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Studies on Producing Yeast Autolysate and its Antioxidant Properties to Enhance Flesh Apple Juice Qualities

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ABSTRACT

Yeast (*Saccharomyces cerevisiae*) has an important role in food industries and it also considers as a powerful probiotic microorganism due to its high content of bioactive compounds. Therefore, we use it as food additives in the form of fresh, dry active, or inactive extract, while these additions to some foods is sensory unacceptable. This problem may be overcome by using yeast autolysate which contains most of the soluble bioactive compounds of the cells. Therefore, yeast autolysate was produced in the current study and the effect of growth phases on its bioactive compounds formation was investigated. The data revealed that, cells had diauxic growth phase and the highest reducing power (RP) and DPPH scavenging activity were obtained after the first exponential phase (cultivation time of 24 hr), however the maximum production of total antioxidant (TAC) and glutathione content (GSH) were observed at the end of the second exponential phase (after 60 hr cultivation). Additionally, thermal treatment of yeast autolysate at 40°C up to 30 min exhibited almost no effect on the bioactive compounds activity. While, the autolysate treatment with $\geq$ 60°C led to sharply decrease of TAC (40% less than control) and a little decrease of GSH, DPPH, and RP contents. A comparison analysis between yeast autolysate and flesh apple juice found that, the IC_{50} of yeast autolysate was 5.6 mg while it was 20 mg for flesh apple juice. Also, yeast autolysate had 53, 6.11, and 2.73-fold higher for GSH, TAC, and RP, respectively, compared with flesh apple juice. Addition of 0.02% thermally treated (at 40°C for 5 or 10 min) yeast autolysate to flesh apple juice was decreased browning index (BI) and hydroxymethylfurfural (HMF) to 12 and 18%, respectively, this decrease reached 25 and 40% for BI and HMF, respectively, when the addition level was 0.08% which also was sensory accepted. So, it could be concluded that, addition of yeast autolysate into flesh apple juice is sensory accepted, enhanced its antioxidant properties, and decreased enzymatic as well as nonenzymatic browning.

Keywords: Yeast autolysate– Antioxidant– thermal treatment– Flesh apple juice – Browning– Sensory evaluation.

INTRODUCTION

Antioxidants are substances that able to delay or inhibit the oxidation process which occurs under atmospheric O_2, reactive oxygen species (ROS) or reactive nitrogen species (RNS), (Pisoschi and Negulescu, 2011 and Wu *et al.*, 2011). They are classified into two major groups, endogenous and exogenous antioxidants. Endogenous antioxidants are nonenzymatic substrate (eg. albumin, uric acid, glutathione, thioredoxin, and metallothionenis) or enzymes such as glutathione peroxidase (GPx), catalse, superoxide dismutase (SOD), glutathione reductase (GR), and thioredoxin reductase (Munhoz and Netto, 2004 ; Pisoschi and Negulescu, 2011 and Ignea *et al.*, 2013). Exogenous antioxidants are vitamins (C, E, D, K_3), minerals, phenolic, flavonoids, or synthetic compounds (Pisoschi and Negulescu, 2011 and Ignea *et al.*, 2013). Nowadays, there is a great concern about antioxidants in pharmaceutical, cosmetics, and foodstuffs (Molyneux, 2004). Therefore, natural antioxidants derived from plants and microbes especially probiotic microbes such as *Lactobacillus plantarum* (Li *et al.*, 2018) and *Saccharomyces cerevisiae* (Mohamed *et al.*, 2017 ; Shahat, 2018 and Haile and Kang, 2019) were received more attention.

Yeast, *Saccharomyces cerevisiae*, is a unicellular eukaryotic organisms and it is belong to fungi in the classification (El Moualij *et al.*, 1997). *Saccharomyces cerevisiae* is one of the most explored organisms because it plays an important role in industrial applications as well as genetic studies (Ilowefah *et al.*, 2017 ; Li *et al.*, 2018 and Haile and Kang, 2019). Several bioactive compounds such as glucans, mannan, glutathione, and vitamins have been reported in *Saccharomyces cerevisiae* (Khan *et al.*, 2016 ; Datta *et al.*, 2017 ; Mohamed *et al.*, 2017 and Liu *et al.*, 2018). Due to its high content of antioxidant, it was used to enhance the antioxidant properties for brown rice flour (Ilowefah *et al.*, 2017), wheat bran (Li *et al.*, 2018), and green coffee bean (Haile and Kang, 2019). Furthermore, dry inactivate yeast was added to wine as a source for glutathione (Gabrielli *et al.*, 2017).

Fruits and vegetables are the widely consumed foodstuffs as natural sources for antioxidant to prevent from chronic diseases. Apples and their derivatives are commonly incorporated in the diet for their phenolic compounds (Dewanto *et al.*, 2002 and Wolfe *et al.*, 2003) which are corresponding for its antioxidant activities, while vitamin C display only 4% of apple antioxidant content (Kahle *et al.*, 2005 ; Oszmianski *et al.*, 2007 and Suárez-Jacobo *et al.*, 2011). The highest fractions of polyphenols are presented in the peels (Khanizadeh *et al.*, 2008) which are discarded in juice manufacturing process. Also, juice clarification removes the phenolic compounds that are presented in flesh tissue (Candrawinata *et al.*, 2012).

Browning of apple takes place during juice manufacturing process or if it subjects to mechanical injury. Browning usually deteriorates the sensory properties due to the associated changes in flavor, color, and texture (Martinez and Whitaker, 1995). When juice is subjected to thermal treatment, a nonenzymatic browning is occurred which is considered to be one of the major factors of quality losses during thermal process, while enzymatic browning is the most important reaction occurred at low temperature and cause damage to both nutrition and sensory qualities (Holderbaum, 2010). Control of enzymatic browning has been reported by using NaCl, ascorbic acid, or by decreasing the pH to be less than 3.0 (Komthong *et al.*, 2007). However, this treatment is unacceptable because the formation of unnatural odor or change in taste (Komthong *et al.*, 2007). Recently, researcher discovered that glutathione react as an inhibitor for polyphenols oxidase and it can be used as natural antioxidant as well as anti-enzymatic and nonenzymatic browning agent (Wu, 2017).

Therefore, the aim of the current study is to produce *Saccharomyces cerevisiae* autolysate, to study the effect of growth phase and thermal treatment on autolysate antioxidant, and to evaluate the impact of autolysate addition to flesh apple juice on their antioxidant properties and sensory quality.

MATERIALS AND METHODS

Materials:

Active dry yeast *Saccharomyces cerevisiae* (Helew El-Shame) and red apple were obtained from the local market at Mansoura city, Egypt.

All chemicals used in the current work were of analytical reagent grade. Ascorbic acid, DTNB (5, 5' dithiobis–2-nitrobenzoic acid), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Tris-buffer, Folin-Ciocalteu reagent, glutathione, and hydroxymethylfurfural were purchased from Sigma-Aldrich, Egypt. Quercetin and gallic acid were purchased from (Biomedical Inc., Orange City, FL, USA). Other chemicals were ordered from Alnasr Company for Chemical and Medical Preparation, Cairo, Egypt.

Methods:

Cultivation conditions:

The yeast was cultivated on yeast-peptone-dextrose (YPD) broth contains 5 g/L yeast extract, 10 g/L peptone, and 20 g/L glucose (Mohamed *et al*., 2017). Cultivation was started by adding one gram of active dry yeast to Erlenmeyer flask (250 ml) contains 100 ml broth and incubated in a rotary shaker incubator at 30◦C with shaking at 200 rpm over night. This 100 ml culture was used as a seed to inoculate two Erlenmeyer flask (one litter) contains 500 ml broth in each and incubated at the same conditions for appropriate time (from 0 to 60 hr).

Preparation of yeast autolysate:

50 ml of culture was collected after appropriate cultivation time from each flask, and then fresh yeast was harvested by centrifugation for 10 min at 6000 rpm. Then, the cells were washed two times with distilled water to remove any culture traces. Afterwards, one part of cells were re-suspended with two part of distilled water then incubated at room temperature at 30◦C for 72 hr (Hassan, 2011). Autolysate was collected after precipitation of cell debris by centrifugation for 10 min at 6000 rpm. The supernatant was store at 4 ◦C and the precipitate was re-extracted again twice with the same procedure. Then, obtained autolysate solutions were combined together and it is further called yeast autolysate.

Thermal stability of fresh yeast autolysate antioxidants:

Fresh yeast autolysate was thermally processed under various temperatures (40, 60, 80, and 100 °C). The samples were holding at each temperature for different time (5, 10, 20, and 30 min). The samples were then withdrawn and immediately put in ice bath for cooling. After collecting all the samples, they are subjected for analyses to evaluate their remaining antioxidant activities.

Preparation of flesh apple juice:

Apples were washed, cleaned, peeled, and cut into strips to remove stems and seeds. 700 g of flesh apple strips was mixed with 1000 ml of cold water, and then the juice was extracted using Braun juicer machine according to Li *et al*., 2018. The cloudy juice was obtained by filtering the juice with cotton cloth filter while the clear juice was obtained after centrifugation of the juice at 6000 rpm for 5 min.

Determination of total solids (°Brix) and pH:

Total solid of yeast autolysate and flesh apple juice samples was measured using hand refractometer (Master refractometer, Tokyo, Japan) in triplicate after washing the refractometer prism with distilled water. The pH was measured using digital pH-meter (pH213 Microprocessor pH meter) after calibration with standard pH 7.0 and 4.0 buffer solutions (Abid *et al*., 2013).

Total antioxidant activity measurements:

Total antioxidant capacity of fresh yeast autolysate was determined spectrophotometrically at λ695 nm based on the phosphomolybdenum method (Kumaran and Karunakaran, 2007). The formation of green phosphate/ Mo (V) compound was measured against blank using Spectro UV-VIS Auto spectrophotometer. Ascorbic acid was used as positive control and standard curve was prepared using different concentrations of ascorbic acid to calculate the concentration of total antioxidant. Finally, total antioxidant content was expressed as ascorbic acid equivalent.

DPPH radical scavenging ability:

Scavenging effect of yeast autolysate and flesh apple juice samples were measured according to the methods of Lee *et al*. (2004). Three different volumes of each sample (0.5, 1.0, and 1.5 ml) was added to individual test tube and the volumes were completed to 2.0 ml with distilled water. Afterwards, 2.0 ml of DPPH (0.2 mM in ethanol) was added, the mixtures were vigorous shaken, left in dark for 30 min, and then it was measured at λ517 nm against control (sample was replaced with distilled water). The DPPH scavenging activity (%) was calculated from the following equation:

$$\textbf{Scavenging activity } \% = \left(1 - \frac{\mathbf{A_{sample}}}{\mathbf{A_{control}}}\right) * \mathbf{100}$$

The concentration that able to scavenge 50% (IC50) was predicted for yeast autolysate, ascorbic acid, and flesh apple juice by interpolation from linear regression curve analysis using the online curve fit website (https://mycurvefit.com/).

Determination of total glutathione:

Total sulfhydryl groups content was measured in yeast autolysate and flesh apple juice using Ellmans's reagent (Sedlak and Lindsay, 1968) with little modification: 250 µl samples/ standard were mixed with 750 µl of Tris-buffer (3mM EDTA, 30 mM Tris-HCl, pH 8.2). Then 250 µl of DTNB solution (3mM in methanol) were added followed by addition of 4.0 ml of methanol. The solution was centrifuged at 4000 rpm for 5 min at room temperature. Then, the absorbance of samples was measured at λ412 nm. Standard curve with acetyl-Cystein with serial dilutions (from 31 to 1000µM) was used for total-SH quantification.

Determination of reducing power:

Reducing power of yeast autolysate and flesh apple juice samples were determined according to the established method (Prabu and Natarajan, 2012). A known volumes (0.5 and 1.0 ml) of sample was mixed with 2.5 ml (0.2M phosphate buffer, pH = 6.6) and 2.5ml of potassium ferricyanide (1 %). Afterwards, incubated at 50◦C for 20 min, then 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 4000 rpm for 3 min. To 2.5 ml of supernatant, 2.5 ml of distilled water was added followed by 0.5 ml of FeCl3 (0.1%) and the absorbance was measured at λ700 nm. Different concentrations of ascorbic acid were used for preparing standard curve to calculate the reducing power as ascorbic acid equivalent.

Determination of total phenolic compounds:

Total phenolic compounds of yeast autolysate and flesh apple juice were determined by Folin-Ciocalteu reagent methods (Singleton *et al.*, 1999) and it was calculated as gallic acid equivalents.

Determination of total flavonoids:

Total flavonoids of yeast autolysate and flesh apple juice were measured using aluminium chloride reagent (Dewanto *et al.*, 2002) with the modification described by Haile and Kang (2019) and it was expressed as quercetin equivalent.

Determination of browning index and hydroxymethylfurfural (HMF):

Browning index measuring methods (Meydav *et al.*, 1977) and HMF determination methods (Keeney and Bassette, 1959) was adapted as described by Zhu *et al.* (2009).

Sensory evaluation:

Samples of flesh apple juice samples were subjected to ten panelists from Food Science Dept., Faculty of Agric., Mansoura University. The juice was evaluated according to the 9-point hedonic scale methods (Wu, 2017). The scale was extremely like (9), like moderately (7), dislike (5), dislike moderately (3), or dislike extremely (1).

Statistical analysis:

Statistical analysis was done using the Statistical analysis system (SAS, 2010) software program.

Results and Discussions:

Cultivation of *Saccharomyces cerevisiae*:

In the current study, the growth curve of *Saccharomyces cerevisiae* was investigated to evaluate the effect of cultivation time on the antioxidants production. A typical diauxic growth curve was observed for yeast growing on YPD-broth (Fig.1). Using the high inoculums ratio (10%) and using the same growth broth, the cells directly started the exponential phase up to 10 hr (Fig. 1). The cells were then entered second short stationary phase which reflects the depletion of broth glucose. Afterwards, a long exponential phase was started at cultivation time of 24 hr. This diauxic phenomenon was reported before for yeast that, when the yeast cultivated on high glucose medium it is rapidly grown and part of the consumed glucose is converted to ethanol and when the glucose was depleted from the culture medium, the yeast start to shift from the rapid glucose growth to the slow ethanol (Stahl *et al.*, 2004). The highest observed OD_{600} was 13.5 at cultivation time of 60 hr.

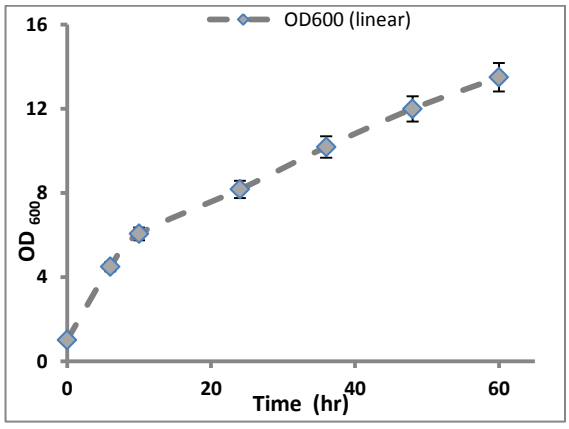

Fig. 1. Growth curve of *Saccharomyces cerevisiae* grown on yeast-peptone-dextrose (YPD) broth at 30°C with shaking (200 rpm) for 60 hr.

Effect of cultivation time on antioxidant production by yeast:

Harmful compounds such as reactive oxygen species (ROS), or reactive nitrogen species (RNS) are produced during the aerobic growth of yeast cells. These compounds may inhibit the cell growth and the cells use its defence system to decrease the deleterious effects of these compounds. The major defence system in the cells is the antioxidant. Due to its varieties, several determinations are used in the current study to evaluate the antioxidant contents in yeast autolysate including total antioxidant content, DPPH scavenging activity, total glutathione content, and total reducing power (Fig. 2).

The total antioxidant content (TAC) of yeast autolysate produced at different cultivation times is spectrophotometrically determined. Data in figure (2.A) display TAC calculated as ascorbic acid equivalents at different cultivation times. TAC was gradually increased by increasing the cultivation time. The lowest content was observed at the beginning of fermentation and it was of 14 µg ascorbic acid equivalents/ ml yeast autolysate solution (Total solid of 0.1%), while it reached the maximum TAC of 83.5 and 86.5 µg ascorbic acid equivalents/ ml at the cultivation time of 48 and 60 hr, respectively. Since further growth of yeast cells produce more harmful compound, therefore TAC should be increased. Previously reported TAC of yeast autolysate was 28 µg ascorbic acid equivalents/ 100 mg yeast autolysate (Hassan, 2011).

Additionally, DPPH free-radical scavenging activity was evaluated in yeast autolysate at different cultivation time. The reduction of the DPPH purple colour reflects the antioxidant ability to quench the free-radical. In comparison with TAC, the maximum DPPH scavenging activity was observed at cultivation time of 24 hr and then it was gradually decreased by increasing cultivation time (Fig.2.B). This data indicate that in the diauxic growth of yeast (Fig.1), the high free-radical scavenging components are produced after the first exponential phase while it was consumed during the second exponential phase. In a previous study, culture content of DPPH and thiobarbituric acid substances (TBARS) after growing the yeast on 4 different media have been reported to be unrelated to each other (*Shahat*, 2018). Also, another study using one measurement to evaluate the antioxidant content of 3 different yeast strains found that the cultivation time corresponding for maximum antioxidant production are varying and it was strain dependent (Lavová and Urminská1, 2013).

The first defence system line presented in yeast against oxidative stress is glutathione (GSH) and its corresponding enzymes such as glutathione peroxidase (GPx) and glutathione reductase (GR) (Jamieson, 1998 and Dordevic *et al.*, 2018). GSH is oxidized under oxidation stress via GPx to glutathione disulfide (GSSG) while the reveres reduction reaction is carried out by GR. These two enzymes are responsible for maintenance the GSH/GSSG ratio inside the cell (Jamieson, 1998). The data in figure (2.C) represented the GSH content at different cultivation times. From these data, it is clear that the highest GSH content was observed at cultivation time of 60 and 48 hr which reached 18 and 19 µg GSH/ml yeast autolysate, respectively. Since GSH content is correlated to the oxidation/reduction potential inside the cells, it is logical that high GSH content is presented during ethanol growth phase than glucose because ethanol is more reduced substrate than glucose. Many researchers previously reported GSH content in yeast to be of 17.7 µg GSH/ml (Hassan, 2011) and 14.8 µg GSH/ ml yeast (Fakruddin *et*

al., 2017). This content of GSH in yeast has been increased to 9-fold by mutation and it was corresponding to increase TAC up to 45-fold (Mohamed *et al.*, 2017). These results may be elucidating the increasing of TAC at the end of cultivation time (Fig.2A).

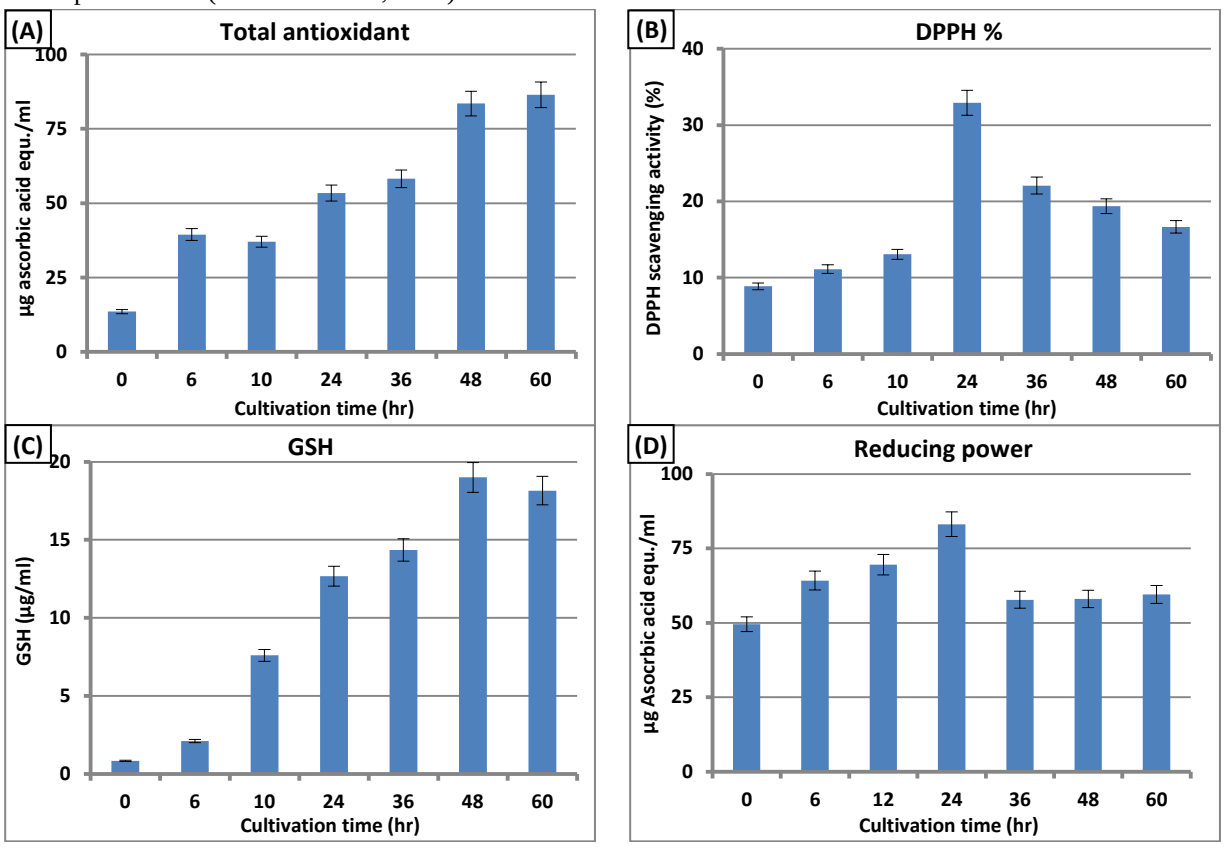

Fig. 2. Effect of cultivation time on antioxidant production by yeast. *Saccharomyces cerevisiae* was cultivated on yeast-peptone-dextrose (YPD) broth at 30°C with shaking (200 rpm). Yeast autolysate was prepared as described in Materials and Methods part. The total solid concentration of autolysate solution was adjusted to be of 0.1%. (A) Total antioxidant content of yeast autolysate obtained at different cultivation times. The total antioxidant was calculated as µg ascorbic acid equivalents/ ml yeast autolysate. (B) Scavenging activity of yeast autolysate calculated as the % inhibition of DPPH. (C) Glutathione content (GSH) of yeast autolysate calculated as µg GSH/ ml yeast autolysate. (D) Reducing capability of yeast autolysate calculated as µg ascorbic acid equivalents/ ml yeast autolysate.

The fourth method used in the current study to evaluate the antioxidant content of yeast autolysate was measuring the reducing power which is calculated as ascorbic acid equivalents/ ml yeast autolysate (Fig.2.D). The reducing power content showed the same phenomenon as DPPH content (Fig.2.B) since both is working on reducing free-radical. The highest observed reducing power was 83 µg ascorbic acid equivalents/ ml yeast autolysate after 24 hr cultivation time while the lowest value was 49 µg ascorbic acid equivalents/ ml at the beginning of cultivation which may be come from the inoculum. These values are in agreement with the previously reported data (Hassan, 2011 and Fakruddin *et al.*, 2017).

Thermal stability of yeast autolysate antioxidant:

Yeast autolysate was subjected to thermal treatment at different temperature degrees (40, 60, 80, and 100 °C) for different periods (5, 10, 20, and 30 min). TAC, DPPH scavenging activity, GSH content, and reducing power were measured to study the thermal treatment effect on yeast antioxidant contents (Fig.3). Data in figure (3.A) show that there is a little decreased at TAC (13% less than control) at 40 °C treatment especially after 30 min holding on 40 °C. This effect was clearly independent on thermal time when the temperature was ≥ 60 °C. At 60 °C the TAC decrease was ≈ 40% (the relative activity to control was 60%), while at 80 or 100 °C this decrease reached the highest value (50% less than control). It is known that endogenous antioxidant depend on enzymes (Pisoschi and Negulescu, 2011 and Ignea *et al.*, 2013) that are heat sensitive, while exogenous antioxidant such as phenolic compounds are heat resistance (Dewanto *et al.*, 2002 and Patras *et al.*, 2009). Destroying of yeast autolysate enzymes by heating may be the reason for decreasing the TAC.

In contrast, there are slight effect of thermal treatment on DPPH and GSH content (Fig.3.B and C). The relative DPPH scavenging activity was around 90% which means that yeast autolysate only lost 10% of their DPPH activity after thermal treatment (Fig.3.B). Several groups have been reported that the major compounds affected on DPPH activity is phenolic compounds which are heat stable (Xu and Chang, 2008 ; Patras *et al.*, 2009 and Yadav *et al.*, 2018). According to GSH (Fig.3.C), there is no effect at 40 °C while the decrease was 14% less than control when the temperature was ≥ 60 °C. Thiol-compounds are heat stable (Ma *et al.*, 2010) but they are oxidized under heat treatment (Tai and Ho, 1998). So, GSH is oxidized at high temperature to GSSG

(Ma *et al.*, 2010). On the other hand, the glutathione reductase which is responsible for reduction of GSSG to GSH is destroyed by heat. These two effects of high temperature on the presence of GSH is the reason for the little decrease on its content after heat treatment (Fig.3.C).

Figure (3.D) presents the effect of thermal treatment on reducing power. The only two thermal treatments that did not affect the reducing power are 40°C/ 5 min and 60°C/ 5 min. Other treatments caused 20% decrease of reducing power than control (relative activity of 80%).

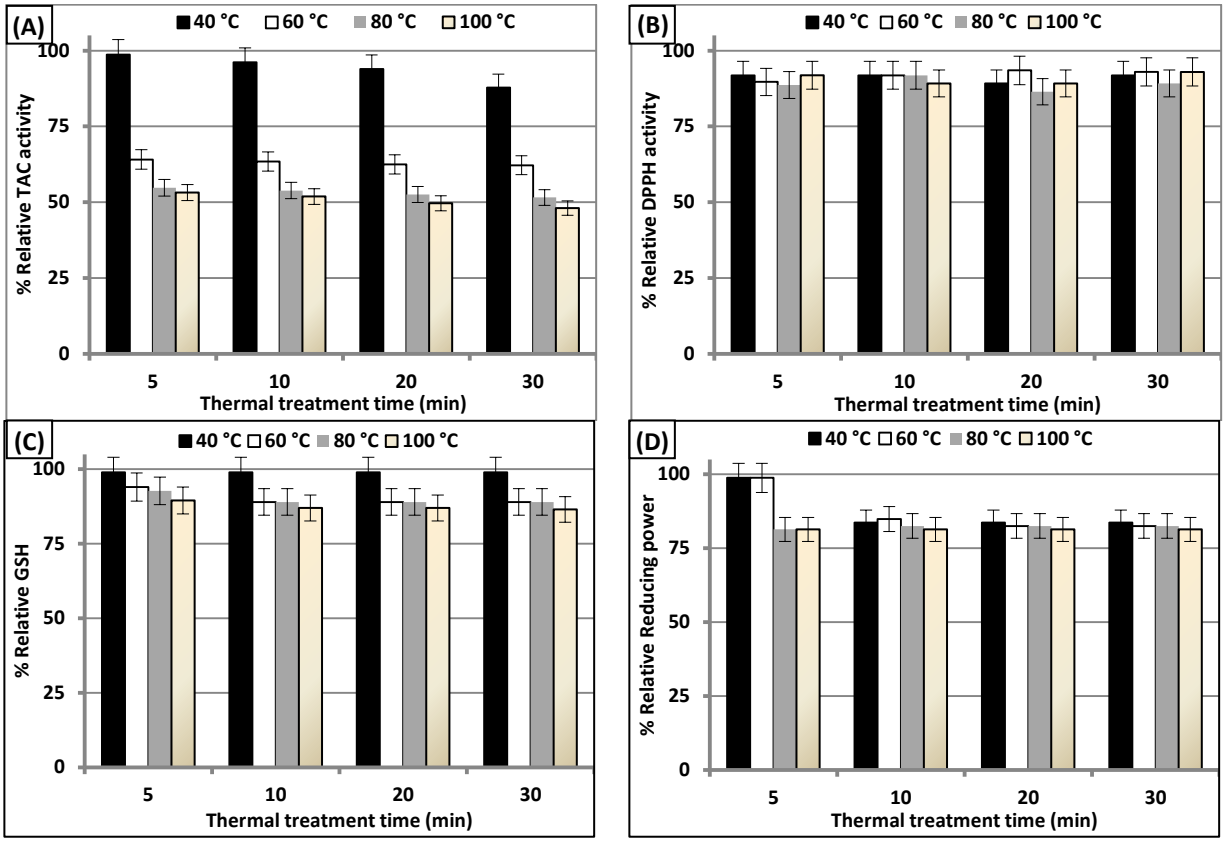

Fig. 3. Thermal stability of yeast autolysate antioxidant. Yeast autolysate was subjected to thermal treatment at 40, 60, 80, and 100 °C for 5, 10, 20, and 30 min. The total solid concentration of autolysate solution was adjusted to be 0.1%. All the values were calculated as relative activity compared to control without thermal treatment. (A) Thermal treatment effect on total antioxidant content of yeast autolysate. (B) Thermal treatment effect on DPPH scavenging activity of yeast autolysate. (C) Thermal treatment effect on glutathione content (GSH) of yeast autolysate. (D) Thermal treatment effect on reducing capability of yeast autolysate.

IC_{50} of ascorbic acid, flesh apple juice, and yeast autolysate:

For accurate comparison between the ability of ascorbic acid, flesh apple juice, and yeast autolysate for scavenging free-radicals, the concentration that scavenged 50% of DPPH (IC_{50}) was calculated (Fig.4). The results display that ascorbic acid (Fig.4.A) has the highest scavenging ability with IC_{50} of 0.12 mg/ml followed by yeast autolysate with IC_{50} of 5.6 mg/ml (Fig.4.C). The lowest obtained IC_{50} (20 mg/ml) was recorded for flesh apple juice (Fig.4.B). Previously study has been reported that IC_{50} for yeast culture between 2.5 to 3.5 mg/ml depending on the culture composition (Shahat, 2018) while it was > 10 mg/ml for commercial apple (Beh *et al.*, 2012).

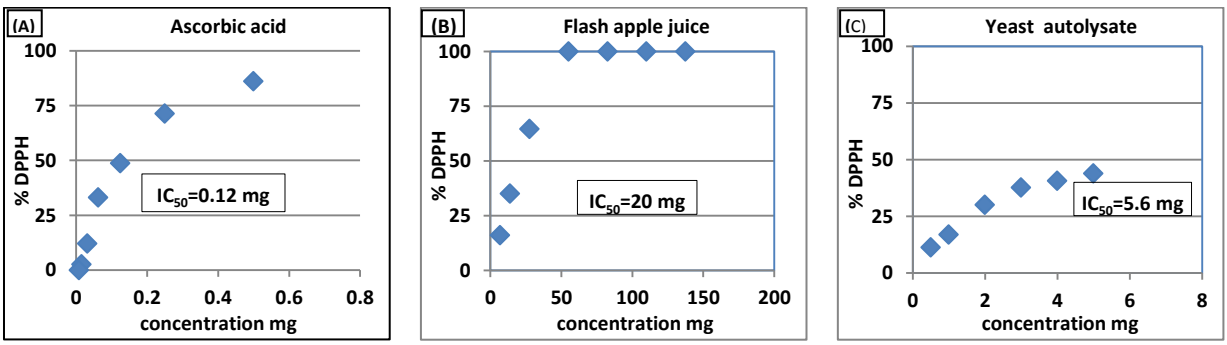

Fig. 4. IC_{50} of ascorbic acid, flesh apple juice, and yeast autolysate. The concentration (mg/ml) of ascorbic acid (A), flesh apple juice (B), and yeast autolysate (C) that scavenged 50% of DPPH radical activity. The IC_{50} was predicted by linear regression analysis using the online curve fit website (https://mycurvefit.com/).

Antioxidant properties of flesh apple juice and yeast autolysate:

For further discovering the difference between prepared yeast autolysate and flesh apple juice, antioxidant, phenolic, and flavonoids were determined (Table, 1). Both of them had almost the same concentration of TPC, while flesh apple juice had 4-fold higher of TFC concentration than yeast autolysate. On the other side, yeast autolysate showed high content of GSH (957 µg/g wet cell) which is considered 53-fold higher than flesh apple juice. For further studies the effect of TPC, TFC, and GSH content on antioxidant properties, TAC and RP were determined. Data in table (1) revealed that, yeast autolysate had 6.11 and 2.73-fold increased in TAC and RP than flesh apple juice, respectively. These results indicated that, yeast autolysate is rich in antioxidant than flesh apple juice. This could be illustrated by the majority of antioxidant activity in apple comes from the combination of phytochemicals while ascorbic acid contribution is only 4% (Dewanto *et al.*, 2002). Also, 45-fold increase in GSH formation by mutated *Saccharomyces cerevisiae* than wild type strain is corresponding to 9-fold increase of the total antioxidant activity (Mohamed *et al.*, 2017). So, the high antioxidant activity of yeast autolysate could reflect the contribution of GSH as well as its other antioxidant factors.

Table 1. Antioxidant properties of flesh apple juice and yeast autolysate

	pH	TAC (µg/g)		GSH (µg/g)		RP (µg/g)		TPC (µg/g)		TFC (µg/g)	
Flesh apple juice	4.0	266	±3	18	±0.2	435	±13	676	±15	397	±5.8
Yeast autolysate	4.5	1625	±72	957	±11.5	1180	±34	716	±19	95	±6.3
Enrichment (yeast/apple)		6.11		53.17		2.73		1.06		0.24	

TAC, total antioxidant content (µg ascorbic acid equivalent/ g); GSH, reduced glutathione; RP, reducing power (µg ascorbic acid equivalent/ g; TPC, total phenolic content (µg gallic acid equivalent/ g); TFC, total flavonoids content (µg quercetin equivalent/ g). All of them were calculated based on wet weight of apple or yeast cell not for juice or yeast autolysate.

Effect of yeast autolysate addition on flesh apple juice browning:

Effect of yeast autolysate to delay the browning reaction of flesh apple juice was studied. Fresh yeast autolysate as well as thermally treated yeast autolysate (treated for 5 or 10 min at 40, 60, 80, and 100°C) was added to flesh apple juice with fixed concentration (0.02%) and the effect of these additions on both browning index and HMF were evaluated after 4 hr incubating at 25 °C (Fig.5). From the data presented in figure (5), it is clear that treated autolysate at 40°C for 5 or 10 min were the best treatment which they reduce the relative BI (12% decrease) and relative HMF (18% decrease) compared with control. Treated autolysate at 60°C for 5 or 10 min only could reduce relative HMF (14% decrease) with very little effect on relative BI (3% decrease 5% increase, respectively) compared with control. In contrast, thermally treated autolysate at 80°C or 100°C regardless the time increased both BI and HMF reading which could explain by the formation of hydroxymethylfurfural at these temperatures.

For further differentiation of yeast autolysate effect on enzymatic and nonenzymatic browning, an additionally experiment has been done (Fig.6) in which the polyphenols oxidase browning was stopped via several factors such as decrease the pH to 3.0, adding 2.0% NaCl, or adding 0.2% ascorbic acid (Komthong *et al.*, 2007). The results showed that, effect of yeast autolysate on browning index depends on the adding concentration (Fig.6.A and B). The highest decreasing on BI after 4 hr incubation at 25°C was almost 25% and it was achieved with a concentration of (0.08%, Table.2). These data indicated that, thermally treated autolysate able to decrease both enzymatic and nonenzymatic browning with the same level which may be due to its GSH content as previously reported (Wu, 2017).

According to the effect of yeast autolysate on HMF, data were presented in figure 6.C and D as well as table (2). Under acidic conditions (pH=3), in which the enzymatic reaction was stopped, the decrease on HMF formation after 4 hr incubation at 25°C was almost the same for all adding concentration of yeast autolysate, NaCl, and ascorbic acid (Fig.6.D and Table.2). This decrease was almost 30% comparing with control (without any addition). At pH=4, which both enzymatic and nonenzymatic browning is occurred, the decrease in HMF was noticed to be a concentration dependent (Table, 2). The highest decrease (70%) was observed with NaCl and ascorbic acid, however, yeast autolysate contribution was 40% with a concentration of 0.08%. Several groups were reported the use of NaCl and ascorbic acid as anti-browning agent for enzymatic and nonenzymatic browning (Zhu *et al.*, 2009 and Holderbaum, 2010). Also, addition of pure glutathione with a ration of 0.08% has been reported to inhibit apple polyphenols oxidase activity by 99.8% (Wu, 2017), which may reflects the anti-browning effect of our yeast autolysate to its glutathione content.

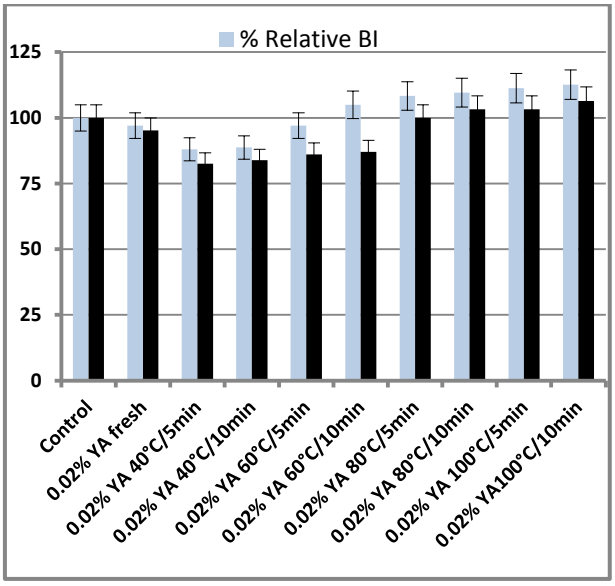

Fig. 5. Effect of adding thermally treated yeast autolysate on flesh apple juice browning. Yeast autolysate (fresh or thermally treated at 40, 60, 80, and 100 °C for 5 or 10 min) was added to flesh apple juice in a concentration of 0.02%, and then incubated for 4 hr at 25°C. The relative browning index (BI) as well as relative HMF were calculated depending on the reading of control.

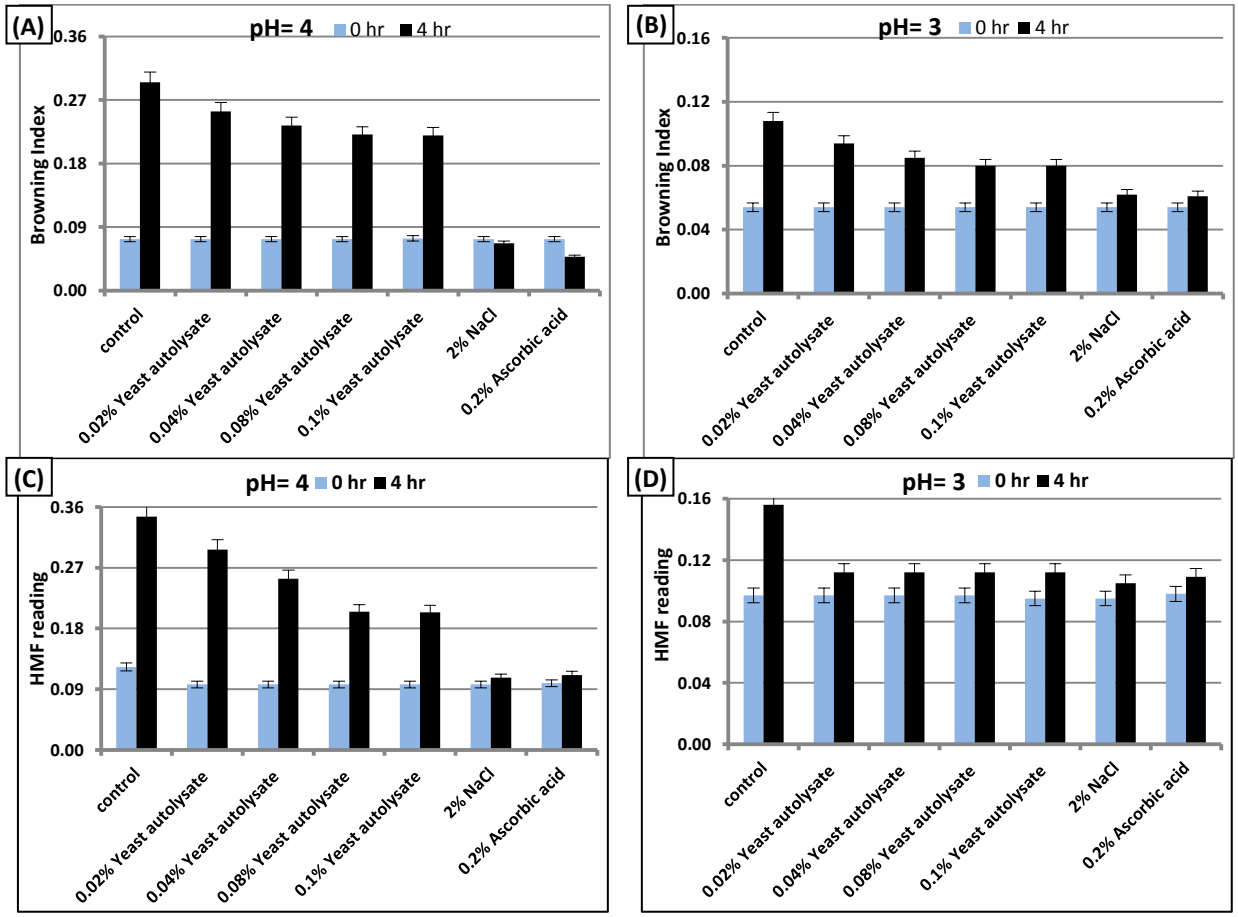

Fig. 6. Effect of adding different concentration of yeast autolysate (thermally treated at 40°C for 10 min) on flesh apple juice browning index and HMF. Yeast autolysate was added to flesh apple juice in varying concentration (from 0.02% to 0.1%). The browning index reading (BI) and HMF reading were plotted. (A) Addition effect of yeast autolysate to flesh apple juice (pH =4) on BI. (B) Addition effect of yeast autolysate to flesh apple juice after adjusting its pH to 3 on BI. (C) Addition effect of yeast autolysate to flesh apple juice (pH =4) on HMF reading. (D) Addition effect of yeast autolysate to flesh apple juice after adjusting its pH to 3 on HMF reading.

Table 2. Effect of yeast autolysate addition on flesh apple juice browning index and HMF

Samples	Addition	% BI		% HMF	
		pH= 4	pH= 3	pH= 4	pH= 3
Flesh apple juice	control	100.0	100.0	100.0	100.0
Flesh apple juice + Yeast autolysate	0.02%	86.1	87.0	85.8	71.8
	0.04%	79.3	78.7	73.4	71.8
	0.08%	74.9	74.4	59.2	71.2
	0.10%	74.6	74.1	59.0	70.8
Flesh apple juice + NaCl	2.0%	22.7	57.4	30.9	67.3
Flesh apple juice + Ascorbic acid	0.2%	16.3	56.5	32.1	69.9

Sensory evaluation of flesh apple juice:

Fresh flesh apple juice with or without any addition was subjected to sensory evaluation to know the addition effect on the juice acceptability (Table, 3). From the results presented in table (3), addition of 0.04% and 0.08% of yeast autolysate improve the acceptability of flesh juice. The observed problem with higher concentration of yeast autolysate is that there are deterioration effect on odor and taste. The color scale was extremely like with ascorbic acid and like moderately with 0.08 and 0.10% of yeast autolysate. Also, odor and taste were extremely like with 0.02%, 0.04%, and 0.08% yeast autolysate, while dislike moderately with ascorbic acid which also has been reported previously (Komthong *et al.*, 2007).

Table 3. Sensory evaluation of flesh apple juice supplemented with yeast autolysate and ascorbic acid

Samples	Color (9)	Odor (9)	Taste (9)	Overall (9)
Control	5.21±0.21	8.72±0.23	8.35±0.11	6.42±0.41
0.02% Yeast autolysate	5.83±0.15	8.61±0.16	8.13±0.32	7.22±0.29
0.04% Yeast autolysate	6.71±0.31	7.71±0.11	8.11±0.26	8.11±0.38
0.08% Yeast autolysate	7.52±0.34	7.12±0.44	7.89±0.25	8.12±0.41
0.1% Yeast autolysate	7.71±0.16	4.81±0.25	5.41±0.41	5.71±0.33
0.2% Ascorbic acid	8.92±0.43	3.62±0.33	4.22±0.37	4.73±0.24

The presented values are mean± *SD* obtained from 10 independent panelists. The scale was extremely like (9), like moderately (7), dislike (5), dislike moderately (3), and dislike extremely (1).

CONCLUSION

In the current work, *Saccharomyces cerevisiae* autolysate was prepared and its antioxidant properties were analyzed. The antioxidant contents of yeast autolysate were not affected at 40°C up to 30 min but treatment at ≥60°C had a deterioration effects on total antioxidant content. Yeast autolysate showed higher antioxidant, GSH, DPPH, and reducing power than flesh apple juice. Addition of yeast autolysate to flesh apple juice up to 0.08% could enhance its antioxidant properties, inhibit both enzymatic and nonenzymatic browning, and improve its sensory acceptability.

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دراسات علي انتاج مستخلص الخميرة الناتج بالتحلل الذاتي وخصائصه المضادة للاكسدة لتعزيز جودة عصير لب التفاح

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خميرة الخباز لها دوراً مهماً في الصناعات الغذائية وكذلك تعتبر من الكائنات البروبيوتيك الفعالة وذلك لارتفاع محتواها من المركبات النشطة الفعالة. لذلك يتم استخدامها كمضافات للأغذية في صورة طازجة ، جافة نشطة أو مستخلص غير نشط ، بينما هذه الاضافات الي بعض الاغذية تكون غير مقبولة حسياً. لكن يمكن التغلب علي تلك المشكلة عن طريق استخدام مستخلص الخميرة الناتج بالتحلل الذاتي للخلايا والذي يحتوي علي معظم المواد النشطة الفعالة الذائبة بالخلايا. لذلك تم في هذه الدراسة انتاج مستخلص الخميرة وتم فحص تأثير مراحل النمو علي المركبات الفعالة المتكونه فيه. ولقد أظهرت النتائج ان منحني النمو لخلايا الخمير له طورين نمو لوغاريتمي و أن اعلي طاقة اختزال وكذلك نشاط الكسح لمركب الـDPPH تم الحصول عليهم بعد طور النمو الاول (24 ساعة من التحضين) لكن اقصي انتاج لمضادات الاكسدة الكلية و الجلوتاثيون قد لوحظ في نهاية طور النمو الثاني (بعد 60 ساعة من التحضين). إضافة لذلك، لم تظهر المعاملة الحرارية للمستخلص علي درجة 40 °م حتي 30 دقيقة اي تأثير علي نشاط المركبات الفعالة به. لكن المعاملة علي درجة 60 °م فاكثر أدت الي انخفاض حاد في محتوي مضادات الاكسدة الكلية (40% اقل من الكنترول) وانخفاض قليل لكلا من الجلوتاثيون، قوة الاختزال، و الـ DPPH. وفي دراسة مقارنه بين المستخلص وعصير لب التفاح اتضح ان الجرعة المثبطة لـ 50% من الـ DPPH هي 5.6 مجم للمستخلص بينما كانت 20 مجم لعصير لب التفاح. وايضا احتوي المستخلص علي 53 ،6.11 ، و 2.73 مرة ضعف لـ الجلوتاثيون، مضادات الاكسدة الكلية، و قوة الاختزال، علي التوالي مقارنة بعصير لب التفاح. وقد اتضح ان إضافة 0.02% من المستخلص المعامل حراريا علي درجة 40°م لمدة 5 او 10 دقائق لعصير لب التفاح ادي الي انخفاض معامل التلون البني و الهيدروكسي مثيل فورفيرال بنسبة 12 و 18%، علي التوالي، وصل هذا الانخفاض الي 25 و 40% علي التوالي، عند مستوي إضافة 0.08% من المستخلص والذي كان مقبول حسيا. لذلك نستنتج ان اضافة هذا المستخلص الي عصير لب التفاح مقبول حسياً ، دعم خواصه المضادة للاكسدة وخفض تفاعلات التلون البني الانزيمي وغير الانزيمي به.

الكلمات الدالة : مستخلص التحلل الذاتي للخميرة – نشاط مضادات الاكسده– المعاملة الحرارية– عصير لب التفاح– التلون البني – التقييم الحسي.