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Use of biopreservation technique to increase shelf life for some types of meat

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The results showed that the hot aqueous extract of Welsh onion *Allium fistulosum* L include flavonoids, terpenes, glycosides, saponins, alkaloids and tannins, killer toxin was purification from commercially bakery yeast *Saccharomyces cerevisiae* through Precipitation 30-70% (NH₄)₂So₄ with inhibition zone 26 and 22 mm for *Escherichia coli* and *Staphylococcus aureus*, respectively, and Sephacryl S-200 with inhibition zone 31 and 27 mm for *E. coli* and *S. aureus*, respectively. The highest inhibition zone of *E. coli*, *Salmonella ssp.*, *Pseudomonas aeruginosa* and *S. aureus* was 32, 30, 23, and 26 mm respectively, at a rate of 28 mm for Welsh onion extract (50%) + killer toxin (50%) which use for extending Shelf life and of minced beef, The total bacterial count for control was 8.2×10², 9.1×10² and 9.8×10² CFU.g⁻¹, while it be 8.2×10², 3.4×10² and 2.1×10² CFU.g⁻¹ for treated minced beef by Welsh onion extract (50%) + killer toxin (50%) during storage period for 0, 3 and 6 day at 4°C. the peroxide value for control was 2.2, 3.1 and 4.3 mEq/kg while it be 2.2, 2.9 and 3.8 mEq/kg for same treated and storage period.

Keywords: Bio-preservation, bioactive compounds, *Allium fistulosum* L, killer toxin, *Saccharomyces cerevisiae*, extending shelf life, minced beef, total bacterial count, peroxide value

INTRODUCTION

Due to the increase in consumer awareness and the community's interest in the toxicity of chemicals added to food to extending shelf-life, which was indicated by some studies related to food safety (Al-Soufi, 2015; Alsoufi and Aziz, 2021), the global interest was increased to find alternative safe sources for use in food industry (Alsoufi and Aziz, 2019), such as some plants and herbs that began to be widely used in many foods and their products, as use of marjoram (*Origanum majorana* L.) leaves extract as a preservative agent of beef meat (Oribi, 2017), or potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* L.) peels extract to extending shelf life of Awassi lamb meat during stored in low temperatures (AL-Masri, 2016), or pomegranate (*Punica granatum* L.) peel extract for improvement quality and chemical characteristics for minced frozen chicken meat (Al-Qazzaz, 2014).

Presently, herbal plants extracts were use in wide type of industrial application such as food preservation due to their content of bioactive compounds (Aziz and Al-Niaame, 2013; Al-Niaame and Aziz, 2013). Welsh onion *Allium fistulosum* L. have been used in classical

medication since old times due to phytotherapeutical (antifungal, antimicrobial, anti-inflammatory and antioxidant) properties represented by the presence of many bioactive compounds such as polyphenols, flavonoids, polycarboxylic acids, allicin, sterols, ajoene and alliin which are responsible for the biological effects of this plant (Tigu et al. 2021)

Killer toxin is a type of proteins that produce from *Saccharomyces cerevisiae* which is used to inhibition or retard the growth of other microorganisms, this substance considered safety to consume from consumer due to the absence of toxicity in the products of this yeast and its use mainly in the manufacture of bread and pastries, these deadly substances were used to prolong the shelf life of food (Alsoufi and Aziz, 2017a; Alsoufi and Aziz, 2017b).

the oxidative rancidity in meat it responsible for unacceptable taste, flavor and change in color that result in losing of the nutritional value of meat and its effect on consumer health and formation of toxic compounds (Al-Qazzaz, 2014), so it is required to add industrial antioxidants such as BHA and BHT to prevent of fat oxidation (Shelyaya et al. 2014), but recently, many doubts have recorded about the toxicity and side effects

on human health, which requires searching for natural sources that are safer (AL-Masri, 2016), so this study aimed to use some extracts of green onion (Welsh onion) *Allium fistulosum* L. and killer toxin produce form bakery yeast *Saccharomyces cerevisiae* to extend the shelf life of minced beef.

MATERIALS AND METHODS

Welsh onion

Allium fistulosum L. were obtained from markets in Baghdad during January 2021. The onion were washed, dried and cut to small pieces for extraction of bioactive compounds.

Extraction of bioactive compounds

The hot aqueous extract of Welsh onion was prepared according to the method of Aziz and Al-Niaame (2013), by mix 50 g of onion with 100 mL of distilled water and put in water bath at 100°C for 1 h, then cooling at room temperature and filtered (Whatman No.1), the filtrate extract was dried by freeze dried and kept at 4°C until use.

Preparation of Welsh onion solution

Welsh onion solution was prepared according method of Alsoufi and Aziz (2021) by dissolved 1 g of dried Welsh onion extract with amount of distilled water and heat up to 80°C in a water bath with stirred till to completely dissolve then add distilled water to make a final volume of 10 mL and sterilizing at 117°C for 10 min and kept at 4°C till use.

Detection of phytochemicals

phytochemicals (bioactive compounds) in such us flavonoids, terpenes, glycosides, saponins, alkaloids and tannins were detection in Welsh onion hot aqueous extract solution according to method of Al-Niaame and Aziz (2013).

Yeast

Commercially bakery yeast (Pack-maya, Turkey).

Activation

Yeast activation and growth through a method of Azizet al. (2014b).

killer toxin Extraction

Killer toxin was extraction from yeast according to the method of Alsoufi and Aziz (2017b) by put 100 mL of yeast extract glucose peptone broth and 3 mL of activated yeast in 250 mL conical flask and adjusted pH to 5.5 then incubation in shacking incubator at 30°C and 125 rpm/min for 24 h, the yeast cells were separated by cooling centrifuge at 4°C and 5000 rpm/min for 20 min, supernatant was considered as a crude extract for killer toxin and dried by freeze dried and kept at 4°C till use.

Type of bacteria

Four isolates (*Escherichia coli*, *Salmonella* ssp., *Pseudomonas aeruginosa* and *Staphylococcus aureus*) were obtained from college of science, university of Al-Mustansiriah, Iraq. Nutrient agar slants was used to kept isolates at 4°C.

protein

Protein (mg/mL) was estimation through method of Bradford (1976).

Preparation of killer toxin solution

Killer toxin solution was prepared through method of Alsoufi and Aziz (2017b) by dissolved 0.1 g of dried Welsh onion extract with amount of distilled water and heat up to 80°C in a water bath with stirred till to completely dissolve then add distilled water to make a final volume of 10 mL and sterilizing at 117°C for 10 min and kept at 4°C till use.

Purification of killer toxin

Killer toxin was purified according to the method of Aziz et al. (2104a) using ammonium sulfate saturation 30-70% to precipitate killer toxin from 200 mL of crude extract, then centrifugation at 5000 rpm for 30 min at 4°C, the precipitate was dissolved in 0.1 M Tris-HCl buffer pH 7.4 and dialyzed against same buffer overnight at 4°C, then concentrate by polyethylene glycol (PEG 20000) up to 10 mL (1.48 mg/mL) which loaded on a Sephacryl S-200 column (1.5x60 cm), the equilibration of column and fractionation of crude extract killer toxin using 0.1 M Tris-HCl buffer pH 7.4 at a flow rate of 18 mL/h (3 mL/fraction). Active fractions were collected, dialyzed against distilled water overnight at 4°C and lyophilized by till use.

Antibacterial activity

The antibacterial activity of killer toxin and Welsh onion was estimation using well diffusion (Table 1) as in the method of Alsoufi et al. (2020).

Table 1: Distribution of antibacterial solutions

Treatment No.	antibacterial solution	Rate
1	Welsh onion	1
2	killer toxin	1
3	Welsh onion + killer toxin	0.75+0.25
4	Welsh onion + killer toxin	0.50+0.50
5	Welsh onion + killer toxin	0.25+0.75
6 (Control)	Distilled water	1

Estimated of bacterial total count

The total count of bacteria for minced meat samples was estimated by preparing the dilutions by adding 5 g of the minced beef to 90 mL of sterile peptone water (0.1%) and shaking well to prepare the first dilution 10⁻¹, which the other dilutions from it up to 10⁻³. The Petri dishes were cultured by pour plate method and incubation at 37°C for

24-48 h, and the number of growth bacteria was expressed as CFU.g⁻¹ (Oribi, 2017).

Extending shelf life of minced beef

The effect of extending shelf life of minced beef was estimated by add 5mL of Welsh onion + killer toxin (1:1) antibacterial solutions for 1 kg of the minced beef and storage for 10 day at 4°C (Awad and Hindi, 2016).

Peroxide value

The number of peroxide value of minced beef that treated with 5 mL of Welsh onion + killer toxin (1:1) antibacterial solutions and storage for 10 day at 4°C was estimated according method of AL-Masri (2016).

RESULTS AND DISCUSSION

Phytochemicals (bioactive compounds)

The results showed that the hot aqueous extract of Welsh onion include flavonoids, terpenes, glycosides, saponins, alkaloids and tannins. This result similarly to Bhat (2020) who explained that the *Allium* sp have bioactive phytochemical compounds such as organosulfur, flavonoids, Polyphenols, alkaloids, tannins, saponins, and others, therefore, this plants has traditional uses as a herbal medicine, and what referred Sung et al. (2018); Yamamoto et al. (2005); Chen et al. (2000) about the plant possessing antimicrobial, antifungal, anti-inflammatory, antioxidant, anticancer activities and much more.

Purification of killer toxin

The results showed an increase activity of the killer toxin after precipitation and dialysis, which indicates the importance of this step in removing of the other proteins present in the crude extract and concentration of the killer toxin. The inhibition zone by the crude extract of yeast was 19 and 16 mm for *E. coli* and *S. aureus* respectively, while its highest to be 26 and 22 mm, respectively, after precipitation and dialysis. These results were confirmed by the results reached by Aziz et al. (2104a); Castaliuolo et al. (1999), who indicated an increase in the activity of killer toxin after precipitation and dialysis that considered one of the important steps that must be during purification due of its positive properties such as reducing the size, getting rid of the largest amount of water and other proteins and increasing of inhibition activity (Al-Soufi, 2016; Al-Soufi, 2016), the results in (Figure 1) shows several protein peaks appear during killer toxin purification using a gel filter Sephacryl S-200 as an additional purification step, peak 4 showed a high inhibition activity 31 and 27 mm against *E. coli* and *S. aureus*, respectively. killer toxin purification steps were showed in (Table 2). the results of similar studies showed an increase in activity through increasing purification steps (Aziz et al. 2104a; Busseyet al. 1988; Pfeiffer and Radler, 1982; Palfree et al. 1979).

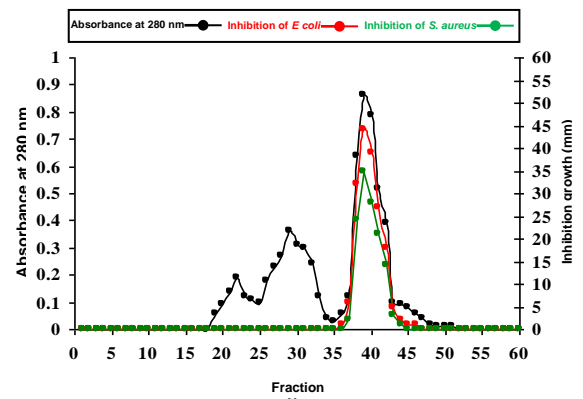


Figure 1: Purification of killer toxin from *S. cerevisiae* by Sephacryl S-200 column (1.5x60 cm), the equilibrated of column and fractionation of crude extract killer toxin using 0.1 M Tris-HCL buffer pH 7.4 at a flow rate of 18 mL/h (3 mL/fraction).

Effect of antibacterial solutions on inhibition growth of some bacterial strains

The inhibition zone (mm) of *E. coli*, *Salmonella* ssp., *P. aeruginosa* and *S. aureus* was 27, 26, 23 and 24 mm respectively, at a rate of 25 mm for Welsh onion extract (100%). It was 26, 14, 18 and 21 mm respectively, at a rate of 20 mm for killer toxin (100%). It was 30, 26, 22 and 25 mm respectively, at a rate of 26 mm for Welsh onion extract (75%) + killer toxin (25%). It was 32, 30, 23, and 26 mm respectively, at a rate of 28 mm for Welsh onion extract (50%) + killer toxin (50%). It was 28, 25, 20 and 23 mm respectively, at a rate of 24 mm for Welsh onion extract (25%) + killer toxin (75%) (Table 3).

The insurance of food safety and extending of shelf life of food represent the main challenge for the growth of worldwide food industry, due to the health concerns expressed by consumers for getting a healthy and safe food which free of chemical preservatives and exchange it with natural ingredients to extending of shelf life of food (Petrová et al. 2015; Sadaka et al. 2013), in this regard, Premanathan et al. (2011) succeed to inhibit of *Candida albicans* and *Yersinia ruckeri* using killer toxin purified *S. cerevisiae*. While, Al-Niaame and Aziz (2013) found that the methanolic extract of lavender *Lavandula officinalis* L showed the highest inhibition zone (15-18) mm at 200 mg/mL against *Acinetobacter baumannii* and *Enterococcus faecalis*. Also, all of Aziz et al. (2014a; 2014b) observe that crude extract and purified killer toxin form *S. cerevisiae* was active antimicrobial against *E. coli*, *Salmonella enterica*, *S. typhimurium*, *Shigell aflexneri* and *S. sonnei*. However all of Alsoufi and Aziz (2017b); Alsoufi and Aziz (2019); Alsoufi and Aziz (2021) Observe that The inhibition (%) of *E. coli*, *S. typhimurium*, *Aspergillus* ssp., *Penicillium* ssp., *C. albicans* and *Pichia jadinii* was 71, 62, 52, 59, 68 and 76% at a rate of 64.6%,

Table 2: Purification steps of killer toxin from bakery yeast *S. cerevisiae*.

Purification Step	Volume (mL)	Protein (mg/mL)	Total Protein(mg)	Yield (%)	Inhibition(mm)	
					<i>E coli</i>	<i>S. aureus</i>
Yeast Crude extract	200	0.16	32	100	18	11
Precipitation 30-70%(NH ₄) ₂ SO ₄	10	1.48	14.8	46.25	26	22
Sephacryl S-200 Peak 4	15	0.15	2.25	7.03	31	27

Table 3: Effect of hot aqueous extract of Welsh onion *Allium fistulosum* L. and killer toxin on inhibition of growth (mm) of some bacterial strains

Bacterial strains	Inhibition of growth (mm)				
	Welsh onion extract (100%)	killer toxin (100%)	Welsh onion extract (75%) + killer toxin (25%)	Welsh onion extract (50%) + killer toxin (50%)	Welsh onion extract (25%) + killer toxin (75%)
<i>E. coli</i>	27	26	30	32	28
<i>Salmonella ssp.</i>	26	14	26	30	25
<i>P. aeruginosa</i>	23	18	22	23	20
<i>S. aureus</i>	24	21	25	26	23
Average	25	20	26	28	24

Table 4: Total bacterial count (CFU.g⁻¹) and peroxide value (mEq/kg) of minced beef treated minced beef by Welsh onion *Allium fistulosum* L. extract (50%) + killer toxin (50%) during storage period for 0, 3 and 6 day at 4°C.

Treatment	Storage period (day) of minced beef at 4°C					
	Total count (CFU.g ⁻¹)			Peroxide value (mEq/kg)		
	0	3	6	0	3	6
Control	8.2×10 ²	9.1×10 ²	9.8×10 ²	2.2	3.1	4.3
Welsh onion extract (50%) + killer toxin (50%)	8.2×10 ²	3.4×10 ²	2.1×10 ²	2.2	2.9	3.8

It was 70, 69, 40, 52, 58 and 61% at a rate of 58.3% and it was 68, 65, 44, 50, 59 and 57 at a rate of 60.125% for killer toxin, Lavander and Rosemary, respectively.

Shelf life and peroxide value of minced beef

The total bacterial count for control was 8.2×10², 9.1×10² and 9.8×10² CFU.g⁻¹, while it be 8.2×10², 3.4×10² and 2.1×10² CFU.g⁻¹ for treated minced beef by Welsh onion extract (50%) + killer toxin (50%) during storage period for 0, 3 and 6 day at 4°C. the peroxide value for control was 2.2, 3.1 and 4.3 mEq/kg while it be 2.2, 2.9 and 3.8 mEq/kg for same treated and storage period. The results of this study agreed with a many of studies in this field. Oribi (2017) refer that use of *Origanum majorana* L. leaves showed a decrease its value in peroxide and total bacterial count for minced beef during storage for 0, 7 and 10 day at 5°C. The peroxide value of beef kofta and chicken burger treated with herbs was decrease during storage at frozen temperature (Shelyaya et al. 2014; Darwis et al. 2012). Also, Awad and Hindi (2016) observed that use of carotenoids extracted from shrimp

shell that the peroxide value of minced beef treated 4% carotenoids was stable after 6 days of storage at 4°C, and the total bacterial count was decrease during same period of storage.

Herbs and plant extracts have been used for add flavor and extending shelf life of food and due they contain bio active compounds against wide types of microorganisms that cause food spoilage, therefore, the trend has increased recently towards using natural alternatives to preserve food and avoiding the use of chemicals in preservation due they represent potential sources of danger for consumer health (Bhat, 2020; Sung et al. 2018; AL-Masri, 2016).

CONCLUSION

This study showed the ability of Welsh onion *Allium fistulosum* L. extract and killer toxin produced from bakery yeast *Saccharomyces cerevisiae* in decrease of total bacterial count and peroxide value of minced beef during storage.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Mohammed A Alsoufi: designed the study, purification steps, interpreted the data, and drafted the manuscript .

Raghad A Aziz: conduct microbial and chemical analysis and involved in collection of data.

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