and enzymes often has a lethal result and deactivating action [13]. When high power ultrasound propagates into liquid, the micro-bubbles which are commonly present in it or that may form from the presence of suspended particles, will oscillate according to the pressure wave. High acoustic pressure will determine their growth and violent collapse, which is accompanied by a sudden increase of the temperature and the pressure in the surrounding area. The shear force and rapidly changing pressure created by ultrasound waves are effective in destroying microbial cells, especially when combined with other treatment, including heating [14] and combined heat and ultrasound treatment under pressure [15]. It is, therefore, likely that ultrasound reduces the heat resistance of microorganism by physical damage to cell structures, cause by extreme pressure changes and disruption of cellular protein molecules. This makes them more sensitive to denaturation by heat. Similar change of protein structures can cause enzyme inactivation [15].

This work focused on the application of non-thermal processing (e.g. high electric field pulse (HELP), high hydrostatic pressure (HP), supercritical carbon dioxide ($ScCO_2$), and ultrasound (US), for preservation of orange juice.

2. MATERIALS AND METHODS

2.1 Materials

Sterilized orange juice (soluble solids content=11° Brix) was from Malee, Sampran Company, Thailand. Yeast (*Saccharomyces cerevisiae*) was from Kasetsart University, Thailand. Potato

dextrose agar (Criterion Company, USA) and Sodium Chloride (NaCl, Carlo Erba Company) were used for colony counting. Inoculums with yeast cells of approximately 10^7 CFU/ml were used to inoculate sterile juice to obtain yeast cells of approximately 10^6 CFU/ml.

2.2 Heat treatment (HT)

To investigate the effect of temperature alone on *S. cerevisiae*, thermal treatments of orange juice were applied at room temperature (30°C), 50, 70, 90°C for 15 min, respectively. Juice (5 ml) was placed in a glass tube and heated in the water bath at constant temperatures.

2.3 High electric field pulse (HELP)

The specification of HELP equipment (Figure 1) used in this study was as follows: Power supplier (15 kV, 400 W, LECIP NEON Transformer, Model EX230A, Japan), Capacitance (Sprada, 2 µF, 4 kV-DC, Germany), High voltage switch (3 kV, EPCOS Company, Germany). The treatment chamber was made of stainless steel (size: L = 15 cm B = 10 cm, gap of chamber was 2mm constant).

High electric field pulses were applied at 2 µF and 15 kV/cm field strength. The samples were treated at 20, 40, 60 pulses and 0.067 Hz (1 pulse/15s) pulse frequency. For all HELP experiment 10 ml of orange juice was inoculated with yeast inside the treatment chambers under sterile condition. After treatment the samples were collected in a sterile test tube and immediately cooled on ice bath.

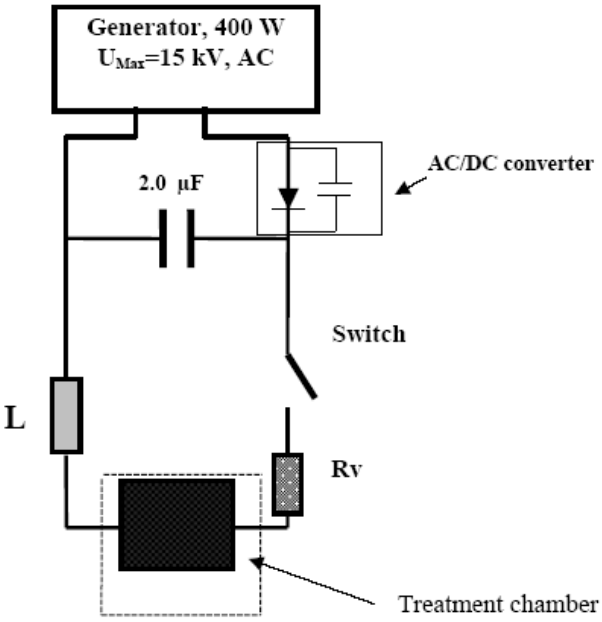

Figure 1 Scheme of HELP equipment

2.4 Supercritical carbon dioxide system ($ScCO_2$)

A $ScCO_2$ equipment with working pressure up to 1000 bar and high pressure vessel volume of 1 litre (Dunze GmbH, Germany) was used in this study. Liquid CO_2 (purity > 99.8 %, TIG company, Thailand) was used for all $ScCO_2$ experiments.

For each experiment 10 ml of orange juice inoculated with yeast were poured in a sterile test tube and covered with sterile cotton. The test tube was inserted inside the $ScCO_2$ vessel and then pressurized by $ScCO_2$. The samples were treated at given pressure (6, 10, 20, 25 MPa), 30 to 50 °C and constant treatment time (15 min), respectively.

2.5 High hydrostatic pressure (HP)

High pressure treatment was carried out in high pressure equipment with 400 MPa maximum working pressure and 500 ml vessel volume (NOVO, Germany). For high pressure treatment 10 ml of orange juice inoculated with yeast was sealed in a polyethylene bag and inserted in the high-pressure vessel.

Orange juices were treated at pressure 100, 200, 300 MPa and temperature 50 °C as well as at 200, 300 MPa and temperature 30°C respectively. The time for all HP experiments was 15 min constant.

2.6 Ultrasonic system (US)

a Ultrasound equipment (Sonopulse 3200, 20 kHz, Bandlin, Germany) with 200 watt maximum power (100%), 150.38/cm^2 power density and automatic amplitude compensation was used for ultrasound experiments. Treatment were carried out in a 100 ml double-wall cylindrical glass vessel (diameter 3 cm; height 20 cm) connected to a thermostatically-connected water bath (Fisher Scientific ISOTEMP 2150, Pittsburgh PA U.S.). 50 ml of yeast inoculated orange juice was poured into the vessel.

The ultrasound treatment at 20, 40, 60, and 80 % power was applied. The temperature of sample during ultrasound treatment maintained constant by recycling water from water bath. After the ultrasonic treatment, the samples were taken out, poured in sterile glass tube, cooled in cold water and immediately carried out for total count measurement.

2.7 Counting

Before and after each treatment viable counts of the surviving yeasts were determined by standard plating technique. Every sample was serially diluted in sterile 0.5 % saline solution (dilution 1:10), plated in petri dishes containing Potato Dextrose agar and incubated at 30 °C for 3 days before counting. Each experiment was carried out three times at least and the arithmetic mean was reported as final result.

3. RESULTS AND DISCUSSION

3.1 Effect of heat treatment

Heat treatment at 50 °C and 15 min slightly inactivated the yeast in orange juice (less than 1 log). Increasing the temperatures up to 70 or 90 °C decreased the microorganism rapidly so that all yeast (up to 6 log) could be inactivated at temperature ≥ 70 °C (Figure 2).

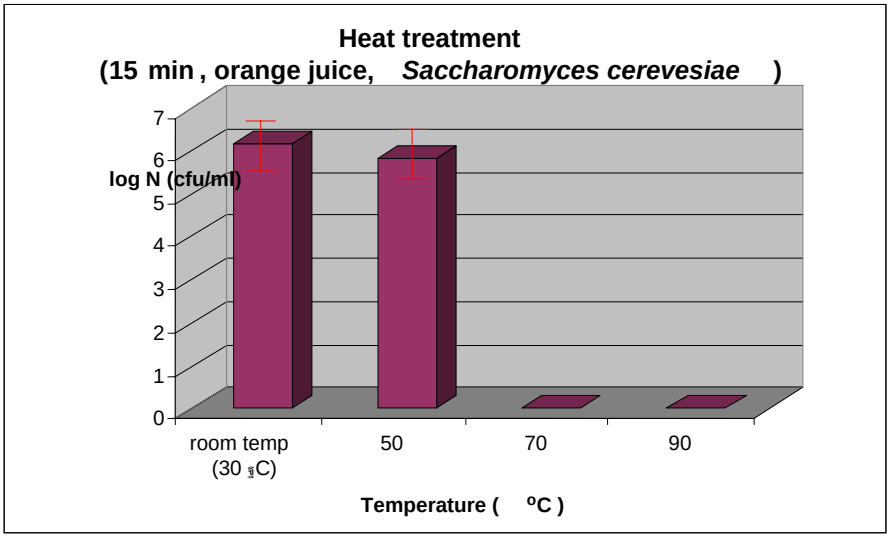

Figure 2 The number of *Saccharomyces cerevisiae* at different temperatures after heat treatment for 15 min.

3.2 Effect of high electric field pulses

Increasing the pulse number resulted in increasing the inactivation of microorganism. Yeast inactivation up to 2 logs could be achieved after 60 pulses. The increase of temperature during this process was low because of very low energy of each pulse and relatively long time between each pulse (every 15 sec 1 pulse, 0.067 Hz) (Table 1).

Table 1 Total count of *Saccharomyces cerevisiae* in orange juice after HELP treatment at room temperature, 15 kV/cm field strength and different pulse number

Pulse number (n) 15 kV/cm; 2 μF	**Total count (cfu/ml)**
Blank (no pulse)	3.8×10^{5}
20	1.3×10^{4}
40	5.7×10^{3}
60	4.4×10^{3}

Toepfel *et al*. [16] have investigated the effect of process parameters such as electrical field strength, total pulse energy input and treatment temperature on inactivation of *Listeria inocua, E. coli*, *Bacillus megaterium* and *S. cerevisiae*. They have found that temperature higher than 40 °C can strongly increase the lethality of the HELP process. Cells like *Listeria* could be affected easily by HELP even at a field strength as low as 16 kV/cm if the process temperature is >40 °C. For inactivation of *S. cerevisiae* a very low input of specific energy in the range of 10 and 30 kJ/kg is required for a 5 log cycle reduction at 55 °C initial treatment temperature.

3.3 Effect of supercritical/liquid carbon dioxide

$ScCO_2$ treatments at 50 °C and 15 min caused up to 6 log inactivation of yeast in orange juice at moderate temperature (50 °C). The $ScCO_2$ process is suitable method to produce pasteurised orange juice at moderate temperature (50 °C). The inactivation effect was independent on pressure level within applied pressure in this study (6 to 25 MPa).This indicates that 6 MPa is sufficient to inactivate *S. cerevisiae* at moderate temperature using liquid CO_2 (Figure 3). At 30 °C and low treatment pressure (6 MPa) only slight inactivation up to 50 % of total count could be observed after 15 min treatment time (Figure 4). It is also necessary to combine liquid CO_2 treatment with moderate temperature of 50 °C to ensure effective yeast inactivation. Spilimbergo *et al.* [17] examined the effect of $ScCO_2$ gas on inactivation of *S. cerevisiae* in apple juice. Pasteurisation of *S. cerevisiae* in apple juice with $ScCO_2$ was efficiently performed at 36 °C in a laboratory scale equipment at different pressure values (100-200 bar). A pressure increase has a positive effect on CO_2 process to reduce the processing time. Parton *et al.* [18] have found that an increase of process temperature leads to higher yeast inactivation in fruit juice by $ScCO_2$ treatment at 38 °C and 90 bar.

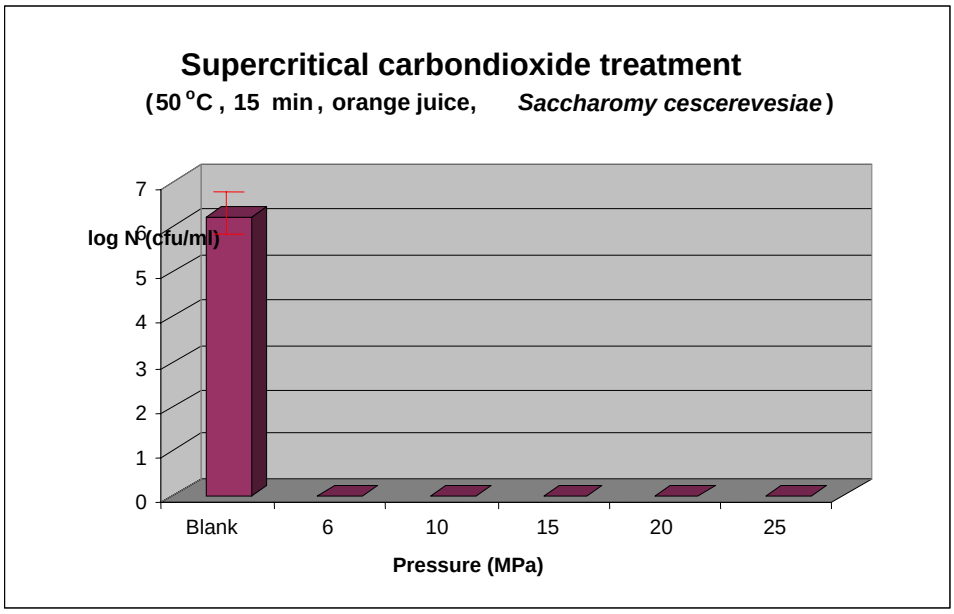

Figure 3 The number of *Saccharomyces cerevisiae* after $ScCO_2$ treatment for 15 min at 50 °C

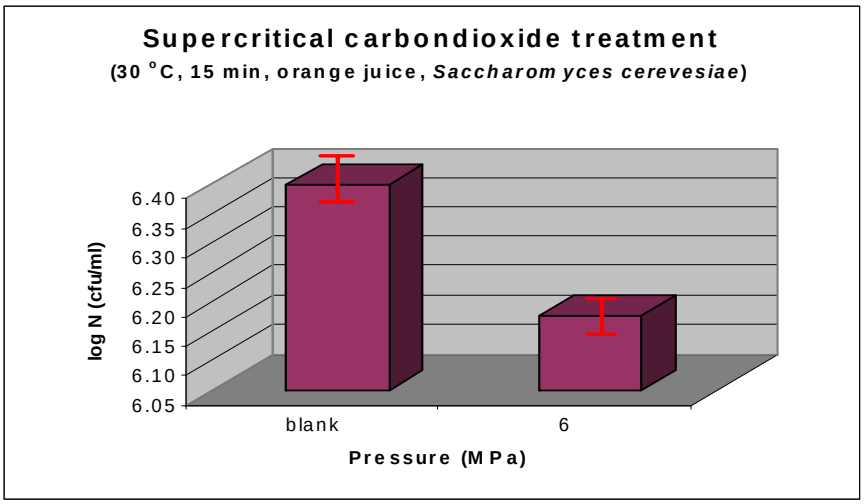

Figure 4 The number of *Saccharomyces cerevisiae* after $ScCO_2$ treatment for 15 min at 30 °C

3.4 Effect of high hydrostatic pressure

Yeast inoculated in orange juice was completely inactivated after 15 min at 200 and 300 MPa pressure at 50 °C. In contrary, 100 MPa pressure was not sufficient for total inactivation of yeast. Reduction of yeast up to 2 log could be achieved at 100 MPa and 15 min at 50 °C (Figure 5).

At 30 °C, 15 min and 200 MPa up to 5 log of microorganism was inactivated. Increasing the pressure up to 300 MPa resulted in the total inactivation of yeast at 30 °C and 15 min (Figure 6).

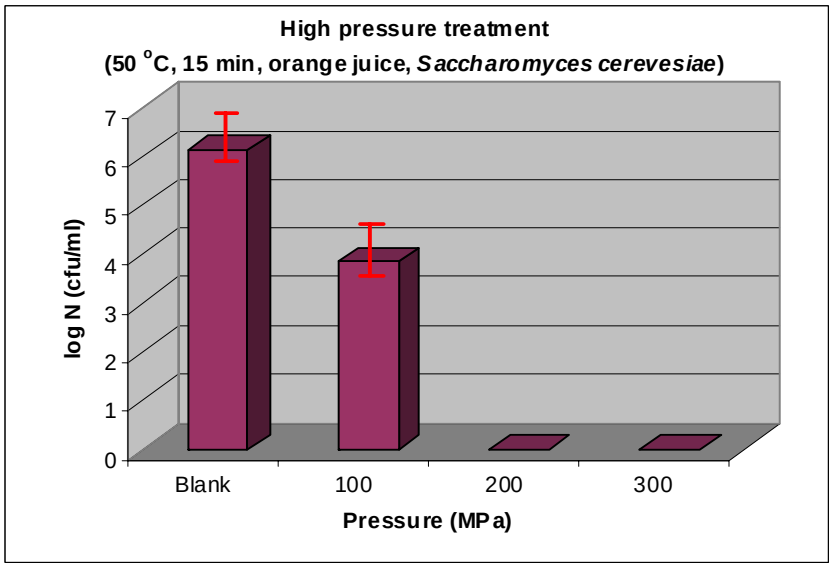

Figure 5 The number of *Saccharomyces cerevisiae* at different pressures after treatment for 15 min at 50 °C

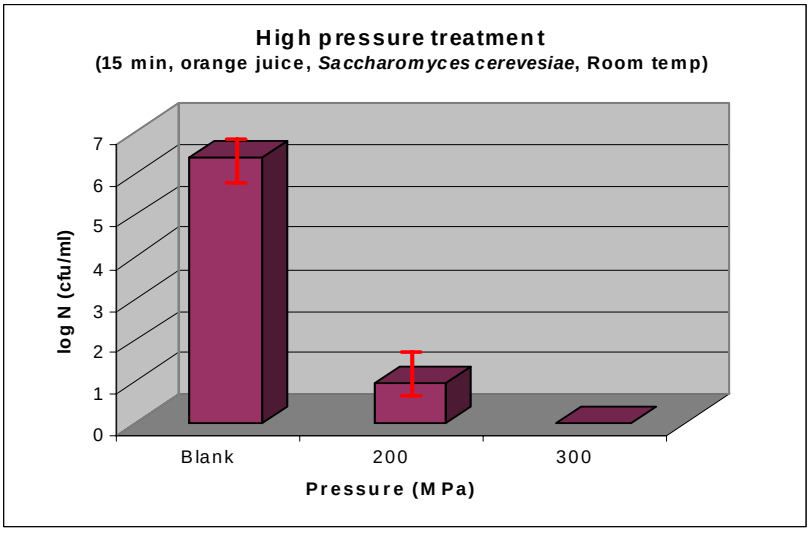

Figure 6 The number of *Saccharomyces cerevisiae* at different pressures after treatment for 15 min at 30 °C

Chen and Tseng [19] have studied the inactivation kinetics of *S. cerevisiae* CCRC 20271 at the combination of different hydrostatic pressure (0.1 –300 MPa) and temperature (35-55 °C). They have observed that the decrease in survival fractions of cells within 40 min was small when cells were maintained at temperature between 35 to 55 °C and under pressure of < 100 MPa. However, the viable fractions of the pressurized cells fell significantly as the pressures were increased further to ≥ 150 MPa. The higher the temperatures, the greater the rate of decrease of viable cells occurred with time.

3.5 Effect of ultrasonication

In our primary experiments at room temperature we could not detect any major effect of ultrasound on yeast inactivation (data not shown). The effective method for microorganism inactivation in orange juice is only combination of ultrasound with moderate temperature (50 °C). Ultrasonication at 20% amplitude and treatment time 15 min showed nearly no effect on microorganism inactivation. Increasing the ultrasound amplitude higher than 40% induced inactivation of *S.cerevisiae* in orange juice (up to 7 log). At ultrasound amplitude ≥ 40 % a completely inactivated of yeast at 50 °C and 15min could be achieved (Table 2).

Table 2 Effect of amplitude during ultrasound treatment at 50 C and 15 min treatment time on inactivation of *Saccharomyces cerevisiae* in orange juice.

Amplitude (%) during ultrasound treatment	Total count (cfu/ml)
0% (Blank)	7.4×10^7
20%	1.2×10^7
40%	not found
60%	not found
80%	not found

According to Raso *et al*. [20] the amplitude of ultrasonic wave has a major effect on microbial inactivation. The intensity of ultrasound is directly related to the amplitude of the ultrasound wave [21].

Guerrero *et al*. [22] have studied the effect of ultrasound on the survival of *S. cerevisiae* in Sabouraud broth at different temperature (35 to 55 C), pH 9 (3-5.6) and amplitude. The resistance of the yeast decreased as ultrasound wave amplitude increased. Structural studies performed in cells sonicated at 45 °C and 95.2 μm of wave amplitude indicate the treatment provoked puncturing of cell walls with leakage of content as well as damage at subcellular level.

4. CONCLUSIONS

It is obviously that HELP treatment at high pulse number (60 pulses and 15 kV/cm field strength) is a suitable non-thermal processing for inactivation of microorganism and reduction of total count in orange juice. The field strength of 15 kV/cm used in this study was high enough to reduce the number of yeast in orange juice up to 2 log after 60 pulses. $ScCO_2$ is very effective non-thermal method for inactivation of yeast in orange juice. All the tested pressures (6 MPa –250 MPa) showed that the total yeast could be inactivated at 50 °C and 15 min. Other suitable non-thermal technique for yeast inactivation is high pressure. High pressure at ≥ 200 MPa and 50°C could inactive yeast in orange juice up to 6 log within 15 min. Ultrasound at 40% (80 W) and higher power combined with moderate temperature (50°C) and 15 min treatment time could be applied to inactive yeast in orange juice. It has been shown in this study that using non-thermal processing technique orange juice could be preserved without any excess heat treatment. These no-thermal techniques could be helpful for food industry to produce fruit juice with high sensory and nutrient quality.

5. ACKNOWLEDGEMENTS

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