Innovations

Ability of Baker's Yeast (*Saccharomyces Cerevisiae*) to Increase Apple Vinegar Production and to Suppress its Associated Fungi

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Abstract: *The production of apple vinegar through Baker's Yeast fermentation technique using three concentrations; 1%, 3% and 10% from fruit cubes or juice of three apple fruits: red fruit; Red Delicious, green fruit; Granny Smith and yellow fruit; Golden Delicious was evaluated for their suitability for vinegar production. The analysis was done using apple cubes and apple juice, then evaluated through several parameters including microbial growth and sensorial evaluation by color, odor and taste. More apple vinegar was produced from fruit juice than from fruit cubes and with higher quality and in a short time within 1 month compared to more than 80 days using cubes. The other concentrations of yeast (1% and 3%) are suitable where 1% yeast is the most suitable and was sufficient to combat undesirable formed fungal microflora that are associated with apple fruit and recorded in this study (Aspergillus niger, Penicillium digitatum, P. expansum, Alternaria solani, and Rhizopus stolonifer) The molds were found associated with apple fruit cubes or juice but their frequency was reduced gradually as the concentration of yeast was gradually increased to 10% to less than 5%. Among the three apple cultivars used, Golden Delicious gave better odor and taste than Granny smith or Red Delicious when apple juice was used as a substrate for vinegar production but when fruit cubes were used; Red Delicious was the best without obvious variation in the amount of vinegar yield.*

Keyword: *Baker's Yeast, Saccharromyces cerevisiae, apple cultivars, food industries.*

Introduction

Vinegar is an aqueous solution of acetic acid and trace chemicals that may include flavorings. Vinegar typically contains 5–8% acetic acid by volume. Usually, acetic acid is produced by the fermentation of ethanol or sugars by acetic acid producing bacteria, Acetobacter (Bhat et al., 2014). Human has been using vinegar as a condiment and food preservative for thousands of years. In recent years, vinegar consumption is increasing due to its antibacterial activity, its ability to lower blood pressure and to reduce cardiovascular diseases, also it has antioxidant activity and can promote nutrient metabolism (Akarca et al., 2020).

Apple is a horticultural fruit rich in vitamins, minerals, phenolic substances, organic acids and antioxidants (Budak et al., 2014). In the processing industry, apples are often used for the production of sauce and juice but can also be processed into other products such as cider and vinegar (Heikefelt, 2011).

Saccharomyces cerevisiae (also known as "Baker's Yeast") is a unicellular fungus responsible for alcohol production and bread formation (Moyad, 2008; Struyf, 2017). The use of *S. cerevisiae* in alcoholic fermentation converts sugars in fruits into acetic acid and that may improve the flavor and quality of apple vinegar, thereby indicating additional economic benefits of fermentation (Cousin et al., 2017).

At the normal case, production of apple vinegar occurs spontaneously via yeasts and bacteria naturally found on the surface of an apple fruit. The problem with the natural fermentation that the desirable yeast has to be at high concentration, if its amount is lower than the limit, there might be a risk to develop other microorganisms (molds and bacteria) unwanted which produce off-flavors and toxins (Morgan &Mosawy, 2016). Mycotoxins, as secondary metabolites of filamentous fungi e.g. *Penicillium*, *Alternaria* and *Aspergillus* that occur naturally in food and feed, were detected in apple vinegar especially patulin and ochratoxins (Fernández-Cruz et al., 2010). Apple vinegar produced through spontaneous fermentation is unpredictable and may contain mycotoxins that threaten human health. Furthermore, little information about the use of baker's yeast in vinegar fermentation and production and a few reports available about fungal microflora that can be associated with apple vinegar. Therefore, the objectives of this study:

1. To evaluate the effect of different gradual concentrations of Baker's yeast on apple vinegar fermentation and production (through natural acetobacter use).
2. To isolate and characterize fungal microflora associated with spontaneous apple vinegar production.

3. To analyze quantity and quality (sensory traits) of apple vinegar produced from different apple cultivars.

Materials and Methods

The following ingredients were used in this study: 3 types of commercial apple cultivars (Red: Cultivar red delicious /Green: Granny Smith / Yellow: Golden delicious) that are usually common and available in local Jordanian market (Figure 1), prepared aqueous solutions (1%, 3% and 10%) of Baker's yeast (Figure 2) bought from the local market (Astrico) and ready to use as treatments in vinegar production process. About 0.5 ml of each solution was used as a starter (Figure 3)

Figure (1):Selected Apple fruits; Green fruit: cv. Granny Smith, Red fruit: cv. Red Delicious, and Yellow fruit: cv. Golden Delicious.

Figure (2): Commercial Baker's bread yeast (*Sacharromyces cerevisiae*) used by the study.

Figure (3): Prepared aqueous solutions (1%, 3% and 10%) ofBaker's yeast used by the study.

To prepare Aqueous-yeast solutions, Baker's active dry bread yeast (*Sacharromyces cerevisiae*) was used, the yeast was obtained from the local market and prepared into three solutions at 1%, 3% and 10% w/v freshly prepared and used as starter treatments.

The three types of apple fruits were washed gently in the water, air-dried, chopped into small 2cm-in-diameter cubes (cubes) and mixed thoroughly with 5 ml of each yeast solution. After drainage of the access of solution, the cubes were packed into 250 ml-in-volume clear plastic containers. A negative control (distilled water alone) that does not contain yeast was used. The weight in grams of each container was taken using a laboratory top-loading balance.

The containers were labeled and assigned (four replicates per a treatment, 4 (Treatments + Control) X 4 Replicates X 3 Cultivars = 48 containers as experimental units) and then put into an incubator at a temperature of 27°C in darkness for about 60 days (Figure 4). No acetobacter culture was used. Acetobacter was expected to occur naturally thus visual observations were done to detect the occurrence of its

mother layer at the surface of produced vinegar. At the end of the experiment, apple vinegar was extracted by manual squeezing through sterile cheesecloth from each container and racked off to new bottles and vinegar yield was taken in milliliters The samples were then pasteurized at 77 °C for 10 min and cooled at room temperature to remove any traces of alcohol then were kept at room temperature.

Figur

Apple cubes experiment during fermentation.

Other apple fruits were first squeezed to obtain juice that was then mixed with the yeast at three concentrations 1%, 3% and 10% w/v and subdivided into 100ml flasks. A negative control that does not contain yeast was used (apple juice alone). Apple fruit juice was prepared by cutting apple fruit into small pieces by a sterile knife, grinding them into a blender and filtering the juice using a sterile cheesecloth. Three flasks per a treatment were used as replicates. All flasks were labeled (3 Reps X 4 Trts+Crl X 3 CVs = 24 experimental units) and put into an orbital incubator at an orbital shaking speed of 70 rpm and a temperature of 33 °C in darkness for three weeks with periodical checking. One milliliter volume from a commercial vinegar (at 5% concentration) product was then added into each flask. Then the flasks were kept at room temperature for one week. The samples were then pasteurized at 77 °C for 10 min (Wheeler et al,.1987) and cooled at room temperature to remove any traces of alcohol (Figure 5).

Figure (5)

Apple juice experiment during fermentation using an orbital incubator

The amount of apple cider vinegar produced after the fermentation process of apple juice was evaluated by calculating the vinegar in ml (ml) divided by the weight of the apple sample (g) and then taking the percentage of yield for the apple cubes experiment. The percentage of juice for each cultivar was taken. The percentage of vinegar yield was considered the same as the percentage of juice since no reduction in the amount of juice during experiment was expected.

For microbial analysis, Potato Dextrose Agar (PDA) medium was prepared and used for the isolation of fungi that may associated with apple fruit under the laboratory conditions. The fungal isolation was done by transferring one milliliter from vinegar (before the pasteurization procedure) with a sterile pipette and spreading it on the surface of a medium containing Petri dish. All culture Petri dishes were then incubated at an incubator in darkness at 25 °C for one week. Then cultured fungi were identified into Genera and Species using fungal description keys. Frequency of each isolated fungus found as a mold associated with vinegar in Petri dishes were counted and recorded.

The statistical analysis of obtained data was done using SPSS software. Completely randomized design (CRD) was used in the two experiments. Mean separation was done using LSD test using factorial arrangement. All two-tailed P-values of less than 0.05 were considered significant. Main effects and their interactions and simple correlation among measured parameters used in this study were statistically analyzed.

Results

The amount of apple vinegar was significantaffected by the type of treatment (Figure 6).There were significant differences in the amount of vinegar yield obtained using the gradual concentrations of baker's yeast. Ten percent of yeast gave the highest amount of about 36% vinegar from fruit cubes. However, the other two concentrations (1 and 3% yeast) gave higher vinegar yield but did not significantly different from that of the control. The results revealed a significant increase in amount of apple vinegar produced from fruit cubes by using 10% yeast, regardless of the type of apple cultivar (Figure 6). The amount of apple vinegar produced was ranged from about 27 to 36% depending on the cultivar or yeast concentration.

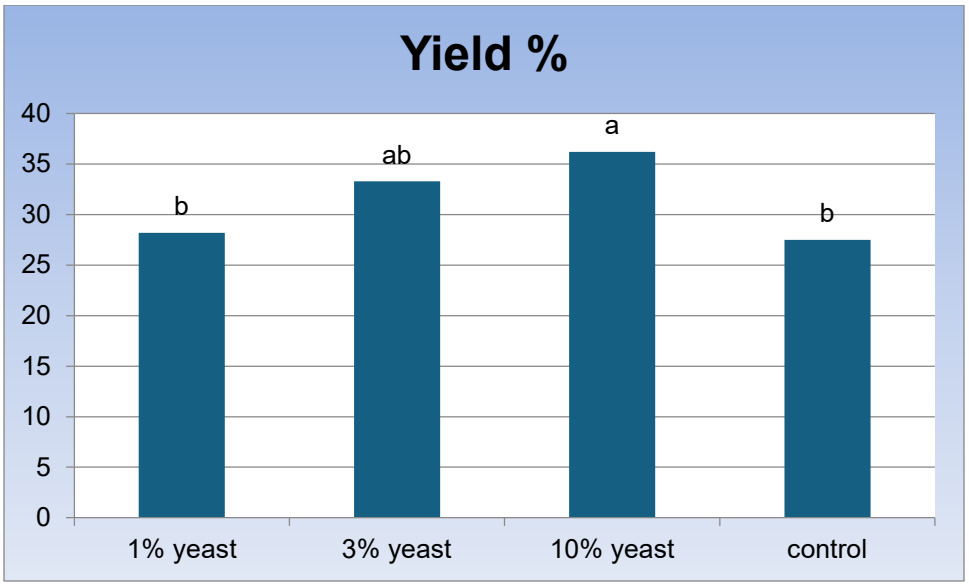

Figure 6: Amount of vinegar yield produced from fruit cubes by different treatments of bread yeast regardless the type of apple cultivars used.

The amount of apple vinegar (yield %) produced from fruit cubes and juice, respectively by using different types of apple cultivars regardless of the type of yeast treatment. The results indicated no significant differences in vinegar yield obtained from the different types of apple cultivars (data not shown). The average amount of vinegar produced was about 31% from cubes out 72 % from juice.

There were no significant differences in the amount of apple vinegar produced from apple juice using different yeast treatments. The amount of vinegar was almost similar to the amount of apple juice regardless the treatment or cultivar used.

Figure (7) indicates no significant variation in color of vinegar made from apple juice or fruit cubes between the three apple cultivars regardless of concentration of yeast added. The color slightly ranged from tan yellow to orange as shown in the control.

The sensorial analysis of apple vinegar (figure 8) showed significant reduction in the typical odor and taste of vinegar especially when 10% yeast was used for its production using either juice or fruit cubes. Using 1% yeast as a treatment did not significantly affect the odor and taste as there was no significant variation from the control.

Among the three applecultivar used, Golden Delicious gave better odor and taste than Granny smith or Red Delicious when apple juice was used as a substrate for vinegar production but when fruit cubes were used; Red Delicious was the best (Figure 8).

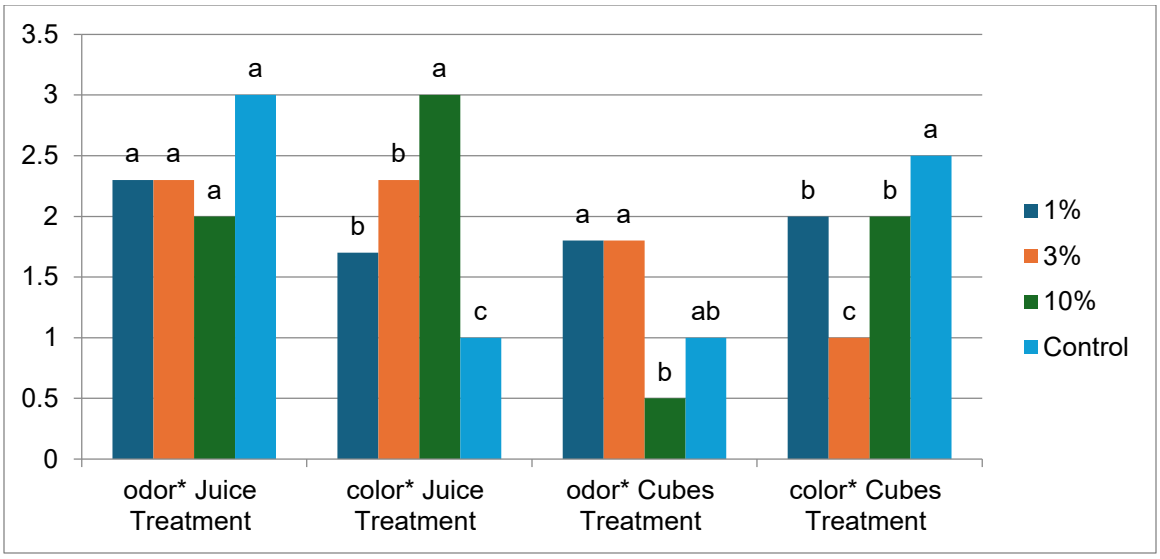

Figure (7)
The sensory analysis of vinegar with different treatments regardless of apple cultivar.

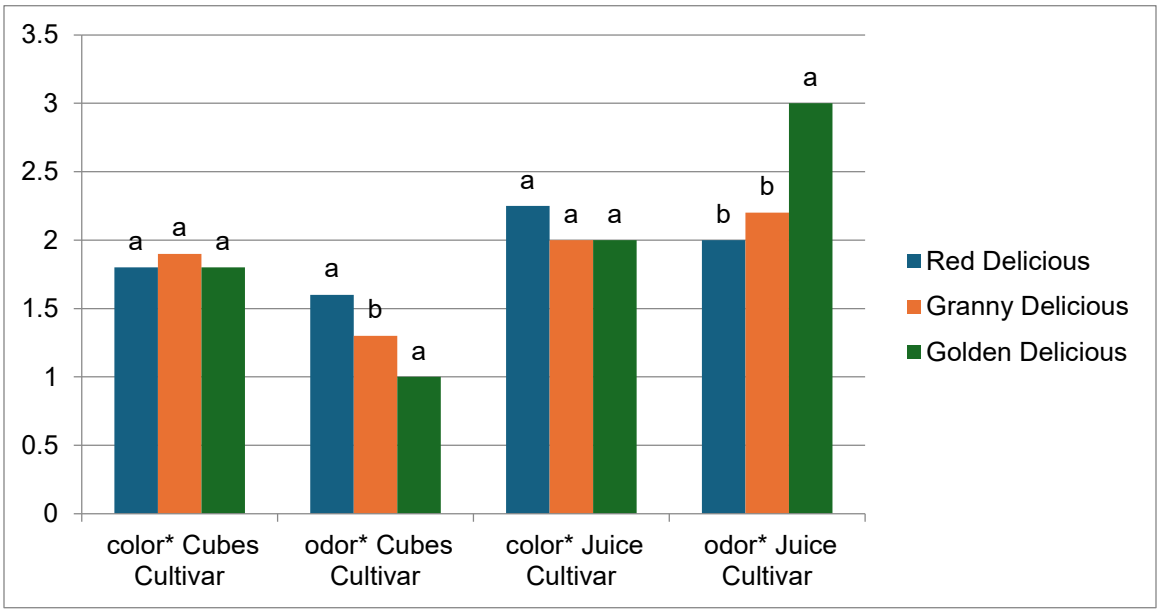

Figure (8)
The sensory analysis of vinegar with different cultivar regardless of treatments

Five types of fungal molds were found associated with apple fruit and was isolated and cultured on PDA medium during the process of vinegar production;*Penicillium degitatum*, *Aspergillus niger*, *Rhizopus stolonifer*, *Penicillium expansum* and *Alternaria solani*. All of these molds were successfully isolated from

vinegar produced from apple fruit cubes. The molds were found associated with apple fruit cubes or juice but their frequency was reduced gradually as the concentration of yeast was gradually increased to 10% to less than 5% (Table 1).

Table (1)

Frequency % of different molds found associated with vinegar production from apple fruit cubes or juice using different concentrations of Baker' yeast

	Mold frequency %			
	1% yeast	3% yeast	10% yeast	Control
Apple Cubes	34 b*	16 c	4 c	100 a
Apple Juice	11 b	3 b	1 b	30 a

*Means within the same row followed by the same letter are not significantly different at 0.05 probability level using LSD test.

Discussion

Vinegar is manufactured by using different processes and different materials (Hasan et al., 2021) and it is prepared by a two steps of fermentation process, ethyl alcohol and acetic acid fermentation of raw materials containing starch and/or sugar(Akarca et al., 2020), three cultivars of apple fruits in this study were used as a raw materials;(red fruit of cultivar Red Delicious, green fruit of cv. Granny Smith and yellow fruit of cv. Golden Delicious) and this type of fruit was used because apple is sugary food and one of raw materials used to produce vinegar (Bamforth, 2005). Baker's bread yeast (*Sacharromycescerivisiae*) was used as artificial treatment for fermentation instead of spontaneous fermentation by fungal microflora associated with apple fruit which produce off-flavors and toxins (Morgan &Mosawy, 2016). Mycotoxins, as secondary metabolites of filamentous fungi e.g. *Penicillium*, *Alternaria* and *Aspergillus* that occur naturally in food and feed, were detected in apple vinegar especially patulin and ochratoxins (Fernández-Cruz et al., 2010).

The first experiment was carried to manufacture vinegar from apple cubes when the amount of vinegar was affected by concentrations of yeast (*S. cerivisiae*), and the use of 10% yeast produced more vinegar than other treatments. This is due to increase an in alcohol amount where (*S. cerevisiae*) has an active role in alcoholic fermentation (Calugar, et al., 2021), as the alcoholic fermentation by yeast usually produces ahigher alcohol content than spontaneous fermentation in fruit (Ubeda et al., 2011).

The second experiment was carried out to produce vinegar from apple fruit juice where the amount of vinegar was similar to amount of juice but there was a

difference in the appearance of vinegar produced from apple juice. More apple vinegar was produced using fruit juice than using cubes and with higher quality and in a short time within 1 month compared to more than 80 days using cubes.

The other concentrations of yeast (1% and 3%) are suitable where 1% yeast is the most suitable and was sufficient to combat undesirable formed fungal microflora that are associated with apple fruit and recorded in this study (*Aspergillus niger*, *Penicillium digitatum*, *P. expansum, Alternaria solani*, and *Rhizopus stolonifer*). The sensory characteristics for vinegar produced from apple cubes were affected by different treatments because the different of processing techniques affected on the color of apple vinegar (Heikefelt, 2011) and the difference the color of vinegar attributed to influence the color of raw materials and chemical reactions during preparation (Liu et al., 2019), as the color degree of vinegar when using different concentrations of Baker' yeast was less than the color degree of vinegar when using spontaneous fermentation by fungal microflora, and the odor was affected by the different treatments and when using 10% yeast as a treatment, the typical odor of vinegar was not obvious. No variation in color of vinegar made from apple juice or fruit cubes between the three apple cultivars regardless of concentration of yeast added. The color slightly ranged from tan yellow to orange. The yeast treatment makes the color of produced vinegar reddish brown especially at 10% concentration. Notice that when the percentage of yeast increases, the odor of vinegar decreases, as the difference in the preparation process led to the difference in the odor of vinegar, and this is explained by (Morales et al., 2001) the

The frequencies of different molds found associated with vinegar production from apple fruit cubes or juice using different concentrations of Baker' yeast was reduced gradually when the concentration of yeast increased due to its ability to completely inhibit their growth. Baker's yeast was found effective in controlling blue mold disease of apple fruit during its cold storage (Karajeh et al., 2018).

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