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The influence of plant oil on the production of lipases enzyme from *Staphylococcus aureus* isolated from imported cheeses

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Abstract

Milk and dairy products are very important as food sources. However, they are usually contaminated with members of the genus *Staphyloccocus* especially that liberate lipolytic enzymes. Thus the goals of this research were the, production of lipolytic enzymes at 37°C and 20°C by *Staphyloccocus aureus* that isolated from imported cooked cheese, lipases enzymes activity screened at different plant oils, activation or inhibition of lipase production using different plant oils. Rhodamine B agar, tween 80 agar and Baird- Packer containing sodium tolerite agar were used for the detection of lipase, esterase and lecithinase enzyme respectively. Lipase enzyme was estimated by titration method using olive oil as a substrate. The results showed that fourteen isolates representing to 70% were lipase and lecithinase producer they gave orange florescence color on the top of rhodamine B plate and turbid zone on bed parker telurite agar respectively, while esterase enzyme could detected from only two isolates that represented to 10 %. The production of lipase and esterase was depended on time and temperature, both enzymes secreted after 18 h incubated at 37°C and 72h at 20°C; their activity were increased with time to reach the maximum activity after 72h at 37°C and 96h at 20°C. The best lipase producing isolate was *S. aureus* KK1; its lipase was more active in hydrolyzing garlic oil followed by fingureek and castor oils at both 37°C and 20°C. The results cleared that castor oil, tween 80 and seasam were supported maximum lipase production which reached to 23.3, 20 and 13.2 U ml^{-1} respectively after 48 hr in compares with 6 U/ml produced at nutrient broth. Otherwise inhibition of lipase production recognized among other plant oils, from wheat germ 3.5 to mint 0.33U ml^{-1}.

Keywords: Cooked cheese, *Staphylococcus aureus*, Lipase, Plant oil.

Introduction

Milk and dairy products are very important food for human beings. However, they are usually contaminated with many microorganisms especially members from the genus *Staphyloccocus* that inhabiting specific ecological niches (Heikens *et al.,* 2005). This contamination depends on many factors including milk quality, processing methods, temperature and duration of storage, season and general sanitation in manufacture area (Torkar and Teger, 2006). *S. aureus* pose a health risk not only for humans, but also, as etiological agents of mastitis in veterinary through the production of toxins and enzymes which requires a high number of bacteria (10^5- 10^6 cfu gm^{-1} of food) at a pH value more than 5.5(Anonimus,1994). Thus in the last decades, staphylococci and their toxins had also emerged as important and potential pathogen for human and animal (Heikens *et al.,* 2005). Although, extracellular enzymes may cause dairy ripening; lipases produced from *S. aureus* could increase the rate of food deterioration through their action on lipids causing accumulation of intermediate and end products that change the flavor of foods (Soares *et al*., 2011; Saxena and Gomber, 2010). The aims of this research were screening of lipases activity from *S. aureus* isolated from imported cheeses; lipases activity on different plant oils; activation or inhibition of lipase production by plant oils

Materials and Methods

Screening of lipases produced from *S. aureus*: Twenty isolates were isolated and identified as *S. aureus* by central health laboratory since October 2015- February 2016.

Rhodamine B method was achieved for screening of true lipases according to (Kouker *et al*., 1987). Briefly, nutrient agar was supplemented with 2.5% olive oil, little of Arabic gum and sterilized, 1% solution of 1 mg ml^{-1} Rhodamine B stain was added, mixed and poured in petridishes. Each isolate of *S. aureus* were streaked on the top of Rhodamine B agar, incubated at either 37°C or 20°C for 72 h and visualized under UV

transilluminator. Lipase producing isolates gave florescence orange color.

Also tween 80 method was applied for screening of both lipases and esterases. The appearance of a halo zone around growth of *S. aureus* isolates on the top of 1% tween 80 agar was indicated for esterases activity while development of obscure region around growth indicated for lipases activity (Slifcan, 2000). Measurement of zone diameter to the growth diameter was used for qualitative screening of enzyme. The isolate *S. aureus* KK1 was chosen and used through the experiment. Phospholipases (licithinase) was screened using Baird- Packer agar containing potassium tellurite and egg yolk. Opaque region around growth was indicated for licithinase enzyme. Also, nutrient agar supplemented with 1% egg yolk was used and clear zone indicated for licithinase enzyme (O,toole, 1987).

The activity of *S. aureus* lipase on plant oils: Lipases activity of *S. aureus* KK1 was determined on different plant oils (triglycerides) including: olive, castor, whete germ, seasam, amla, minut, sunflower, garlic, fingrueek, ketan, corn and synthetic tween 80. Nutrient agar with 1% of separated oil was prepared, sterilized and poured into petridishes. The isolate *S. aureus* KK1 was streaked on the top of agar and incubated at either 37 ℃ or 20℃. Clear zone of oil hydrolysis was screened each 18 h until one week.

The influence of plant oil on the lipase production by *S aureus*KK1: The activation or inhibition of lipases production by plant oils was study using nutrient broth nourished with 1% plant oil. Broth with individual oil was prepared, sterilized and inoculated with *S. aureus* KK1 and incubated at 37℃ for 72h using shaker incubator. Cultures were spinet each 24h using cold centrifuge at 5000 rpm for 10 min, cell free supernatants were used as crude enzymes to determine the lipase activity. Lipase assayed by titration method using sodium hydroxide and phenonphthaline as an indicator according to (Sethi, 2014). Briefly, substrate was prepared by mixing 50 ml of olive oil with 50 ml of distilled water and 7 g of Arabic gum for only 3 min at 25 ℃. Reaction mixture was prepared by combining 5 ml of substrate emulsion, 4 ml of phosphate buffer (0.2 M pH 7.2) and 1 ml of cell free supernatant, incubated at 37℃ for 30 min. Meanwhile, 10 ml of stopping solution was added (vol: vol acetone: ethanol) and finally titrated against sodium hydroxide. Estimation of lipase unit ml^{-1} was done according to the definition: lipase activity= the amount of enzyme wich librates 1M of fatty acids under test parameters (1 ml of 0.05 N NaOH = 100 mM fatty acid min^{-1}) using equation bellow:

Lipase activity= vol (NaOH) consumed × molarity (NaOH)/vol (enzyme sample) × reaction time.

Statistical analysis: Duncan multiple range test was used for statistical analysis using ANOVA two way no blocking analysis to evaluate the interfering among treatments. Three replicate were used at each treatment and the means were estimated using Genstat computer design.

Results and Discussion

The screening of lipases activity from twenty *S. aureus* isolates showed that only fourteen isolates representing to 70% were lipase producer they gave orange florescence color on top of Rhodamine B plate and turbid zone on tween 80 agar, while esterase enzyme could detected from only two isolates which represented to 10 % (figure1 A, B). While, results indicated that all fourteen lipase producing isolates of *S. aureus* were produced licithinase enzyme. Opaque region appeared around colony on Baird- Packer agar nourished with egg yolk and potassium tolerite, while clear zone around growth on nutrient agar contained egg yolk clarified licithinase production as presented in figure (1C, D). The fluorescent dye Rhodamine B were used extensively for the detection of true lipases produced by microorganisms due to its specificity, reliability and easy. Rhodamine B in the presence of oils forms a fluorescence complex with free fatty acids only that librated from the action of lipase only.While, tween 80 had the ability to detect lipase (forming precipitated complex) and esterase (halo zone).

The existing of lipase producing *S. aureus* had been identified in diverse habitats containing oils which results from significance bacterial lipid metabolism or their involvement in bacterial pathogenesis (Kalyani and Saraswathy, 2014).Some strains called “endemic strains” that are existing in some processing plants, that led to contaminate food or their products during or after processing; this assumption agreed with al- Khafaji (2013) that could isolate lipase producing *S. aureus* from imported meat in Iraq, also each Gundogan *et al.* (2006), Soras (2011) and Rodrigues *et al* (2014) deled with the isolation of *S. aureus* from different food, food product, meat and cheese. On the other hand, Soares (2011) spotlighted on the importance of contamination the cheese manufactures by members of *Staphyloccocus* bacteria in health concern and Diary industry especially that could produce licithinase enzyme which may related with human pathogenicity.

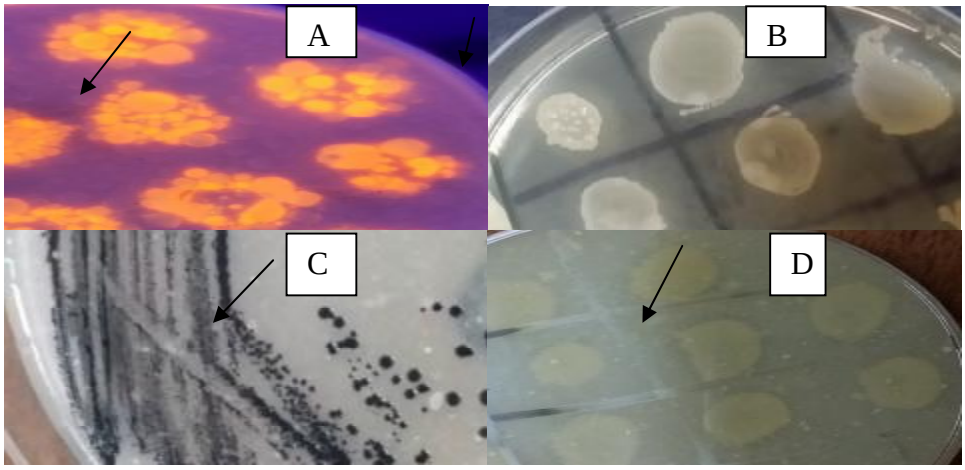

Figure (1): Screening of lipase, esterase and licithinase enzymes from *S. aureus* isolated from cheese
A= rhodamine B method, B= Tween 80 method, C= Bedparker with egg yolk, D= nutrient agar with egg yolk.

The production of lipase and esterase were seemed to be dependent on time and temperature, both enzymes secreted after 18 h and 48h at 37℃ and 20 ℃ respectively. Lipase activity increased with time to reach its final activity after 72h at 37℃ and 96h at 20℃. Lipase activity differed among isolates in which turbid zone ranging from restricted zones recognized in five isolates (table1) to 10mm and 8 mm of diameter recognized with *S. aureus* KK1 when incubated for 72h at 37℃ and 20° C respectively.

Table 1: Qualitative determination of lipase activity produced by *S. aureus* at different temperature

Isolate	Lipase activity (mm) 37℃				
	Lipase activity (mm) 20℃				
	18 h	24h	48h	72h	96h
Kk1	6	7	8	10	0
	-	-	6	8	8
Kk2*	5	5	6	6	6
	-	-	4	5	5
Kk3*	5	6	6	7	7
	-	-	RZ	RZ	RZ
Kk4	4	5	5	6	6
	-	-	RZ	RZ	RZ
Kk5	4	5	5	5	5
	-	-	RZ	RZ	RZ
Kk6	4	5	6	6	6
	-	-	RZ	RZ	RZ
Kk7	4	5	6	6	6
	-	-	RZ	RZ	RZ
Kk8	4	5	5	6	6
	-	-	RZ	RZ	RZ
Kk9	4	5	5	5	5
	-	-	RZ	RZ	RZ
Kk10	RZ	RZ	RZ	RZ	RZ
	RZ	RZ	RZ	RZ	RZ
Kk11	RZ	RZ	RZ	RZ	RZ
	RZ	RZ	RZ	RZ	RZ
Kk12	RZ	RZ	RZ	RZ	RZ
	RZ	RZ	RZ	RZ	RZ
Kk13	RZ	RZ	RZ	RZ	RZ
	RZ	RZ	RZ	RZ	RZ
Kk14	RZ	RZ	RZ	RZ	RZ
	RZ	RZ	RZ	RZ	RZ

*Isolates with esterase activity (tween 80 method), RZ= restricted zone

Results indicated that lipase produced by *S. aureus* KK1 could degrade different plant oils with different ranges of transparent zones around bacterial growth when incubated at 37° C. While, at 20° C lipase activity reduced for all oils tested. Lipase enzyme produced from *S. aureus* KK1 was more active in hydrolyzing garlic oil, fenugreek and castor oils at 37 C that gave 6.96, 5.93 and 5 mm transparent radius respectively, While, olive and amla oils were little affected by bacteria and restricted zone of oil hydrolysis was recognized on ketan oil as presented in table (2) such activity of microbial lipase recognized early for *Asperigillus* and *Mucor* strains which could degraded palm, seas am, olive and soy bean oils (Qiao et al. 2017).

Table (2): The radius of oil hydrolysis by lipase activity produced by *S. aureus* KK1

Oil type	temperature 20C	temperature 37C	means of media
1	1hi	1hi	1g
2	5c	3.5def	4.25b
3	4d	2.967fg	3.483c
4	6.967a	2.5g	4.733a
5	3efg	2.5g	2.75d
6	2.5g	1hi	1.75f
7	3.233ef	1.233h	2.233e
8	5.933b	1.333h	3.633c
9	2.5h	1.5h	2ef
10	0.667i	0.667i	0.667g
11*	4.967c	3.533de	4.25b
mean	3.615	1.976	

*precipitated zone, incubation time 72h, (1-sesam,2-castor,3-wheat germ,4-garlic,5-mint,6- amla,7-loz,8-fingueek,9-0live,10ketan, 11- tween 80)

The statistical analysis of recent results cleared that two out of plant oils were activate lipase production by *S. aureus* KK1, they were castor (23.3 U/ml) after 48 h, seasam (16.6 U/ml) after 72h and the synthetic Tween 80(20 U/ml) after 48 h. Otherwise inhibition of lipase production recognized with other oils. Also, there were some interactions between time period and type of oils. The estimation of lipase activity cleared that the increasing incubation time (48 h) caused increase in lipase activity when culture supplemented with tween, sesam, castor, wheat germ, loz, fingukee, corn, olive and ketan. The increment of lipase activity continued to 72 h with culture supplemented with sesam (16.66 U/ml) and wheat germ (7.4U/ml). Reduction in lipase activity was recognized after only 24 h with cultures of nutrient broth alone or nutrient broth that nourished with garlic or amla table(3). This finding was disagreed with (Ghaima et al., 2014) which find that olive oil was the best inducer for *Staphylococcus* sp LP12 through submerged fermentation.

Table (3): The production of lipase enzymes from *S aureus* KK1 by different oil type.

Media/	time	1	2	3	mean of media
	1	12e	5.3j	6ij	7.8
	2	10.7f	20b	10f	13.6
	3	6.6hi	13.3d	16.6c	12.2
	4	10.7f	23.3a	6.6hi	13.5
	5	1.7lm	3.6k	7.5gh	4.3
	6	8.3g	1.9l	0.5lmno	3.6
	7	0.3no	0.3no	0.3no	0.3
	8	1.4lmno	1.2lmno	0.6lmno	1
	9	0.8lmno	1.1lmno	0.1o	0.7
	10	0.7lmno	1.6lmn	0.1o	0.8
	11	0.8lmno	1.1lmno	0.1o	0.7
	12	0.6lmno	1.1lmno	0.3no	0.7
	13	1lmno	1.4lmno	0.4mno	0.9
	mean of time	4.3	5.8	3.8	

Incubation temp. 37C; Time: 1=24h, 2=48h, 3=72h, (1-NB, 2-tween 80, 3-sesam, 4- castor, 5-wheat germ, 6-garlic, 7-mint, 8-amla, 9-loz, 10- fingueek, 11- olive,12- ketan, 13- corn).

Triglyceride oils content in the plant oils might became a substrate for lipase enzyme produced by *S. aureus* KK1 at early stages of growth which led to liberate many type of monoglyceride as oleic, linoleic, capric acids. Each monoglyceride might effect on lip A gene causing an induction or inhibition of the gene. The inhibition observed of lipase production could represent a negative feedback regulatory system control by the product. This assumption explained that control culture (nutrient broth) gave its high activity after only 24h while lipase activity may increase or decrease with time at cultures augmented with plant oils. On the other hand plant oils might provide carbon sources for bacterial growth or some of them had an inhibitory growth parameters that may effect on *Staphylococcus* growth. The inhibitory activity might related to tannins, phytate and antibiotic like substances as referred to the chemical analysis of garlic oil (Oluwatoyin, 2014; Bagudo and Acheme, 2014) ; lipase inhibitors and anti-bacterial effect that contained in fingueek (*Tigonella*) (Fernando *et al*., 2012; , Akbari *et al*. 2012).

The inducer action of castor and seas am oils might due to their high content of oleic acid which cause to induce lipA gene for high lipase production in a cascade manner. This assumption in agreement with (Long *et al.* 1992) that referred to the role of oleic acid in induction of lipase enzyme from a pathogenic *S. aureus* which secrete two kind of lipase enzyme. The first enzyme called lipase modifying enzyme which inhibit by oleic acid while the second enzyme induces by oleic acid thus to overcome the inhibition action of oleic acid *S. aureus* secrete more quantity of Lipase enzyme. Also, (Xie et al., 2012) referred to feedback regulatory system for some monoglyceride as oleic, linoleic and merestic and their action on lipase gene production.

The conclusions in the present aspect clarified that the presence of *S. aureus* strains at different cheese samples may became from human due to their high frequency of lipolytic activity observed among these isolates especially at 37°C (O,toole, 1987; Owens and John,1975) both already reported that most of the *S. aureus* isolated from humans had the ability to produce lipase. Also, the contamination of cheese products by *S. aureus* producing lipase and lecithinase demonstrates the need to control this important bacterium in diary processing environment because lipase enzyme enhances pathogenicity to consumers and food spoilage of the product. As a treatment of lipase activity, the addition of many plant oils may delayed of cheese spoilage and inhibit lipase production from contaminated *S. aureus*.

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