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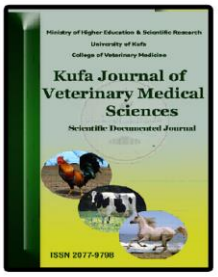

Effect power levels in microwave of *E.Coli O 157:H7* from bovine milk and Soft Cheese samples in Babylon Province

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Abstract:

Colonies of *E.Coli O157:H7* were isolated from 80 locally produced Cow's and Buffalo's soft cheese samples that were collected randomly at weekly intervals (5 samples/week) from various retail markets in different locations of babylon province during two climatic periods(40 samples /species/season) where the first periad was extended from the begining of December 2015 to the end of February 2016 while the second period was extended from the begining of July to the end of September 2016.In addition,50 fresh cattle faecal samples were collected from different farms in babylon province for the isolation of *E.Coli O157:H7*.The identification of *E.Coli O157:H7* isolates were confirmed based on their cultural, biochemical and seralogical characteristics using the commercial latex agglutination test kit .Data revealed that there was a significant (P≤ 0.05) seasonal variation in the prevalence of *E.Coli O157:H7* where all Cow's and Buffalo's soft cheese samples had significantly (P≤ 0.05) higher prevalence of *E.Coli O157:H7* in summer season (50% and 40% respectively) than in winter season (25% and 15% respectively).It was found that all of the 80 bovine soft cheese samples had significantly (p≤0.05) higher prevalence of *E.Coli O157:H7* in summer (45%) than in winter (20%) seasons.

In addition to that, all the 50 bovine faecal samples had significantly (P≤ 0.05) higher prevalence of *E.Coli O157:H7* in summer (72%) than in winter (40%) seasons. Complete elimination of viable *E.Coli O157:H7* was achieved when the inoculated milk was subjected to the microwave power level of 900 watts after 30 seconds of exposure. Complete elimination of viable *E.Coli O157:H7* was acheived when the contaminated soft cheese samples were subjected to the microwave power level of either 300 or 450 watts for 60 seconds of exposure.

KEYWORDS: ***E. Coli O157:H7, Milk, Soft cheese,*** **microwave , *Babylon***.

تاثير مستويات مختلفة من الموجات الصغرى الكهرومغناطيسية للاشريشيا القولونية *E.Coli O157:H7* والمعزولة من عينات الحليب والجبن المحلي للابقار والجاموس في محافظة بابل

الخلاصة

عزلت الأشريشيا القولونية *E.Coli O157:H7* من 80 عينة من عينات جبن الأبقار والجاموس محلي الصنع والتي جمعت بشكل عشوائي لفترات أسبوعية (5 عينة/ أسبوع) من أسواق البيع في مناطق مختلفة من محافظة بابل خلال فصلين (40 عينه/لكل نوع /فصل) حيث أمتدت الفترة الاولى من شهر كانون الأول 2015 الى نهاية شباط 2016 بينما الفترة الثانية أمتدت من بداية شهر تموز الى نهاية شهر أيلول 2016 بالاضافة الى ذلك

جمعت 50 عينة براز من الابقار من مختلف الحقول في محافظة بابل لغرض عزل الاشريشة القولونية *E.Coli O157:H7* .تم عزل وتشخيص الاشريشية القولونية *E.Coli O157:H7* أعتمادا على الزرع والخصائص الكيموحيوية والسيرولوجية باستعمال أختبار تلازن اللاتكس التجاري . تشير البيانات على وجود فرق معنوي($P\leq 0.05$) باختلاف الفصل في أنتشار الاشريشيا القولونية *E.Coli O157:H7* في كل عينات جبن الابقار والجاموس وكان الأنتشار عالي للاشريشيا القولونية *E.Coli O157:H7* في فصل الصيف (50%و 40%على التوالي) مقارنة بفصل الشتاء (25% و 15% على التوالي). وجد بأن في جميع عينات الجبن الطري المحلي (80 عينة لها أنتشار عالي المعنوية من الاشريشيا القولونية *E.Coli O157:H7* خلال فصل الصيف 45% مقارنة بفصل الشتاء 20% , بالاضافة لذلك وجد ان جميع عينات البراز البقري لها أنتشار عالي المعنوية للاشريشيا القولونية خلال فصل الصيف 72 % مقارنة بفصل الشتاء 40% . فقدت الأشريشيا القولونية نموها *E.Coli O157:H7* في الحليب بعد تعرضه للموجات الصغرى الكهرومغناطيسية (microwaves) بقوة 900 واط بعد 30 ثانية . تم القضاء على أعداد الأشريشيا القولونية *E.Coli O157:H7* في عينات الجبن الطري تماماً بعد تعرضه للموجات الصغرى الكهرومغناطيسية (microwaves) بقوة 300 أو 450 واط لمدة 60 دقيقة .

الكلمات المفتاحية:الاشريشيا القولونية *E.Coli O157:H7* ,حليب ,جبن,الموجات الصغرى الكهرومغناطيسيه, محافظة بابل

Introduction:

Since the identification of *E.coli O157:H7* as a human pathogen in 1982 (Fratamico and Smith,2006), *E.coli O157:H7* has become a pathogen of major concern for the food and dairy products because of its ability to cause severe illness,in particular,hemorrhagic colitis,hemolytic,uremic syndrome and thrombotic thrombocytopenic purpura(Govaris et al.,2001; Maher et al.,2001). .Most of the food borne outbreaks of *E.coli O157:H7* have been associated with the consumption of foods originated from cattle, especially foods contaminated with cattle faeces,because *E.coli O157:H7* has been found regularly in healthy cattle faeces,which is known to be an asymptomatic carrier **(**Öksüz et al.,2004).In Iraq,similar to other countries,domestic cheeses are still very popular which usually produced from raw milk with insufficient hygienic quality.A large amount of traditional cheeses are manufactured from raw milk and consumed freshly or after ripening in salt brine.Over the last several years, detection methods of STEC in foods have been significantly developed from culture-based methods into DNA-based and immune assays with each method having its strengths and weaknesses (Derzelle *et al.*,2011).

Microwave heating is caused by the ability of the food materials to absorb microwave energy and convert into heat .The presence of moisture or water causes dielectric heating due to the dipolar nature of water .when an oscillating electric field is incident on the water molecules , the polarized dipolar molecules try to realign in the direction of the electric fieled.Because of the high frequency electric fieled ,this realignment occurs at a million times per second and causes internal friction of molecules resulting in the volumetric heating of the food materials (Chandransekarn et al.,2013).The temperture and time of the heating during the microwave process depend on a number of factors such as composition ,shape,density,size,quantity and physical state of the food materials

Materials and methods:

A total of 80 locally produced cow's and buffalo's soft cheese samples (250 gm each)were collected randomly at weekly intervals (5 samples /week) in a sterile 500ml polyethylen plastic bags from various retail markets in diffrenet locations of Babylon provinces during two climatic periods (20 samples /species/ season),where the first period was in winter that extended from the begining of December 2015 to the end of February 2016 while the second period was in summer that extended from the begining of July to the end of September 2016.All the collected

cheese samples (250 gm each)were transported to the laboratory of veterinary public health department at the collage of veterinary medicine inside a protable ice-cooled box.All the microbiological tests were performed on arrival of samples in order to isolate and identify the *E.coli* O157:H7 from the samples.

Preparation of *E.coli* O157:H7 inoculums:

By using a sterile platinum loop pure five colonies of *E.coli*O157:H7 were transferred from overnight old culture (18-24) hours on nutrient agar to a tube containing 5ml of sterile nutrient broth and the count of approximately 1×10^6 cfu/ml was determined after aerobic incubation for 24 hours at 37°C.The bacterial counts were confirmed by preparing serial ten-fold dilutions of an inoculums in sterile peptone water and pour plated in duplicates for each dilution with VRB agar.The colonies were counted after incubation for 24 hours at 37 °C under aerobic condition (Khudhir.,2011).

CHROMagar™ E .coli O157:

According to the desired quantity ,the chromogenic medium powder was weight out based on the proportion of 29 gm per one liter of purified distilled water. The chromogenic powder was dispensed in distilled water slowly by rotating the mixture until swelling of the agar and dissolved by heating to not more than 100 °C (boiling) with stirring regalarly and then cooled inside a water bath to 48 °C.The chromogenic medium at 48 °C was poured onto sterile disposable petri dishes and let to solidify. The chromogenic petri dishes were kept under refrigeration (4 °C) storage for several days until used.

Rapid Latex test kit:

Latex agglutination test kit as Remel Wellcolex Diagnostic Kit was imported from Remel Europe Ltd clipper Boulevard wet, cross ways Dartford, Kent,UK.

Results:

This study includes detection of the prevalence of verotoxin- producing *E.Coli O157:H7* (VTEC O157:H7) in collected samples from locally made soft
cheese after isolation on selective media and identification by biochemical tests, latex agglutination test.The seasonal variation in the prevalence of *E.coli O157:H7* in the locally produced bovine soft cheese samples collected from different local retail markets in Babylon province is shown in Table 1, 2 and 3 .The results established the statistically significant ($p\leq0.05$) influence of the season on the prevalence of *E.coli O157:H7* in the bovine soft cheese samples.Data revealed that there was a significant ($p\leq0.05$) seasonal variation in the prevalence of *E.coli O157:H7* where all the cow's and Buffalo's soft cheese samples had significantly ($p\leq 0.05$) higher prevalence of *E.coli O157:H7* in summer season (50% and 40% respectively) than in winter season (25% and 15% respectively).It was also found that all of the 40 cows and Buffalos soft cheese samples that were collected for each season had significantly ($p\leq0.05$) higher prevalence of *E.coli O157:H7* in summer season (45%) than in winter season (20%).

Table (1):The prevalence of *E.coli O157:H7* in locally produced bovine soft cheese samples collected from Babylon province during the summer season.

Source of cheese	Number of sampels examined	Number of postive sampels	Percentage of positive isolating samples
Cows	20	10	50%
Buffaloes	20	8	40%
total	40	18	45%

Table(2):The prevalence of *E.coli O157:H7* in locally produced bovine soft cheese samples collected from Babylon province during the winter season.

Source of cheese	Number of sampels examined	Number of postive sampels	Percentage of postive samples
Cows	20	5	25%
Buffaloes	20	3	15%
total	40	8	20%

Table (3):Seasonal variation in the prevalence of *E.coli O157:H7* in the locally produced bovine soft cheese samples collected from retail markets in Babylon province

Source of cheese	Number of samples examined per season	Percentage of positive samples	
		summer	winter
Cows	20	50%	25%
Buffaloes	20	40%	15%
Total	40	45%	20%

The viability of *E.Coli O 157:H7* in milk subjected to different power levels in microwave:

The mean values of the total survivars of *E.Coli O 157:H7* that were enumerated in inoculated milk immediately after the exposure to each of the four power levels in microwave for 30 and 60 seconds are shown in Table 4. .Microwave power level for different exposure times had significantly ($p \leq 0.05$) influenced the inactivation degree of *E.Coli O 157:H7* in milk.Microwave power level of 300 watts for 30 and 60 seconds of exposure produced a significant ($P \leq 0.05$) reduction of *E.Coli O 157:H7* where the starting initial count of 7.60 log cfu/ml was reduced to 5.4 and 0.0 log cfu/ml after 30 and 60 seconds of exposure respectively .Increasing the microwave

power level up to 450 watts for 30 and 60 seconds of exposure increased the inactivation degree of *E.Coli O 157:H7* in milk to 3.77 and 0.0 log cfu/ml respectively . Microwave power level of 600 watts for 30 and 60 seconds of exposure resulted in a further increase in the inactivation degree of *E.Coli O157:H7*where additional significant ($p<0.05$) reduction of *E.Coli O 157:H7* in milk to 2.30 and 0.0 log cfu/ml respectively .Complete elimination (inactivation) of viable *E.Coli O 157:H7* was achieved when the inoculated milk was subjected to the microwave power level of 900 watts after 30 seconds of exposure.

Table(4):The viable counts of *E.Coli O157:H7* in milk subject to different power levels and times in microwave.

Exposure time (seconds)	Counts of E. Coli O157:H7 log CFU/ml Mean ± SE			
	300 watts	450 watts	600 watts	900 watts
0	7.6000±.01155 a	7.6067±.01202 a	7.6000±.0057 a	7.6000±.01732 a
30	5.4000±.01732 Ab	3.7700±.00577 Bb	2.3000±.0057 Cb	.0000±.00000 Db
60	0000 ± .00000 Ac	.0000 ± .00000 Ac	0000 ± .00000 Ac	.0000 ± .00000 Ab

- LSD=0.009
- Different small letters in column denote significant ($p<0.05$) differences among incubation times.
- Horizontal different capital letters denote significant ($p<0.05$) differences between the control and the different power levels in microwave .
- SE=Stander Error.

The viability of *E.Coli O 157:H7* in contaminated soft cheese subjected to different power levels of microwave.

The mean values of total survivors of *E.Coli O 157:H7* that were enumerated in contaminated soft cheese immediately after the exposure to each of the two power levels in microwave (300 and 450 watts) for 30 and 60 seconds are shown Table 5. Microwave power levels for two different exposure times had significantly ($p\leq0.05$) influnced the inactivatoin degree of *E.Coli O 157:H7* in contaminated soft cheese.Microwave power level of 300 watts for 30 and 60 seconds of exposure produced a significant ($p\leq0.05$) reduction of *E.Coli O 157:H7* counts at the rate of 96% and 100% respectively where the starting initial count of 6.69 log cfu/gm was reduced to 5.30 and 0.0 log cfu/gm after 30 and 60 seconds of exposure respectively .increasing the microwave power level up to 450 watts for 30 and 60 seconds resulted in a further increase in the inactivation degree of *E.Coli O 157:H7* in soft cheese and produced a significant ($p\leq0.05$) reduction of *E.Coli O 157:H7* counts at the rate of 99% and 100% respectively where the starting initial count of 7.30 log cfu/gm was reduced to 4.95 and 0.0 log cfu/gm after 30 and 60 seconds of exposure respectively.Complete inactivation of viable *E.Coli O157:H7* was acheived when the contaminated soft cheese was subjected to either 300 watts or 450 watts for 60 seconds of exposure.

Table(5):The viable counts of *E.Coli O 157:H7* in soft cheese subjected to two power levels of microwave for 30 and 60 seconds.

Microwave power (watts)	E. Coli O157:H7. Counts (log cfu/gm) Mean±SE				
	Exposure time (sec.)				
	O Seconds	30 Seconds	% of reduction	60 Seconds	% of reduction
300	6.6900± 00577 Ab	5.3000±.00577 Ba	96%	.0000±.00000 C	100
450	7.3000±.01155 Aa	4.9500±.01155 Bb	99%	.0000±.00000 C	100

- LSD=0 .01
- Horizontal different capital letters denote significant ($p<0.05$) differences among exposure times.
- Different small letters in a column denote significant ($p<0.05$) differences between microwave power.
- SE= Stander Error.

DISCUSSION:

Chromagar medium is considered very sensitive and highly selective for identifying *E.coli O157:H7*.Typical *E.coli O157:H7* colonies appeared as mauve colour on Chromagar while other bacteria appeared as blue colonies.Typical colonies of *E.coli O157:H7* appeared white on the nutrient agar and on the Eosin Methylene Blue agar appeared as green metallic sheen (Macfadden.,1985).*E.coli O157:H7* isolates were cultured on selective media to confirm their identification for motility and biochemical tests. Variation in the microbiological finding occurred due to different factors such as the quality of raw milk samples, sensitivity of isolation and identification methods, species of animal, number of animals on the dairy farms and the management practices in these farms (Jayarao *et al*., 2006). Milk Utensils or the hands of the milkers and manufacturers played a larger role in the contamination of products made from raw milk and /or re-contamination of the products made from pasteurized milk (Post Processing contamination) (Lourde et al., 2005).Source of many foodborne outbreaks mostly attributed to the contaminated dairy products with the fecal materials (CDC 2006).The Tables 1,2 and 3 indicated that the locally produced soft cheese samples were contamination with Eentrohaemorrhagic Escherichia coli(*E.coli O157*) higher in summer compared to in winter seasons, where the proportion of isolation during the summer was (45%) and in the winter was (20%), and this percentage was very high compared to the rates globally documented isolation that had pointed out by Mora et al. (2007) who isolated the bacteria in 8 out of 102

samples of bovine soft cheese in Peru (7.8%) and (7.6%) of soft cheeses made from raw cow's milk in Canada (Honish et al., 2005) also had been isolated from 4% of soft cheeses in Turkey (Oksuz et al 2004).Dunn et al.,(2004).investigated that STEC were excreted at higher frequency in the warmer months and at lower frequency in cold months.Cases of *E.coli O157: H7* outbreaks in humans were seasonal with the majority occurring between June and September (Besser et al .,1999). Flies have also been found to carry *E.coli O157: H7* and can be responsible for transmission on farms (Ahmed et al.,2007; Alam et al .,2004).Both the mastitic udder and the fecal contamination are regarded as the important routs for the *E.coli O157: H7 to* enter the milk supply (Lira et al.,2004). Contaminated ground with feces has been identificd as the source of infection in 48 out of 196 *E.coli O157: H7* outbreaks documented in USA between 1982 to 1998 (Menget et al., 2001).

The viability of *E.Coli O 157:H7* in milk subjected to different power levels in microwave:

In this study the mean values of the total survivars of *E.Coli O 157:H7* that were enumerated in inoculated milk immediately after the exposure to each of the four power levels in microwave for 30 , 60 and 90 seconds are shown in Table 4. *E.coli O157:H7* grows best within a temperature range of 30 to 42°C and the optimal temperature being 37°C (Association, N. A. M. 2010).but *E. coli O157:H7* does not grow well at 44 to 45.5°C,(CDC. 2009).In the present study *E.Coli O 157:H7* counts reduced to 5.4 , 3.77, 2.3 and 0.0 log cfu/ml after 30 seconds of exposure to microwave power levels of 300,450,600 and 900 watts respectively.Complete elimination (inactivation) of viable *E.Coli O157:H7* was achieved when the inoculated milk was subjected to the microwave power level of 900 watts after 30 seconds of exposure.

Several investigators had studied the comparison of heat resistance at 55 ^{0}C and 60^{0}C of log phase and late stationary phase cultures growen at 37 ^{0}C and confirmed that late stationary phase cultures had greater heat resistance as seen earlier in *E.Coli O 157:H7* (Todd et al.1993) and many other bacteria (Beuchat and Lechowich 1968; Griffiths and Haight 1973; Hurst et al.1974).Increased thermotolerance by varying the heating rate has been reported for several bacteria (Stephens et al.1994).The presence of sugers and /or salt in a food product or due to bacteria dispersing into fatty components (e.g. as in comminuted meat production),might increase their survival during the cooking process .Heat resistance of some bacteria increases on exposure to temperatures slightly above their optimum for growth (Foster and Spector 1995),or when they are heated slowly as might happen in slow cooking of food (Mackey et al 1987). Microwaves have been applied in a broad range of food processing such as drying ,tempering cooking, pasteurization and sterilization (Puligundla et al .,2014).Microwave heating has gained popularity in food processing due to its ability to achieve high heating rates,reduction in cooking time,uniform heating,safe handling,ease of operation and low maintenance(Zhang et al.,2006).In addition ,microwave heating might change flavor and nutritional qualities of food in a lesser extent as opposed to the conventional heating during cooking or reheating process.(Vadivambal and Jayes,2010).Besides that, the conventional heating methods required higher energy consumption and relatively longer processing time(Varith et al.,2007).

The viability of *E.Coli O 157:H7* in contaminated soft cheese subjected to different power levels of microwave .

The mean values of total survivors of *E.Coli O 157:H7* that were enumerated in contaminated soft cheese immediately after the exposure to each of the two power levels in microwave (300 and 450 watts) for 30 and 60 seconds are shown Table 5. Microwave power level of 300 watts for 30 and 60 seconds of exposure produced a significant ($p \leq 0.05$) reduction of *E.Coli O 157:H7* counts at the rate of 96% and 100% respectively where the starting initial count of 6.69 log cfu/gm was reduced to 5.30 and 0.0 log cfu/gm after 30 and 60 seconds of exposure respectively.These results were similar to those reported by Jackson et al. (1995), who observed between 4 and 6 logs reductions of *E. coli* O157:H7 in 114 gms ground beef patties cooked to 68.3°C (155°F) on a snap action grill.Complete inactivation of viable *E.Coli O157:H7* was acheived when the contaminated soft cheese was subjected to either 300 watts or 450 watts for 60 seconds of exposure. Spano, et al.(2003) indicated that *E. coli* O157:H7 disappeared completely during stretching of curd for 5 minutes in hot water at 80°C during the manufacturing of Mozzarella.In microwave heating ,the heat is generated inside the food in ashort time during the microwaves penetration (Decareau,1985). Microwaves have graeter penetration depth leading to rapid heating rate within short processing time and contribute to the minimization of temperature difference between the surface and the interior of food materials (Withkiewicz and Nastaj.2010).Due to the high ferquency electric field in microwaves realignment of polarized dipolar molicules occurs at a million times per second and causes internal friction of molecules resulting in the volumetric heating of the food materials (Chandrasekaran et al.,2013).

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