LIPOLYTIC AND PROTEOLYTIC ACTIVITIES OF PSEDOMONAS SPP ISOLATED FROM TABLE BUTTER

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ABSTRACT

Twenty five samples of table butter were collected randomly in their retail containers from different localities in El-Dakahlia Governorate, Egypt. Collected samples were examined bacteriologically for occurrence and behavior of Pseudomonas organisms. Pseudomonas spp. Was isolated only from 5(20%) of 25 table butter samples, with a count ranged from 2x10 to 2.4x10^4 cfu/ml.

The incidence of Pseudomonas organisms isolated from table butter samples using Pseudomonas selective medium (PSM) was Ps. fragi (10%), Ps. fluorescens (6%) and Ps. aeruginosa (2%).

The proteolytic activity of isolated Pseudomonas spp on skim milk agar plates proved that all tested Ps. fragi (5) and Ps. fluorescens (3) and Ps. aeruginosa (1) produced proteolytic activity (100%).

Lipolytic properities of isolated Pseudomonas spp from table butter proved that all tested Ps. fragi (5) and Ps. fluorescens (3) produced lipolytic activity . On the other hand Ps. aeruginosa failed to produce lipase enzyme.

The public health and economic importance of isolated Pseudomonas spp as well as suggested control measures for improving the quality of the product were discussed.

الملخص العربى

قدرة الزوائف المعزولة من زبـد المائدة على تحليل الدهن والبروتين

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أجريت الدراسة على ٢٥ عينة من زبد المائدة والتى جمعت بطريقة عشوائية من أماكن متفرقة بمحافظة الدقهلية وذلك لدراسة مدى تواجد الزوائف (ميكروبات السيدوموناس) وتصنيفها واختبار قدرتها على إفراز إنزيمات محللة للبروتين والدهون، وقد دلت النتائج على تواجد ميكروب السيدوموناس Pseudomonas فى ٢٠٪ من عينات زبد المائدة وجاء العدد الكلى للميكروبات يتراوح بين ٢٠ و ٢٫٤ × ٤١٠ ميكروب/ جم من زبد المائدة.

وقد كانت نسبة تواجد السيدوموناس فى زبدى المائدة باستخدام المستنبت الزرعى كالتالى Pseudomonas Selective Medium (PSM) : كالتالى : ٢٠٪ Ps.fragi و ١٢٪ Ps. fluorescens و٤٪ Ps. aeruginosa .

وقد أوضحت النتائج أن كل عترات السيدوموناس التى تم عزلها من زبد المائدة كانت لها القدرة على إفراز الإنزيم المحلل للبروتين بنسبة ١٠٠٪، Ps.fragi(٥) ، Ps. fluorescens(٣)، Ps. aeruginosa(١).

وباختبار قدرة تلك العترات على إفراز الإنزيم المحلل للدهون دلت النتائج على أن ٥ عترات من Ps.fragi و ٣ عترات من Ps. fluorescens لها القدرة على إفراز هذا الإنزيم أما عترات Ps. aeruginosa لم تنجح فى إفراز هذا الإنزيم.

وقد تم مناقشة الأهمية الاقتصادية والصحية لميكروبات السيدوموناس المعزولة والاجراءات الواجب اتخاذها للحد من تواجد تلك الميكروبات فى اللبن ومنتجاته وذلك لتحسين جودة تلك المنتجات وحماية لصحة المستهلك.

INTRODUCTION

Butter is exposed to contamination with several types of microorganisms from different sources, under suitable conditions, these contaminant found way to grow and multiply in butter leading to undesirable changes which render the product of inferior quality and unmarketable leading to economic losses, unfit for human consumption or may posses public health hazards **(Kraft, 1992)**.

Pseudomonas spp are the most important psychrotrophs, because many strains were elaborated extracellular hydrolytic enzymes as proteases and lipases. These enzymes can survive pasteurization, unlike the producing organism, causing arrange of organolyptic defects in butter **(Gyllenberg et al., 1963 and Cousin, 1982)**. Several cases of food poisoning due to Pseudomonas aeruginosa have been reported due to consumption of contaminated dairy products **(Ahmed et al.,1989)**.

The present work was planned to study the proteolytic and lipolytic activities of Pseudomonas spp isolated from table butter samples.

MATERIAL AND METHODS

Twenty five samples of table butter were collected randomly in their retail containers from different dairy shops, supermarkets and groceries in Mansoura city, El-Dakahlia Governorate, Egypt. Collected samples were examined bacteriologically for occurrence and behavior of Pseudomonas organisms.

Preparation of samples

Each sample was melted in water bath adjusted at $40^{o}C \pm 1^{o}C$ and thoroughly mixed with periodical shaking until a homogenous

semi-solid mass was obtained.

Preparation of serial dilution

Serial dilutions were prepared following the procedures described by **APHA (1992)**.

Incidence of Pseudomonas species:

The technique recommended by **(APHA, 1992)** was performed using Pseudomonas Selective agar base **(Oxoid, 1990)** supplemented by CN supplement **(Oxoid,1990)**.

Identification of Pseudomonas isolates:

The representative suspected colonies were purified and then identified according to **Bailey and Scott's (1978)** and Bergey's Manual of Systemic **Bacteriology (1982)**.

Determination of the proteolytic and lipolytic activities of Pseudomonas species:

Nine strains belonging to Pseudomonas species isolated previously from table butter samples were investigated for proteolytic and lipolytic activities as described by **Harrigan and McCance (1976)**.

Preparation of isolates:

Pseudomonas isolates were sub cultured onto nutrient agar plates and incubated at 30^{o}C for 24 h. Pure cultures were inoculated into nutrient broth and incubated overnight at 30^{o}C prior to testing.

Proteolytic activity using skim milk agar:

Overnight cultures were spot inoculated onto milk agar, slandered plate count agar supplemented with 10% sterile skim milk. The inoculated plates were incubated at 25^{o}C for 48 hrs, and subsequently flooded with 10% v/v acetic acid solution. Clear zone around the colonies after one minute exposure were regarded as positive **(Harrigan and McCance,1976)**.

Lipolytic activity using Victoria blue butter fat agar:

A sugar-free nutrient agar medium (pH 7.5) with emulsified butter fat and Victoria blue B as indicator was used for determination of lipolytic activity. Overnight cultures were streaked onto pre-poured plates and incubated at 25^{o}C for up to 7 days. Bright blue colonies were regarded as lipase positive **(Harrigan and McCance,1976)**.

RESULTS AND DISCUSSION

Pseudomonas spp. are the most important group of Psychrotrophes associated with spoilage, they grow rapidly at refrigeration temperatures and often dominate the microbial population. Also many investigators indicated that some Pseudomonas spp could survive heat treatment used in pasteurization of milk **(Abad et al.,1993)**. Higher temperatures for shorter times were less effective in destroying the Pseudomonas spp than the pasteurization of milk **(Cousin, 1982)** .

Pseudomonas spp was isolated only from 5(20%) of 25table butter samples (Table,1).

Pseudomonas spp can gain access as post-pasteurization contaminants and cause spoilage of dairy products **(Walker,1988).** Moreover, its presence in milk or its products can be indicative of fecal contamination **(Al-Ashmawy, 1990)**.

Pseudomonas count in examined table butter samples ranged from 2x10 to $2.4x10^4$ cfu/ml. The incidence of individual Pseudomonas isolated from table butter samples using Pseudomonas Selective medium (PSM), Ps.fragi (20%), Ps.fluorescens (12%) and Ps.aeroginosa (4%) (Tables 1,2). These findings were nearly similar with those obtained by **Chezhova (1975)** and **Kraft (1992)**.

The proteolytic activity on skim milk agar plates of isolated Pseudomonas spp from table butter proved that all tested Ps.fragi (5), Ps.fluorescens (3) and Ps.aeroginosa (1) were produced proteolytic activity (100%). (Table, 3).

Extra cellular proteinases and lipases from psychrotrophic Pseudomonas are recognized to be the primary microbial spoilage enzymes of dairy products **(Sorhauge and Stepaniak, 1991** and **Vyletelova et al., 1999)**.

Lipolytic properities of isolated Pseudomonas spp from table butter proved that all tested Ps.fragi (5), Ps.fluorescens (3) were produced lipolytic activity. On the other hand, Ps. aeruginosa failed to produce lipolytic activity (Table, 3).

Pseudomonas are spoilage bacteria which elaborated extra cellular lipases which can decompose lipid and produce free fatty acids (FFAs) and having characteristic odors and flavor. The optimum temperature for Pseudomonas lipases is mostly in the range 30°C to 50°C they are still able to act at refrigeration temperatures and even at temperatures used for frozen storage of food (-10°C).

Therefore, strict hygienic measures should be imposed during butter manufacture, handling and storage, also, periodical inspection of dairy plants by specialists for hygiene should be applied to safeguard the consumer from being infected and to safe a lot of products from being spoiled on the market.

Table (1): **Prevelance and count of Pseudomonas spp. in examined table butter samples**

No. of samples	*Positive samples*		*Min.*	*Max.*	*Mean*	*±S.E.M.*
	No.	*%*				
25	**5**	**20**	**2x10**	**$2.4x10^4$**	**$1.3x10^3$**	**$±1x10^3$**

Table (2): **Incidence of identified Pseudomonas isolates from examined table butter samples in single or combined form.**

Pseudomonas isolates	*Positive samples*	
	No	*%*
Ps.fragi	**5**	**20**
Ps.fluorescens	**3**	**12**
Ps.aeroginosa	**1**	**4**

Table (3): **Proteolytic and lipolytic activities of Pseudomonas spp. isolated from Table butter samples:**

Pseudomonas isolates	*No. of tested isolates*	*Positive protyolytic isolates*		*Positive lipolytic isolates*	
		No.	*%*	*No.*	*%*
Ps.fragi	**5**	**5**	**100**	**5**	**100**
Ps.fluorescens	**3**	**3**	**100**	**3**	**100**
Ps.aeroginosa	**1**	**1**	**100**	**0**	**0**
Total	**9**	**9**	**100**	**8**	**88.8**

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