Canadian Journal of Applied Sciences. 1(3): 399- 412; January, 2013
ISSN 1925-7430; Available online http://www.canajas.ca

Original Research Article

INHIBITORY ACTIVITY OF ALLYL ALCOHOL DERIVED FROM ALLIIN IN GARLIC AGAINST FOOD BORNE PATHOGEN CANDIDA ALBICAN

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ABSTRACT

The objectives of the present study were to evaluate the antimicrobial activity of allyl alcohol produced by the thermal decomposition of garlic against food borne pathogen *Candida Albicans*, and to find out the minimum inhibitory concentration of allyl alcohol in heated garlic. The initial concentration of the organism **"***Candida Albicans"* was estimated by direct microscopic count (DMC) method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests were performed to observe the antimicrobial activity of allyl alcohol-a product of garlic, against *Candida Albicans* at different pH levels and heating times such as: pH 4 and heating time 90 min, pH 4 and heating time 30 minutes, pH 8 and heating time 90 minutes, and pH 8 and heating time 30 minutes respectively in the YMPG broth and PDA agar, by incubating at 35^0C for 24 hours. Similarly HPLC method was used to determine the amount of extracted product of garlic - allyl alcohol at different pH levels. It has been observed that allyl alcohol showed comparatively more anti yeast activity at pH 4, with heating time 90 minutes, which is 1.56% at dilution 1/64. Similarly generation of allyl alcohol from heated garlic extract by HPLC test was found maximum at pH 4, and time 90 minutes that is 8.05 % as compared to other three combined variables of pH, and time. The findings of present study clearly met with the hypothesis that allyl alcohol in heated garlic had anti yeast activity, it was able to inhibit the growth of pathogen such as *Candida Albicans*, by extending heating time at low pH level.

Keywords: antimicrobial activity, allin, allyl alcohol, garlic, *Candida Albicans*,

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INTRODUCTION:

The development of food production methods and sensitive techniques has comparatively minimized microbial contamination in commercial products. However, the numbers of disease outbreaks caused by food products have not decreased; even through a lot of research had been conducted to ensure the increased cleanliness of food products. The outbreaks of food borne illness are still an increasingly important public health problem. Food borne pathogens cause the highest disease burden, The results of a study conducted by Hara-Kudo Y, et al (2012) showed the prevalence of the food borne pathogens in the retail food supplied in Japan, indicating the consumption of raw food associated with risk of contracting food-borne infections. Most of the food borne diseases is periodic and often not reported. These disease outbreaks may take on massive proportions. An outbreak of salmonellosis due to contaminated ice cream occurred in the USA in 1994, affecting an estimated amount of 224,000 peoples. Earlier an outbreak of hepatitis A, in 1988, resulting from the consumption of contaminated clams, affected some 300,000 individuals in China, Facts Sheet N^0 237 (2007). Similarly *E.coli* O157 and listeriosis are important food borne diseases which have emerged over the last decades. Although their incidence is relatively low, their severe and sometimes fatal health consequences, particularly among infants, children and the elderly, make them among the most serious food borne infections.

Many natural products including plants, herbs, and certain foods containing antimicrobial substances have been studied for their antimicrobial activity. These antimicrobial agents can be categorized as naturally occurring compounds and synthetic substances.

Chung I, *et al.,* (2007), evaluated the synergistic activity of garlic oil and Allyl alcohol derived from alliin from Alliin garlic. This study shows that garlic, Allium sativum possess significant antimicrobial activity which leads us to further investigate for the confirmation of this activity against food borne pathogens. Katey, M.L, *et al*., (2005), in a similar kind of study mentioned that garlic was cultivated in India and China before Sumerian civilization and folk used it for spice and medicine.

Earlier researchers Doyle, P.M, et al,. (2001) studied the antibiotic activity of the garlic and found that the growth of both gram positive and gram negative food borne bacteria yeast and mold can be inhibited by garlic, onion, cinnamon. This study proved that garlic and onion possess most antimicrobial activity as compared to other natural herbs. The same study showed that growth and toxin production of many organisms, bacteria, yeast and mold inhibited by the garlic.

Antimicrobial and antibiotic properties have been more extensively studied as compared to its other health benefits.

Inhibitory activity of essential oil of garlic and onion against bacteria and yeast were further investigated by Kim, J.W, *et al,*. (2003). The study shows that garlic possesses antibiotic property due to volatile sulfur compounds derived from S-Allyl – L- Cysteine sulf oxide, a non protein amino acid formed in the vegetable by the action of an enzyme cysteine sulf oxide lase. Alliin (S-Allyl – L- Cysteine sulf oxide) the major S-alkenyl –L-Cystein sulfoxide in garlic is degraded into Allicin (Allyl 2- propenethiosulfnate). The Alliin and Alliinase reaction occurs in injured or crushed garlic.

Choi, M.K, et al,. (2007), conducted a similar kind of study and indicated that Alliin is although first known natural antibiotic agent in garlic, however, other compounds in garlic also exhibit antimicrobial activity such as: acrolein and related aldehyde and derivative of allicin , ajoene also exhibit antimicrobial activity.

The present study is focused over the ant yeast potency of heated garlic due to generation of allyl alcohol from Alliin. The similar work was done earlier by Kim, J. W., *et al,*. (2006). In this research study garlic was heated at 121^0C for 45 minutes or more, then it became a potent growth inhibitor for yeast. The chemical change occurs as the allinase enzyme is denatured due to boiling and alliin (S-allya-L-cysteine sulfoxide) is thermally converted to allyl alcohol, (2-propen-1-ol). Allyl alcohol was found to be 100 to 1000 times more potent against yeast than against bacteria. Allyl alcohol is not a sulfur containing compound like other known antimicrobial compounds in garlic.

Earlier study conducted by Chung, I., et al., (2007), showed that heating of garlic up to 121^0C for 15 minutes is believed to produce the sulfur containing compounds which are to be the primary antimicrobial compound of the garlic. However, ant yeast compound in garlic following prolonged heating is allyl alcohol.

Lemar, K.M., *et al,*. (2005), mentioned an interesting findings that allyl alcohol and garlic (*Allium sativum*) produce an oxidative stress in *Candida Albicans.* Similarly Lemar, K.M., *et al,*. (2007) indicated that diallyl disulfide is an agent triggers the cell death in *Candida.*

Kim, J.W, (2006), mentioned that boiled or autoclaved garlic has lost its germicidal activities in the type of study conducted against bacteria. However the antimicrobial activity of allyl alcohol was tested on eukaryotic microorganisms such as *Candida Albicans* (Harris *et.al*, 2000). The minimum inhibitory concentration (MIC) of Allyl alcohol for the growth of *Staphylococcus aureus* was found to be 6.0%, which is about 3000-fold more than the MIC for *Candida utilis* (0.002%). Kim, J.W., (2006) evaluated that "Allicin" is easily degradable by cooking and ageing process while Allyl alcohol is thermally stable compound, not destroyed by heat or ageing process. While "Allicin" is unstable on heating during a cooking as well as ageing process So, allyl alcohol is a potent naturally occurring ant yeast compound and heated garlic could be used as a natural food preserve.

Kim, J.W, *et al,*. (2004), expressed the findings of their study that yeast can spoil soft drinks and other foods. It has been proved that the growth of yeast is easily controlled by chemical preservatives such as sorbic or benzoic acid, however, consumers prefer natural preservatives for the food safety that enhance the use of plant material as natural food preservatives.

The objectives of the present research study are to evaluate the ant yeast activity of allyl alcohol produced by the thermal decomposition of garlic at different temperature, time, and pH value, and to find out the effects of allyl alcohol against important yeast eukaryotic organism such as: *Candida Albican.*

The Organism *Candida Albicans* has genus *Candida*, contains a number of species, however, *Candida Albicans is among the species that most frequently causes infections* (Candidacies**).** *Candida* occurs in small number in the elementary track mouth, and viginal area. *Candida* is capable of invading every tissue and cavity of the body after infection. These are infections of the skin, vagina, oral cavity, eye, liver and brain. It appears usually as oval yeast like cell. It reproduces by budding. In the infected areas, filamentous hyphae and pseudo hyphae may also

be observed. It is grown at 25 – 35 degree Celsius on Sabouraud's glucose agar. *Candida Albicans* is a diploid fungus (a form of yeast), which is capable of mating but not of meiosis. Rich Winters et al,. (1991).

Direct detection of yeast is possible by direct microscope method. Culture and identification is not usually cultured in the clinical laboratory. Rapid screening test is available on commercial level such as: germ tube test, API -20 C aux yeast identification system, uni-yeast teak system chromogar, Rapid yeast plus system etc,. Biochemical test of the yeast are not available to perform at laboratory level. Forbes B. A., *et al.*, (1998).

YMPG broth (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose) was used for culture, dilution and MIC determination of yeast. YMPG agar was used for colony isolation from original culture through streak plate. PDA agar has been used for determination of colony count for used culture. Phosphate buffer was used for serial dilution of the organism to find the expected count.

The "Minimum Inhibitory Concentration (MIC)" and "Minimum Bactericidal Concentration (MBC)" are experimental methods which were performed in the present study. MIC method was applied to find out the minimum concentration of allyl alcohol, used to inhibit the growth of microorganism. Minimum inhibitory concentration method is a standard against which other methods are assessed. MIC Method uses the lowest concentration of antimicrobial agent that inhibits the visible growth. The use of MIC methods in clinical laboratories is to test the susceptibility of organisms which give equivocal results in diffusion tests, (Collins C.H et al., 1999). In this method, the detection of mixtures is facilitated and isolated colonies are made available for retesting in pure culture. MIC can be employed to determine effective concentration of antibiotic or disinfectant for preventing microorganism's contamination. However, "minimum bactericidal concentration (MBC)" method is a tool for the confirmation of the MIC test. MBC test was performed by applying a loopful streaked from serial diluted tubes on the agar plate divided in several parts. The plates were incubated and microbial growths were checked. Any growth of microbes indicated the negative inhibition. (Seligy, V.L., and Rancourt, J.M., 1999).

High Pressure Liquid Chromatography (HPLC) is the most widely used analytical separation technique. This method is popular because of its non-destructive nature and may be applied to thermally labile compounds. High Pressure Liquid Chromatography (HPLC)) technique was used for the determination of amount of Alliin in heated garlic and Allyl alcohol thermally generated from Alliin. The "Waters Scientific HPLC with C-18 column and UV detector" was the HPLC machine used in the present study. The column (stationary phase) separated the ingredients based upon their polarity, size and other easily understood properties. By changing the different column in a single machine the analysis could be used very fast, and accurately. Here the C-18 column (long C-chain molecules, octadecylsilyl coated silica) was used as non-polar stationary phase and methanol: water was used as polar phase respectively. So, separation was based upon polarity. More polar molecules leave first and less polar molecules leave later (Reverse Phase HPLC). The wide applicability of HPLC as a separation method makes it a valuable separation tool in many scientific fields. (Graham S, 2009).

Literature search has shown a few amount of work done to evaluate the antimicrobial activity of allyl alcohol against food borne pathogens. Kim J.W et al,. (2006), mentioned about the previous research beliefs that thiosulfonate is the principal antimicrobial agent of garlic. However, further research studies are being conducted to find out the antimicrobial and anti yeast activity of Allyl

alcohol. Therefore, the present study was taken to evaluate the anti yeast activity of heated garlic against food borne yeast *Candida Albicans* at different heating time and pH levels.

MATERIALS

Materials:

A. Materials and their Function

a) Garlic:

Whole garlic (*Allium sativum* L.) bulb (200 g) already peeled off was washed with sterile distilled water. The cloves were heated for garlic extract preparation, which are source of anti - yeast compound Allyl alcohol.

b) Yeast Culture

The organism *Candida Abican* (yeast) was used in the present study. The culture was collected from Biotechnology - Microbiology Laboratory of Centennial College, HP center.

c) Other Materials

Media

- YMPG Broth – a special media for broth culture of *Candida Albican yeast*
- YMPG Agar - a special growth media for culture of *Candida Albican yeast*
- PDA (Potato Dextrose Agar – a special growth media)

Chemicals

❖ Phosphate (PO_4) buffer - used for serial dilution

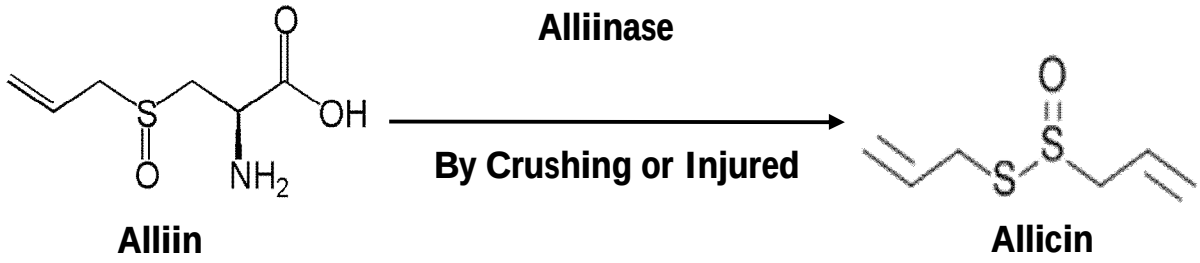
- Alcohol (70%) – used for sterilization of hockey stick
- Sterile distilled water – used for preparation of sample and standard solution
- Allyl alcohol – Antiyeast compound under study extracted from heated garlic
- HPLC grade Methanol and water – used for preparation of mobile phase
- 1M solution of Sodium hydroxide (NaOH) – applied to adjust the PH of Allyl alcohol
- 1M solution of Hydrochloric acid (HCl) - applied to adjust the PH of Allyl alcohol

Equipments

- HPLC (High performance liquid chromatography) – an instrument used for analysis of various compounds
- Centrifuge – a process for separation
- Autoclave - a device to sterilize equipment and supplying to high pressure steam
- Blender - a device to blend and helping in extract formation

- pH meter – an instrument to check pH value

The source used for preparation of media was: (Diffco ™ & BBL Manual 2003).



Chemical reaction showing conversion of Alliin to Allyl alcohol

Standard And sample preparation for HPLC:

Standard Preparation:

Standard solution concentration will be 5.0%, prepared from 100% allyl alcohol mixing with HPLC Grade water.

Sample Preparation:

Sample will be prepared (100% Sample) by filtering with 0.22 µ filter and "Sea-Pak" C-18 cartage.

Chemical reaction showing conversion of Alliin to Allicin

METHODOGY

1. Serial Dilution of the Organism *"Candida Albicans"*

The initial concentration of the organism ***"****Candida Albicans"* was estimated by the instrument haemocytometer – direct microscopic count (DMC). In order to find out the exact cfu / ml, serial dilution was performed and spread plate count was done. Phosphate buffer was used for serial dilution.

2. Minimum Inhibitory Concentration Method (MIC) (Lennette, E.H, et al., 1974)

1. Prepared a 48 hours culture of microorganism. Used 10 ml of YMPG media and inoculated the isolated colony of microorganism
2. Prepared YMPG broth

3. Made a serial dilution of Allyl alcohol extracted from heated garlic, using 08 sterile tubes, with ½ dilution method. Added 5 ml of broth to each tube
4. Divided each dilution in three tubes. Two tubes were used to test microorganism by duplicate. The third tube was the negative control
5. Added inoculums to each tube (0.1 ml aliquot to each tube).
6. Made a positive control (broth + distilled water + inocula). Made a negative control (broth + allyl alcohol)
7. Incubated test and controls tubes for 48 hours at 35 C^0
8. After incubation examined the tubes for growth (MIC)
9. Streaked tubes without growth in YMPG media (MBC)
10. Incubated the plates for 24 hours at 35 C^0, read the highest dilution with no growth along the streak line.

3. HPLC Technique for Analysis of Allyl alcohol

HPLC test was performed to find out the amount of allyl alcohol in the different samples with different variables like temperature and pH value. In HPLC method the column (Stationary phase) separated the ingredient (allyl alcohol) based on its polarity. Here the column is made up of C-18 (long Carbon-chain molecules, octadecylsilyl coated silica) which is non-polar. Allyl alcohol is a polar component of the mixture. More polar molecules left first and less polar molecules left later (Reverse Phase HPLC). A commercially available GPC column (Jaigel W252 column, 50cm × 2cm inner diameter) was used and mobile phase was water/methanol (50/50) acting as an eluting solvent at a flow rate of 1 ml/min. The injection volume was 10 µL of the sample. The pressure recommended was 1200-1400 psi with retention time 10.0 min. A UV detector was used to identify the Ally alcohol at 230 nm spectrum. Kim J.W, (2006).

The amount of allyl alcohol in different samples was determined by applying the formula given below:

$$\frac{\text{Concentration of Standard}}{\text{Peak of Standard}} = \frac{\text{Concentration of Sample}}{\text{Peak of Sample}}$$

RESULTS

I) Results of Control Test for the Media Used

The control test was performed for all the media. The media YMPG Agar, YMPG broth, YMPG Broth and *Candida Albicans*, Potato Dextrose Agar (PDA), and YMPG Broth + Allyl alcohol solution, were incubated at 35^0C temperature for 48 hours. They did not show contamination. The results are given below in Table 1.

All media was tested for control test. No growth was observed in YMPG Agar, YMPG broth, Potato Dextrose Agar (PDA), and YMPG Broth and Allyl alcohol solution respectively. However, YMPG Broth and *Candida Albican* showed positive result with the appearance of turbidity

II) Results of Yeast Strain and Culture Condition

YMPG broth (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose) was used for broth culture, preparation of *Candida Albicans.* Streak plate was prepared by using YMPG agar for isolation of colony from original culture. The plates were incubated at 35^0C for 24 hours. Therefore, YMPG broth and YMPG agar are special media for growth and culture conditions of *Candida Albicans (yeast).* Results of the Yeast Strain and Culture Condition are shown in the table # 2, below:

Table 1. Showing the results of all the control tubes of broth and plates of agar, observation of growth of *Candida Albicans* and of Allyl alcohol solution incubated at 35^0C for 24 hours.

Control Media	Observation
YMPG Agar	No growth
YMPG Broth	No growth
YMPG Broth + *C. Albicans*	Growth (Turbidity)
Potato Dextrose Agar (PDA)	No growth
YMPG Broth + Allyl alcohol solution	No growth

Table 2. Showing the results of streak plate on YMPG agar incubated at 35^0C for 24 hours.

Name of Organism	Colony morphology
Candida Albicans	Creamy white colonies, some of which were flatter, and drier.

Streak plate on YMPG agar incubated at 35^0C for 24 hours showed creamy white colonies, some of which were flatter, and drier.

III) Results of Serial Dilution of the Organism *"Candida Albicans"*

The initial concentration of the organism ***"****Candida Albicans"* was estimated by the instrument haemocytometer – direct microscopic count (DMC). The method serial dilution was performed to find out the exact cfu / ml, and spread plate count was done. Phosphate buffer was used for serial dilution.

IV) Results of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods

Minimum Inhibitory Concentration (MIC) test was applied for the organism *Candida Albicans* by using the half dilution method. A set of eight test tubes was used in which 5 ml YMPG broth was added in each test tube. The half dilution method was performed so that the eight tubes had

dilution from 1/2 to 1/265 and concentration of the heated garlic extract was 50% to 0.39%. The each tube was inoculated with 0.1 ml culture of *Candida Ablicans*. Tubes were incubated at 35^0C for 24 hours. Turbidity showed the growth of organism, and clear solution did not show any growth of the organism.

Table # 3. Showing the results of serial dilution of organism *Candida Albicans*.

Organism	Initial concentration by DMC (cfu / ml)	Spread plate count
Candida Albicans.	4.4×10^7	6.0×10^6

Table 4. Showing the results of MIC and MBC tests with four combinations of pH and heating time for *Candida Albicans,* in the presence of allyl alcohol at 35^0C for 24 hours.

Concentration of Allyl alcohol reducing by ½ dilution method	pH 4 with Heating time 90 min.	pH 4 with Heating time 30 min.	pH 8 with Heating time 90 min.	pH 8 with Heating time 30 min.
	Minimum Inhibitory Concentration (MIC)			
Candida Albicans	1 / 64 1.56%	1/16 6.25%	1/32 3.12%	1/8 12.5%
Minimum Bactericidal Concentration (MBC)				
Candida Albicans	1 / 64 1.56%	1/16 6.25%	1/32 3.12%	1/8 12.5%

Minimum Bactericidal Concentration (MBC) test was performed, potato dextrose agar (PDA) plate was used. The plate was divided into eight parts by marker. One loop full of solution taken from each tube was streaked on the PDA plate with in the specific marking area. The Plate was incubated at 35^0C for 24 hours. The growth showed presence of organism however, the clear area indicated there was no growth of the organism *Candida Albicans*. Result for MIC and MBC tests have been shown in the Table # 4.

The result of minimum inhibitory concentration (MIC) test with four combinations of pH and heating time for *Candida Albicans,* in the presence of allyl alcohol are given above in table # 4. The minimum inhibitory concentration with combination pH 4 and heating time 90 minutes observed was 1.56 %. The minimum inhibitory concentration with combination pH 4 and heating time 30 minutes observed was 6.25 %. The minimum inhibitory concentration with combination pH 8 and heating time 90 minutes was found 3.12 %. The minimum inhibitory concentration with combination pH 8 with heating time 30 minutes was observed as 12.5 % respectively. Similarly MBC test with four combinations of pH and time showed the same results for *Candida Albicans* as observed in MIC tests.

v) Results for HPLC test

HPLC test was performed for the following four treatment combinations: pH 4 with 90 minutes heating time, pH 4 with 30 minutes heating time, pH 8 with 90 minutes heating time and pH 8 with 30 minutes heating time respectively. HPLC test was carried out to determine the amount of allyl alcohol present in heated garlic extract. The garlic extract was heated for 30 and 90 minutes by autoclave along with 4 and 8 pH values respectively. The heating process converted the alliin present in heated garlic extract into allyl alcohol, the compound that inhibits the growth of yeast at very low concentration. The amount of allyl alcohol present in the heated garlic samples was determined by HPLC method. The mobile phase was combination of HPLC grade methanol and water with the ratio of 50:50. A 0.5 % standard sample of allyl alcohol was prepared and the amount of allyl alcohol in the unknown samples was determined by comparing with standard samples. Retention time and peak height of standard and samples with different treatment combinations has been shown in the tables from 5 – 6 given below:

Table 5. Showing the retention time and peak height of standard of Allyl alcohol.

S.N	Peak Name	RT	Area	% Area	Height
1		0.568	7578	12.56	777
2	Allyl alcohol LSf 09	1.471	52749	87.44	5086

Analysis results of standard of Allyl alcohol by HPLC method shown in table 5, indicate that retention time (RT), Area, % Area, and Height of the Allyl alcohol were 1.471, 52749, 87.44, and 5086 respectively.

Table # 6. Showing the retention time and peak height in different samples produced from heated garlic extract after different treatment combinations (pH and heating time) originated by HPLC method.

Different Combinations of pH with Heating time	Retention Time (min.)	Peak height for sample	Amount of Allyl alcohol (%)
Standard Sample (0.5% of Allyl alcohol)	1.471	5086	-
pH 4 with Heating time 90 min	2.270	81904	8.0519
pH 4 with Heating time 30 min	2.001	38710	3.8055
pH 8 with Heating time 90 min.	2.147	48506	4.7685
pH 8 with Heating time 30 min.	2.343	809	0.079

HPLC test results (table 6) showed that the maximum amount of allyl alcohol observed in the sample at pH 4 with 90 min heating was (8.05%), which was followed by 4.77% at pH 8 with 90 min. heating time. Similarly the amount of allyl alcohol determined at pH 4 with 90 min was 3.80%. The lowest amount of allyl alcohol was observed at pH 8 with 30 minutes heating time that was 0.079% respectively.

DISCUSSION

The ant yeast activity of Allyl alcohol produced by the thermal decomposition of garlic was evaluated against *Candida Albicans.* Streaking on YMPG agar was performed for the identification of the microorganism *Candida Albicans.* The microorganism showed creamy white colonies, some of the colonies were flatter and drier see table 1. Similar work was performed by Si H et al, (2013) and Rich Winters et al,. (1991).

The ant yeast activity of allyl alcohol produced from the heated garlic extract by thermal generation was determined by Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods. MIC and MBC tests were performed for the microorganism (yeast) *C. Albicans.* The experimental work was conducted with the combination of two variables of pH and time. The work proceeded with the four combinations such as: pH 4 and heating time 90 minutes, pH 4 and heating time 30 minutes, pH 8 and heating time 90 minutes, pH 8 and heating time 30 minutes respectively. The comparison of the results for the four combinations of "pH and time" has been shown in table # 4.

The result of the first combination at "pH 4 and heating time 90 minutes" shown in table 4, for MIC and MBC tests indicate that minimum inhibitory concentration of Allyl alcohol against *Candida Albicans* was 1/64, that is 1.56% concentration of allyl alcohol. Similarly MBC test showed the same result for *Candida Albicans* as observed in MIC test.

The result of the second combination at "pH 4 and heating time 30 minutes" for MIC and MBC tests given in table # 4 indicate that minimum inhibitory concentration of Allyl alcohol against *Candida Albicans* was 1/16, that is 6.25% concentration of allyl alcohol. Similarly MBC test showed the same result for *Candida Albicans* as observed in MIC test.

Table 4 is showing the results of the third combination at "pH 8 and heating time 90 minutes" for MIC and MBC tests. MIC results indicate that minimum inhibitory concentration of Allyl alcohol against *Candida Albicans* was 1/32, that is 3.12% concentration of allyl alcohol. Similarly MBC test showed the same result for *Candida Albicans* as observed in MIC test.

The result of the fourth combination at "pH 8 and heating time 30 minutes" for MIC and MBC tests are shown in table # 4. MIC results indicate that minimum inhibitory concentration of Allyl alcohol against *Candida Albicans* was 1/8, that is 12.5% concentration of allyl alcohol. Similarly MBC test showed the same result for *Candida Albicans* as observed in MIC test. It has been observed that allyl alcohol showed comparatively more anti yeast activity at pH 4 and heating time 90 minutes that is 1.56% at dilution 1/64. The results of the present research study show that more effective anti yeast activity was generated when pH of the heated garlic extract was adjusted to acidic state at pH 4 and heating time extended to 90 minutes. The present study results were supported by the similar kind of study conducted by Kim J.W et al,. (2006). Similarly the next level of maximum anti yeast activity of allyl alcohol was observed at pH 8 and heating time 90

minutes that is 3.12% at dilution 1/32. This result indicates that allyl alcohol is the anti yeast compound in heated garlic extract that is generated at extended heating time 90 minutes at pH 8. These findings are supported by another research conducted by Choi, J.H, et al,. (2005), mentioning that allyl alcohol is generated through thermal decomposition of Alliin in garlic and observed that yeasts was found very sensitive to allyl alcohol with MIC method.

Similarly findings of present study are favored by Lemar, K.M., *et al*,. (2005), describing that Allyl alcohol and garlic (*Allium sativum*) produce an oxidative stress in *Candida Albicans.*

HPLC test was performed for the four treatment combinations: pH 4 with 90 minutes heating time, pH 4 with 30 minutes heating time, pH 8 with 90 minutes heating time and pH 8 with 30 minutes heating time respectively. HPLC test was carried out to determine the amount of allyl alcohol present in heated garlic extract. Table 5, is showing the results of standard of allyl alcohol. HPLC test for the above mentioned four treatment combinations has been shown in table 6. Table 6 is showing the comparative results of all the four treatments. The generation of allyl alcohol from heated garlic extract was found maximum at pH 4 with 90 minutes heating time that is 8.05 %. The second highest concentration of allyl alcohol was obtained at pH 8 with 90 minutes heating time that is 4.77% respectively. The lowest concentration of allyl alcohol was obtained at pH 8 with 30 minutes heating time that is 0.079%. The results of HPLC indicate that low pH and extended heating time have great impact on generation of allyl alcohol from Alliin in garlic. The results of the present research project are supported by Choi, J.H, et al,. (2005), and Kim J.W et al,. (2006), who mentioned their findings that low pH level and higher heating time convert more amount of alliin to allyl alcohol which shows the maximum inhibition of the microorganism *Candida Albicans*.

CONCLUSION

The findings of present research study clearly met with the hypothesis and showed that formation of ant yeast compound allyl alcohol was positively influenced by extending heating time at low pH.

The yeast inhibition by allyl alcohol occurred when allyl alcohol was thermally generated from alliin at low pH and extended heating time. Findings of the present study lead to the following recommendations concluded as:

- The acidic state at low pH level and higher heating time convert more amount of alliin to allyl alcohol which shows the maximum inhibition of the organism *Candida Albicans* that is 1.56%.
- The higher pH level at pH 8 and less heating time 30 minutes showed the minimum inhibition (12.5%) that indicates the lower amount of allyl alcohol (0.079%) was generated from Alliin.
- Allyl alcohol has more ant yeast activity and could be used as natural preservative in food industry.

FUTURE DIRECTIONS

- Comparative study of antimicrobial activity of allyl alcohol using gram positive and gram negative bacteria could be included in future.

- Synergistic ant yeast activity of alliin and allyl alcohol derived from alliin in garlic could be checked

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