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USE KILLER TOXIN EXTRACTED FROM BAKERY YEAST FOR EXTENDING SHELF LIFE OF FRUITS

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ABSTRACT:
Dry Bakery yeast was used to production of killer toxin. Purification steps included use of $(NH_4)_2SO_4$ 30-70% saturated solution and Sephacryl S-200, giving two peaks that collected to give inhibition zones of 28 and 22mm diameter for *Escherichia coli* and *Salmonella typhimurium* respectively, with 10.6% yield from the total protein 13.7mg\ml of yeast crude extract. The effect of killer toxin and pullulan coating on growth of some selected microorganism's strains showed inhibition of growth of *E. coli, S. typhimurium, Aspergillus ssp., Penicillium ssp., Candida albicans* and *Pichia jadinii* a was 71, 62, 52, 59, 68 and 76% respectively, at a rate of 64.6% for killer toxin (K), and was 68, 70, 42, 48, 61 and 63% respectively, at a rate of 58.6% for pullulan (P), and 74, 68, 65, 68, 71 and 73% respectively, at a rate of 69.83% for 25% P+75% K, and was 83, 76, 72, 81, 76 and 87% respectively, at a rate of 79.16% for 50% P+50% K, and was 67, 72, 54, 56, 64 and 69% respectively, at a rate of 63.66% for 75% P+25% K. The percentage values of weight loss for uncoated and coated apples with 100% (K), 100% (P), 50% K+50% P, 25% K+75% P and 75% K+25% P solutions were 3.2, 4.8, 6.1, 7, 7.2, 8.3 and 9.1%; 3.3, 4.2, 5.1, 5.6, 6.1, 7.2 and 8.3%; 2.9, 3.8, 4.1, 4.6, 5.8, 6.4 and 7.6%; 2.6, 2.8, 3.2, 3.3, 4, 4.7 and 5.1%; 2.9, 3.3, 3.5, 4.1, 5.3, 5.7 and 6%; 3, 3.5, 3.7, 4.4, 5.6, 5.9 and 6.5% respectively, when storage at 25°C for 3, 6, 9, 12, 15, 18 and 21 day respectively, while observe spoilage time (days) during apples storage at 25°C showed that the spoilage began to appear at 4, 14, 7, 19, 9 and 12 day for uncoated and coated apples with 100% (K), 100% (P), 50% P+50% K, 25% K+75% P and 75% K+25% P respectively.

Key words: Bakery yeast, Killer toxin, Purification, Antimicrobial activity, Pullulan, Apples, Extending shelf life.

INTRODUCTION:

Yeast *Saccharomyces cerevisiae* (Bakery yeast) can produce of some types of proteins which working for prevent or delay the growth of other microorganisms in the same growth medium and these proteins her acidic nature and specific reaction which depend of some environ-mental factors such as pH, temperature and ventilation (Aziz, *et al.*, 2014a). Moreover, the yeast has many mechanisms that explain their ability to neutralizing some of microorganisms through the directly influence, or by inhibition of some pathogens, contribution to stimulating the immune system and prevent bacterial toxins from access to receptors in the cells (Shareef and Al-Dabbagh, 2009). So, yeast widely used instead of antibody for the ability to prevent pathogenic bacteria of colonize the gastrointestinal area through competition for nutrients, which prevents or reduces effect these microorganisms (Badia, *et al.*, 2009), several studies proved that yeast hers an important role in providing immune protection against pathogenic infections of the digestive tract by stimulating the increase production of immunoglobulin IgA as well as work on organize of the intestinal environment and reduce division of cells pathogens (Tukmechi, *et al.*, 2011), as it proved high efficiency in the treatment of acute and chronic diarrhea which caused by intestinal bacteria which endemic to intestines of infected adults and children (Kelly, *et al.*, 1994), as well used to prevent diarrhea for the traveler's and relieve abdominal pain of enteric nervous (Desreumaux, *et al.*, 2010), likewise used as therapy for children who suffer from Candidiasis disease which caused by *C. albicans* (Premanathan, *et al.*, 2011).

Pullulan $(C_6H_{10}O_5)$ H_2O is a neutral; water soluble polysaccharide produced from starch by the fungus *Aureobasidium pullulans* by fermentation (Al-Soufi, 2015), pullulan characterized it's a non-toxic for human or animal, low calorie, biodegradable, colorless, odorless, tasteless and have a good adhesive characteristic which enable to use it for coating of food product, so it can be a beneficial to ensure microbiological safety of foods (Chlebowska-Smigiel and Gniewosz, 2009).

In recent years; the increased demand of fresh foods led to the search and finding new method which ensures to keep foods, with freshness and high quality (Krasniewska, *et al.*, 2014). This study aimed to extracted and purify the killer toxin that produce by commercial bakery yeast and used with pullulan that produce from locally isolated *A. pullulans* and use them to extending shelf life of apples.

MATERIALS AND METHODS

Bakery yeast Commercially bakery yeast (Packmaya, Turkey).

Apples: *Malus domestica* type Golden Delicious yellow color.

Pullulan: Pullulan were obtained from a previous study by Al-Soufi, (2015).

Microorganisms strains: Six isolates: *E. coli*, *S. typhimurium*, *Aspergillus* ssp., *Penicillium* ssp., *C. albicans* and *P. jadinii*, were obtained from biological laboratory of market research and consumer protection center, University of Baghdad, Iraq. All isolates were kept in nutrient agar slants at 4°C through of period study as a stock cell culture.

Activation of yeast: Yeast activation and growth by method of Barnett, *et al.*, (2000).

Extraction of killer toxin: Killer toxin was extraction from yeast by method of Aziz, *et al.*, (2014b) as in the following steps:

A). 100ml of YEGP broth was put in 250ml conical flask and inoculum with 3ml of activated yeast and adjusted pH to 5.5 for incubation in shacking incubator at 30°C and 125rpm\min for 24h.

B). yeast cells were separated by cooling centrifuge at 4°C and 5000rpm\min for 20min, supernatant was considered as a crude extract for killer toxin of yeast.

Estimated of protein: Protein was estimation through method of Bradford, (1976).

Antimicrobial activity: Antimicrobial activity of killer toxin was evaluated by using well diffusion as in the method of Gupta, (2009).

Purification of killer toxin: Ammonium sulfate $(NH_4)_2SO_4$ saturation was used to concentration of crude extract for killer toxin of yeast, the precipitate after centrifugation at 5000rpm for 30min in 4°C, was dissolved in 10ml of Tris-HCL buffer 0.1M, pH 7.4 and dialysis against same buffer for 24h at 4°C then dried by freeze dryer and dissolved in same buffer to prepare 5ml killer toxin solution (10mg\ml) that loaded on a Sephacryl S-200 column (1.5×60cm) which equilibrated and collected separate fractions by Tris-HCL buffer 0.1M, pH 7.4 at a flow rate of 18ml\hour. The active fractions which collected were dialyzed against distilled water at 4°C for 24h, and lyophilize by freeze dryer and keep at freezing until use (Aziz, *et al.*, 2014a).

Preparation of pullulan: Dried pullulan was dissolved in a distilled water to obtain 10% solution and heated up to 80°C in a water bath and stirrer to dissolve, the solution was sterilized at 117°C for 10min and storage in refrigerator (Chlebowska-Smigiel, *et al.*, 2007).

Effect of coating on inhibition of micro-organisms: The effect of coating by 100% K, 100% P, 25% P + 75% K, 50% P+50% K and 75% P+25% K on growth of micro-organisms strains and inhibition (%) were determine through method of Chlebowska-Smigiel and Gniewosz, (2009).

Weight loss and spoilage: Apples were been washed, dried, weigh and covering with 100% (K), 100% (P), 25% P+75% K, 50% P+50% K and 75% P+25% K by using a sterile brush, as soon as solution to dryness, apples were weighed again and stored at 25°C for 21 day for monitor the spoilage and calculate of weight loss by relationship with initial weight, which were weighed for the whole period of storage (Krasniewska, *et al.*, 2014).

RESULTS AND DISCUSSION:

Purification of killer toxin: Crude extract of yeast killer toxin showed antimicrobial activity against *E. coli* and *S. typhimurium* with inhibition zone 15 and 11mm diameter respectively (Table 1). The antibacterial activity of *S. cerevisiae* attributable to its ability to production of inhibition materials which have specific effect to destroyed plasma membrane for sensitive cells (Qamar, *et al.*, 2001).

The results in the same table refer to increase the activity of killer toxin that produced from yeast after precipitate by $(NH_4)_2SO_4$ 30-70% saturated solution and dialysis, the average of inhibition zone was increase to be 24 and 16mm diameter for *E. coli* and *S. typhimuram* respectively, so, its important step that must to do before another purification steps because of its ability to remove a large amount of water and some proteins from crude extract to ensure consternate killer toxin that produces from yeast (Janson and Ryden, 2011; Aziz *et al.*, 2014a).

Purification of killer toxin by gel filtration throws Sephacryl S-200 (Figure 1). Sephacryl S-200 giving two peaks that collected to gave inhibition zones of 28 and 22mm diameter for *E. coli* and *S. typhimurium* respectively, with 10.6% yield from the total protein 13.7mg\ml of yeast crude extract (Table 1).

Purification steps were different between studies, but it agreement in get a killer toxin as a highest purity and inhibition activity, for this purpose Soares and Sato, (2000) were used Amicon YM10 membranes to concentration crude extract and gel filtration by Sepharose 6B with Two step to purified killer toxin from *S. cerevisiae* Y500-4L, while, Comitini, *et al.*, (2004) used ultrafiltration and gel filtration on Q-Sepharose to purification killer toxin from *Kluyveromyces phaffii*. İzgü, *et al.*, (2006) used ultrafiltration and gel filtration by a TSK G2000SW column to purify killer toxin from *Pichia anomala* NCYC 432.

Table 1: Purification steps of killer toxin form Bakery yeast.

Sample	Volume (ml.)	Protein (mg\ml)	Total protein (mg\ml)	Yield (%)	Inhibition zone (mm)	
					E. coli	*S. typhimurium*
Crude extract	100	0.137	13.7	100	15	11
30-70% $(NH_4)_2SO_4$	10	0.582	5.82	42.5	24	16
Sephacryl S-200	13	0.112	1.456	10.6	28	22

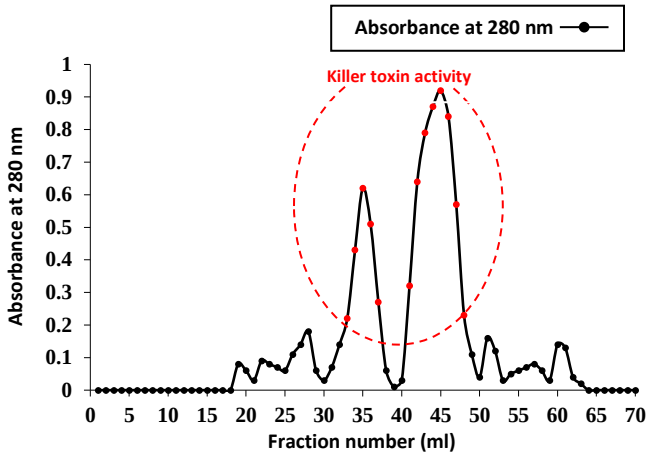

Figure 1: Purification of killer toxin form Bakery yeast through Sephacryl S-200 column (1.5×60cm). Tris-HCL buffer 0.1M, pH 7.4 at a flow rate of 18ml\hour.

Effect of killer toxin and pullulan coating on growth of some selected micro-organisms strains: The inhibition of *E. coli, S. typhimurium*, *Aspergillus* ssp., *Penicillium* ssp., *C. albicans, P. jadinii,* was 71, 62, 52, 59, 68 and 76% respectively, at a rate of 64.6% for (K), and was 68, 70, 42, 48, 61 and 63% respectively, at a rate of 58.6% for (P), and 74, 68, 65, 68, 71 and 73% respectively, at a rate of 69.83% for 25% P+75% K, and was 83, 76, 72, 81, 76 and 87% respectively, at a rate of 79.16% for 50% P+50% K, and was 67, 72, 54, 56, 64 and 69% respectively, at a rate of 63.66% for 75% P+25% K (Table 2).

Table 2: Effect of killer toxin and pullulan coating on inhibition of some selected microorganisms strains

microorganisms	Inhibition (%)				
	killer toxin (K)	Pullulan (P)	25% P+75% K	50% P+50% K	75% P+25% K
E. coli	71	68	74	83	67
S. typhimurium	62	70	68	76	72
Aspergillus ssp.	52	42	65	72	54
Penicillium ssp.	59	48	68	81	56
C. albicans	68	61	71	76	64
P. jadinii	76	63	73	87	69
Average (%)	64.6	58.6	69.83	79.16	63.66

Many research refer to inhibitory effect for killer toxin and pullulan on bacteria, mold and yeast, Chlebowska-Smigiel and Gniewosz, (2009) found that pullulan coating was active as antimicrobial activity through on inhibited 60-100% for all type of bacteria, yeast and mold which used as indicator of pullulan coating, also Jittinan, *et al.,* (2013) was observe that oral film of herbal extract which contain different concentration of pullulan showed antimicrobial activity against *Streptococcus mutans, Streptococcus sanguis* and *Porphyromonas gingivalis*, while Wagner, *et al.,* (2013) explain that *A. pullulans* solution with different concentrations was very active against grey mold *Botrytis cinerea* and *Monilinia fructigena*. On the other side, Premanathan, *et al.,* (2011) was refer to killer toxin form *S. cerevisiae* as a active agent for inhibition of *C. albicans* and *Yersinia ruckeri,* also, all of Aziz, *et al.,* (2014a) and Aziz, *et al.,* (2014b) observe that crude extract and purified killer toxin form *S. cerevisiae* was active antimicrobial against *E. coli*, *Salmonella*

enterica, S. typhimurium, Shigella flexneri and Shigella sonnei.

Weight loss and spoilage: The percentage values of weight loss (Figure 2) for uncoated and coated apples with 100% (K), 100% (P), 50% P+50% K, 25% K+75% P and 75% K+25% P solutions were 3.2, 4.8, 6.1, 7,7.2, 8.3 and 9.1%; 3.3,4.2,5.1,5.6, 6.1,7.2 and 8.3%; 2.9, 3.8, 4.1, 4.6, 5.8, 6.4 and 7.6%; 2.6, 2.8, 3.2,3.3, 4, 4.7 and 5.1%; 2.9,3.3,3.5, 4.1,5.3,5.7 and 6%; 3, 3.5, 3.7, 4.4, 5.6, 5.9 and 6.5% respectively, when storage at 25°C for 3,6,9,12,15,18 and 21 day respectively, while observe spoilage time (days) during apples storage at 25°C. Figure-3 showed that the spoilage began to appear at 4, 14, 7, 19, 9 and 12 day for uncoated and coated apples with 100% (K), 100% (P), 50% P+50% K, 25% K+75% P and 75% K+25% P respectively.

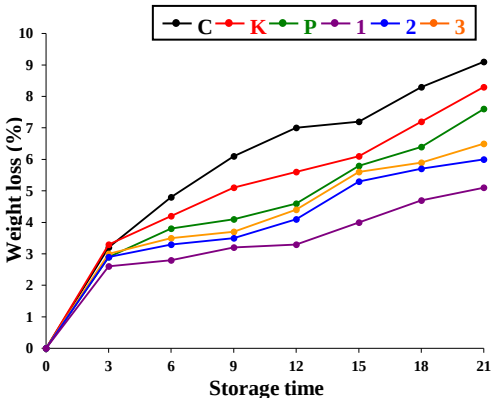

Figure 2: Changes in the apples weight loss stored at 4°C for 10 day by different treatments:
C: Control (uncoated); K: 100% Killer toxin; P: 100% Pullulan; 1: 50% K+50% P; 2: 25% K+75% P; 3: 75% K+25% P.

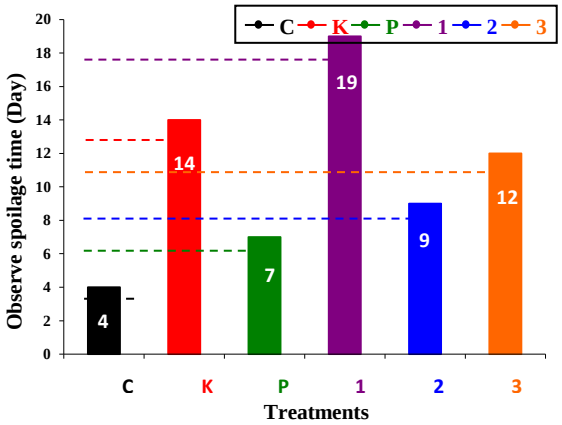

Figure 3: Observe spoilage time (day) in the apples that stored at 25°C for different treatments:
C: Control (uncoated); K: 100% Killer toxin; P: 100% Pullulan; 1: 50% K+50% P; 2: 25% K+75% P; 3: 75% K+25% P.

The results showed that 50% K+50% P treatment giving best results in reducing the weight loss and delay time of spoilage, is due to cover the fruit in equal layer of each of the killer toxin and pullulan, which lead to occurrence of the synergy phenomenon between them. Softening was considered a main problem in fresh fruits and vegetables during marketing from farm to consumer, that occur as a natural process of maturation and water loss which lead to making them loss of economic value, shortening of shelf life and undesirable for consumed by consumers (Al-Soufi, 2015).

Many researches discuss this important parameter by coated fruits with different concentrations of pullulan water solution or mixed with some extracts. Chlebowska-Smigiel, *et al.,* (2007) use pullulan coating to extend apples shelf life stability by covered with 15 and 20% pullulan water solution and storage at 4 and 22°C during 39 day, while Krasniewska, *et al.,* (2014) pointed to the possi-bility of the use of plant extracts (SH) from *Satureja hortensis* L. with pullulan to maintain quality and safety of pepper and apple, so, in this field, Gniewosz, *et al.,* (2014) use pullulan film with meadowsweet flower extracts (*Filipendulae ulmariae flos*) as antimicrobial for *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Salmonella enteritidis* ATCC 13076, *E. coli* ATCC 25922, *Penicillium expansum* ATCC 7861, *Rhizopus arrhizus* ATCC 11145 and *Aspergillus niger* ATCC 9142.

REFERENCES:

Al-Soufi, M.A, Extending the storage life of some fruits by using Pullulan produced from locally isolate *Aureobasidium pullulans*. Iraqi Journal of Market Research and Consumer Protection 7(1): 179-198 (2015).

Aziz, R.A., M.A. Al-Soufi and A.M. Ateia, Purifica- tion and Determination of some Proteins inhi-bitors properties that produced from bakery yeast and study their activity against some types of bacteria that cause diarrhea. First International Scientific Conference, Cihan Uni-versity, Erbil, Kurdistan Region, Iraq (2014a).

Aziz, R.A., M. A. Al-Soufi and A.M. Ateia, Study of bakery yeast extract activity on some enteric bacteria that isolated from some hospital in Baghdad city. Journal of the College of Basic Education 20(82): 143-166 (2014b).

Badia, R., M.T. Brufau, A.M. Guerrero-Zamora, R. Lizardo, I. Dobrescu, R. Martin-Venegas, R. Ferrer, H. Salmon, P. Martinez and J. Brufau, β-Galacto -mannan and *Saccharomyces cerevisiae* var. *boulardii* modulate the immune response against *Salmonella enterica* serovar typhimu-rium in porcine intestinal epithelial and dendritic cells. Clin. Vaccine Immunol. 19(3): 368-375 (2012).

Barnett, J.A., R.W. Payne and D. Yarrow, Yeasts: Characteristics and Identification. 3rd ed., Cambridge University Press, Cambridge. 467 (2000).

Bradford, M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochem. 72: 248-254 (1976).

Chlebowska-Smigiel, A. and M. Gniewosz, Effect of pullulan coating on inhibition of chosen micro-organisms growth. Acta Sci. Pol. Technol. Aliment. 8(3): 37-46 (2009).

Chlebowska-Smigiel, A., M. Gniewosz and E. Swinczak, AN attempt to apply a pullulan and pullulan-proten coatins to prolong apples shelf - life stability. Acta Sci. Pol. Technol. Aliment. 6(1): 49-56 (2007).

Comitini, F., N. Di Pietro, L. Zacchi, I. Mannazzu and M.Ciani, *Kluyveromyces phaffii* killer toxin active against wine spoilage yeasts: purification and characterization. Microbiol. 150:2535-2541 (2004)

Desreumaux, P., C. Neut, M. Cazaubiel, M. Pichelin, P. Justen and F. Pelerin, *Saccharomyces cerevisiae* CNCM I-3856 reduces digestive discomfort and abdominal pain in subjects with irritable bowel syndrome: a randomized double-blinded placebo-controlled clinical trial, abstr. OP-181, 18th United European Gastroenterology Week. Barcelona, Spain (2010).

Gniewosz, M., A. Synowiec, K. Krasniewska, J. L. Przybyl, K. Baczek and Z. Weglarz, The anti-microbial activity of pullulan film incorporated with meadowsweet flower extracts (*Filipen-dulae ulmariae flos*) on postharvest quality of apples. Food Control 37: 351-361 (2014).

Gupta, S., The Short Text Book of Pediatrics. 11th ed., Jaypee Brothers Medical Publishers (P) Ltd. (2009).

İzgü, F., D. Altınbay and T. Acun, Killer toxin of *Pichia anomala* NCYC 432; purification, characteriza-tion and its *exo*-β-1,3-glucanase activity. Enzyme and Microbial Technology 39(4):669-676 (2006).

Anson, J.C. and L. Ryden, Protein Purification: Principles, High Resolution Methods, and Applications, 3rd ed., Wiley-VCH (2011).

Jittinan, C., S. Krisana and V. Pakpachong, The developpment of innovative herbal extract oral film for the unpleasant mouth odour. International Journal of Innovation, Management and Tech-nology 4(2): 265-268 (2013).

Kelly, C. P., C. Pothoulakis and J.T. LaMont, Clostri-dium difficile colitis. New England Journal of Medicine 330(4): 257-262 (1994).

Krasniewska, K., M. Gniewosz, A. Synowiec, J.L. Przybyl, K. Baczek and Z. Weglarz, The use of pullulan coating enriched with plant extracts from *Satureja hortensis* L. to maintain pepper and apple quality and safety. Postharvest Biology and Technology 90: 63-72 (2014).

Premanathan, M., F.A.A. Shakurfow, A.A. Ismail, M.A.Berfad, A.T. Ebrahim and M.M. Awaj, Treat-ment of oral candidiasis (thrush) by *Saccharo-myces cerevisiae*. International J. of Medicine and Medical Sciences 3(3): 83-86 (2011).

Qamar, A., S. Aboudola, M. Warny, P. Michetti, G. Pothoulakis, J.T. Lamont and C.P. Kelly, *Saccha-romyces boulardii* stimulates intestinal immune-globulin A immunorespons to *Clostridium difficile* toxin A in mice. Infect. Immun. 69(4): 2762-2765 (2001).

Shareef, A.M. and A.S.A. Al-Dabbagh, Effect of probiotic (*Saccharomyces cerevisiae*) on perfor-mance of broiler chicks. Iraqi Journal of Veteri-nary Sciences 23(1): 23-29 (2009).

Soares, G.A.M. and H.H. Sato, Characterization of the *Saccharomyces cerevisae* Y500-4L killer toxin. Brazilian Journal of Microbiol. 31: 291-297 (2000).

Tukmechi, A., H. R. R. Andani, R. Manaffar and N. Sheikhzadeh, Dietary administration of beta-mercaptoethanol treated *Saccharomyces cere-visiae* enhanced the growth, innate immune response and disease resistance of the rainbow trout, *Oncorhynchus mykiss*. J. Fish and Shellfish Immunology 3: 923-928 (2011).

Wagner, A., B. Hetman, M. Kopacki, A. Jamioł-kowska, P. Krawiec and T. Lipa, Laboratory effect of boni protect containing *Aureobasidium pullulans* (de Bary) arnoud in the control of some fungal diseases of apple fruit. Acta Agrobotanica 66(1): 77-88 (2013).