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THE ANTI-HELICOBACTER PYLORI ACTIVITY OF CURCUMIN DURING THE MANUFACTURE AND STORAGE OF LABORATORY-PREPARED ICE CREAM

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ABSTRACT

Helicobacter pylori is a foodborne pathogen of major significance resulting in gastric cancer which has become a worldwide problem. So, the present study aimed to evaluate the survivability of this bacterium pre-inoculated into ice cream prior to curcumin addition with different concentrations. From the in vitro study using broth dilution and agar diffusion method, it was obvious that the MIC of curcumin against *H.pylori* growth was 6.25%. Meanwhile, the observed results of the in vivo study about the impact of addition 5, 6 and 7% of curcumin on the survival of *H.pylori* that inoculated in ice cream during manufacture and storage predicted the advantages of curcumin addition, as it inhibited *H.pylori* growth, making it as a potential complementary medicine for curing *H.pylori* related infection in a dose-dependent manner, as the rapid and complete inhibition of its growth was firstly observed with a high concentration of 7% curcumin at the end of the 2nd week of ice cream storage at freezing temperature (-5 C°) versus to control samples. Furthermore, the addition of curcumin to ice cream did not affect the sensory properties of the product, as it acquired a higher score for attributes.

Keywords: curcumin, *H.pylori*, ice cream, anti-*Helicobacter* activity.

INTRODUCTION

From an epidemiological perspective, Helicobacter pylori holds a significant position in relation to human health. According to the socioeconomic status and sanitary conditions, researchers thought to affect more than 50% of the world's population (Otero, 2017). It is a Gram-ve, motile, and microaerophilic bacterium (Kao *et al.,* 2016). According to Dunn *et al.* (1997) and (Paniagua- Contreras *et al.,* 2007), the prevalence is highest in developing nations 90%, like Africa (79.1%), while the lowest prevalence is in industrialized nations 50%, like North America (37.1%). Throughout their lives, people may contract this bacterium (Quaglia and Dambrosio, 2018).

H. Pylori was first discovered in the stomach of patients with gastritis and ulcers in 1982 by Marshall and Warren (Cervantes-Garcia, 2016) who was awarded the 2005 Nobel Prize in Medicine. *H. pylori* became a well-known human pathogen, which infected the stomachs of patients leading to gastritis. This infectious disease can lead to some complications, such as peptic ulcer. Other seldom complications, in some infected

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people (1-5%), are lymphoma of the lymphoid tissue associated with gastric mucosa (MALT) and gastric cancer. It is considered a type 1 carcinogen by the World Health Organization (WHO, 2010), the most frequent factor (44.4%) in the development of gastric cancer, killing nearly 800,000 people worldwide in 2018 (Otero *et al.,* 2018). H.pylori possesses several factors that make it a very tenacious pathogen, due to the production of virulence factors, the ability of biofilm formation and resistance to different classes of antibiotics.

H.pylori routes of transmission are vague (Vale and Vitor, 2010). The human stomach is one of the main reservoirs of this bacterium, considering that the source of infection can be gastro-oral, oral-oral, fecal-oral, and through contaminated food (Otero, 2017; Zamani *et al.*, 2017 and Quaglia *et al.*, 2020). The minimum infectious dose of H.Pylori is low (105 cfu Graham *et al.* (2004). Even small numbers can pose a potential risk to humans (Quaglia *et al.,* 2020). This pathogen has been detected in foods of animal origin including milk and dairy products considering it a food pathogen (Quaglia and Dambrosio, 2018). The hygienic quality of milk and dairy products is very important since they are consumed daily by millions of people around the world (Mousavi *et al.*, 2014).

Due to serious problems caused by *H.pylori* infection, its detection and elimination is a must. However, this is a challenge because the current schemes for this purpose are less effective due to its resistance to antibiotics. So, "the WHO has included this pathogen among those that threaten humanity and that urgently merit measures for its control" as stated by Cervantes-Garcia, (2016). Mousavi *et al.* (2014) studied the antibacterial resistance of *H.Pylori* isolated from raw milk and dairy product samples including ice cream, therefore, several researches have been carried out to control its growth in foods, this area focuses on new therapeutic solutions. One of these approaches was the use of natural antimicrobial compounds without causing a negative impact on food quality. This turns into a practical way for quality control and becomes a strong trend in food production (Fei *et al.,* 2018). Investigation of medicinal plants as a safe alternative is gaining importance among scientists both for human health and the food industry (Doman and Deans, 2000). Turmeric (Curcuma longa), a tropical rhizomatous herb of the family Zingiberaecae, is regarded as the golden spice (Samarasinghe *et al.,* 2003). Curcumin is a yellow pigment, found in turmeric and is generally regarded as safe (GRAS) and is known to possess variable biological and pharmacological activities, such as anti-inflammatory (Verma *et al.,* 2019), antioxidant (Asouri *et al.,* 2013), anti-bacterial (De, *et al.,* 2009), antimutagenic (Araujo and Leon, 2001), anti-arthritis and anti-diabetic (Rathore *et al.,* 2020), In addition, curcumin has considerable clinical safety with relative nontoxicity and has a history of use as an herbal remedy, dietary spice and food coloring agent in East Asia (Lao *et al.,* 2006). Curcumin is considered a major active compound that has an inhibitory activity against 19 strains of H. pylori in vitro with MICs ranging from 6.25-50 ug/ml (Mahady *et al.,* 2002).

Currently, there are no commercial ice creams with anti-*H.pylori* characteristic. Hence, it is interesting to investigate the effect of curcumin when added to ice cream base on the growth of *H.pylori*. So, the objective of this study aimed to study the effect of curcumin against *H.pylori* during the storage of lab-prepared ice cream.

MATERIALS AND METHODS

Bacterial strain:

The H.pylori strain was obtained from the Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, Egypt. The strain was propagated in *H.pylori* special peptone broth for 24 hrs at 37℃ under microaerophilic conditions. 1 millilitre

of the culture was serially diluted in sterile saline to bring turbidity to 0.5 Mcfarland standard (1.5×10^8/ml). The previous suspension is the standard strain suspension of which 1 ml of *H. pylori* suspension may contain approximately $\log10^5$cfu/ml (Quinn *et al.,* 1994). Muller Hinton agar supplemented with 7 % horse blood was used for counting the organism after serial dilution.

Preparation of curcumin extracts (Nostro *et al.*, 2005):

Curcumin powder bottle was obtained from a supermarket in Assiut City, Egypt. Known amounts of powdered curcumin (10 g) were extracted with 100 ml of 95 % ethanol at room temperature for 24hrs (ratio 1:10). The extracts were strained through paper, centrifuged (9000rpm at 4C°) and concentrated to dryness using a rotatory evaporator at 40 C°.

Determination of MIC and MLC of curcumin extract (Jayana *et al.*, 2010):

Different concentrations of curcumin extracts (2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%) were prepared using sterile Muller Hinton broth. Each tube was inoculated by 100 µl from the 48 age culture of *H.pylori* to obtain an inoculum of 1×10^5 cfu/ml broth. The tubes together with the control tubes were incubated under microaerophilic conditions for 72 hrs at 37 C°. The results were interpreted based on the fact that growth occurs in the positive control and any other tube in which the concentration of curcumin extract is not sufficient to inhibit growth.

The lowest concentration of the extract that inhibited the growth of organisms as detected by lack of visible turbidity was MIC. However, in some cases, it was difficult to identify whether the turbidity was due to the growth of bacteria or due to the turbidity of the extract itself, the tubes were subcultured on Columbia agar plate (Oxoid CM271) supplemented with defibrinated horse blood after 24, 48 and 72 hrs of incubation, then they examined for bacterial growth.

Growth of M.os from any incubation period at a particular conc. indicated that the lethal conc. was not achieved. MLC was defined as the lowest conc. of tested substances that killed the tested organism (No growth at the given conc. Within 72 hrs). The mean MIC and MLC were recorded from triple reading in each test.

Preparation of ice cream:

The ice cream powder packets were obtained from a supermarket in Assiut City, Egypt. Then, ice cream was prepared according to the label instructions of the product. The prepared ice cream was divided into 4 groups (each group: 200 ml) and treated with curcumin extract according to the following concentrations:

1st group without curcumin extract.
2nd group was treated with 5% curcumin extract.
3rd group was treated with 6% curcumin extract.
4th group was treated with 7% curcumin extract

Sensory evaluation:

Each group of ice cream was sensory evaluated according to Poste *et al.* (1991). The sensory attributes include appearance**,** body and texture, flavour, mouth feel and overall acceptability (OAA). All samples were scored by a regular score panel. The score was based on a hedonic scale provided in a scorecard comprising the 9-point hedonic scale (9= extremely like, 8= very much like, 7= moderately like, 6= slightly like, 5= nor like neither dislike, 4= slightly dislike, 3= moderately dislike, 2= very much dislike, 1= extremely dislike).

Inoculation with bacterial strain:

Each group of samples (200 ml) was inoculated with *H.Pylori* to obtain a count of log 5 cfu/g.

All groups including the control were kept at domestic freezer (-5 C°). Samples were taken

before treatment (Zero time) or initial count to detect the count of *H.pylori*. Samples from each group were taken to detect the count of *H.pylori* after hardening and for the first 4 days consequently, and then every week for 5 weeks. The count of *H.Pylori* in samples was enumerated by a surface plating method, using a Columbia agar plate with 7% defibrinated horse blood, then the plates were incubated at 37 C°. for 48 hrs in microaerophilic condition (6%O_2, 10%CO_2 and 84%N_2) using an anaerobic jar and gas generating kits (Oxoid BR 56). All analyses were performed in triplicate for each concentration.

RESULTS

Table 1: Count of *H.pylori* (log/g) in ice cream samples fortified with curcumin during manufacture and storage at freezer temperature (-5 C°).

Storage period	Control ice cream samples	Ice cream with curcumin conc		
		5%	6%	7%
Initial count			4.76	
1 st day	4.8	4.71	4.63	4.48
2 nd day	4.81	4.66	4.54	4.38
3 rd day	4.82	4.59	4.45	4.28
4 th day	4.83	4.52	4.3	4.11
1 st week	4.83	4.43	4.15	3.85
2 nd week	4.83	4.32	3.78	0
3 rd week	4.84	4.15	0	0
4 th week	4.83	3.9	0	0
5 th week	4.85	0	0	0

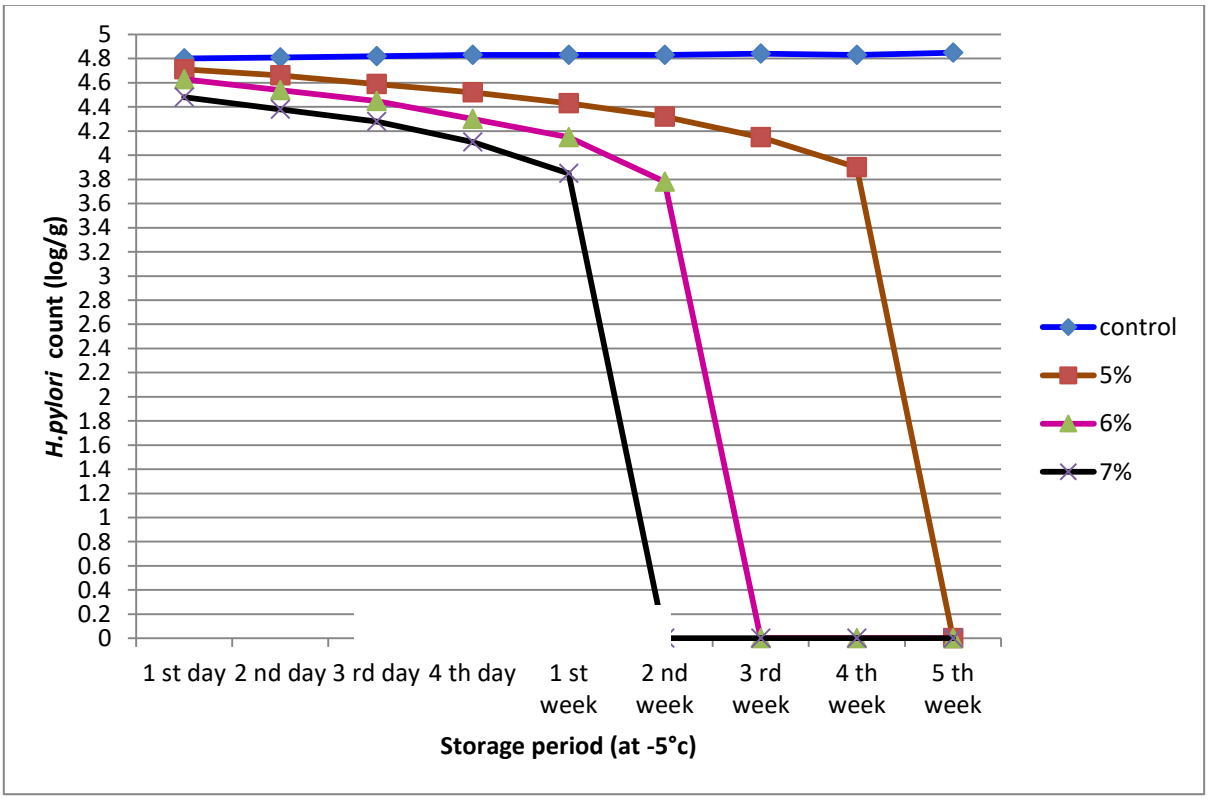

Fig.1: Effect of Curcumin on *H. Pylori* growth during the manufacture and storage of ice cream

Table 2: Sensory evaluation of the prepared ice cream samples treated with curcumin.

Attributes	1st group	2nd group	3rd group	4th group
Appearance	9	8	9	8
Body and texture	9	8	9	8
Flavour	9	8	9	8
Mouth feel	9	8	9	8
(OAA)*	9	8	9	8

***** OAA means overall acceptability

DISCUSSION

Ice cream is defined as the most popular smooth, sweet and cold dessert, prepared from a frozen mix of milk products and flavouring, containing a minimum of 10% milk fat (Karaman *et al.*, 2014). It is enjoyed and consumed by people of all ages all over the world due to its cooling and refreshing effect on the mouth. Nowadays, manufacturers continue to develop the formation of ice cream mix according to consumer demands using functional healthy foods, resulting in the creation of various brand names (Sun-Waterhouse *et al.*, 2013).

The increasing difficulties in the triple therapy of *H.pylori* due to incomplete cure, undesirable side effects, non-compliance among the patients, costs of antibiotics regimens, antibacterial resistance and other factors promote a need to develop efficient non-antibiotic agents against this pathogen that are plant-derived compounds, effective and low cost, have shown their potential as a therapeutic and chemo-preventive agent. Curcumin is known for its medicinal use, dating back to 4000 years ago in the Vedic culture in India, where it is used as a culinary spice and has some religious significance (Prasad and Aggarwal, 2011). It has been indicated considerable anticarcenogenesis (Darvesch *et al.,* 2012). Turmeric's medicinal properties have been attributed to curcuminoids and the main component present in the rhizome includes curcumin (Ammon and Whal, 1991). Curcumin possesses a broad pharmacological impact (Negi *et al.,* 1999) in addition to anti-*H.pylori* activity (Mahady *et al.,* 2002).

Based on the in vitro preliminary study which was conducted for screening of the anti-*H.pylori* activity of curcumin in this study, the minimum inhibitory concentration (MIC) of curcumin was 6.25% to determine the suitable amount of curcumin for adding different concentrations to ice cream using the agar diffusion method. The MIC was defined as the lowest concentration which inhibited the growth of the respective *H. pylori* strain. there are several studies with different outcomes that evaluated the impact of curcumin on growth. For instance, the obtained results support those obtained by Mahady *et al.* (2002 and 2005) who proved that curcumin possesses an in vitro inhibitory activity against *H.pylori* growth with MIC ranged from 6.25-50 ug/ml, while Pattlyathanee *et al.* (2009) revealed that curcumin was capable to inhibit the growth of *H.pylori* at MIC value of 16 ug/ml, moreover, Thong-Ngam and Chatsuwan (2007) assessed curcumin antibacterial activity with MIC of 64 ug/ml. In addition, the data in this study was compared with those obtained by Ali (2020) who evaluated the antibacterial activity of curcumin against *H.pylori* with MIC 12.5%.

The present results in the current study indicated that control ice cream samples (without curcumin) did not show noticeable changes in *H.pylori* count after inoculation with 10^5cfu/g of *H.pylori* at zero time (finished ice cream), then the *H.pylori* count increased gradually till the 5th week of storage at freezing temperature and it

retained its growth, reaching 4.85 log (Table.1 and Fig.1).

Notably, the addition of 5 and 6 % curcumin to ice cream led to a gradual decrease of *H. pylori* count in a rapid manner from the 1st day, until complete elimination at the 5th and 3rd week of storage at domestic freezer temperature (-5) with a log reduction 3.9 and 3.78 log/g, respectively. Meanwhile, when the concentration increased to 7% the growth of *H. pylori* vanished at the end of 2nd week of storage versus control samples as curcumin had a powerful inhibitory effect on its survival. The activity of curcumin against *H. pylori* was observed in concentration concentration-dependent manner. Hence, ice cream manufactured with the addition of curcumin does not create risk for consumers these results confirmed what was published by Pathlyathane *et al.* (2009) who showed that curcumin had anti *H.pylori* activity. Curcumin possesses bio-protective properties due to its content of phenolic compounds named curcuminoids (Goel, 2009).

Many authors, indicated that humans may become infected with *H.pylori* through food (Angelidis *et al.,* 2011), as it could be detected in food of animal origin including ice cream (Quagilia and Dambrosio 2018). In a study, Mokhtar (2004) could detect *H. pylori* in 2% of the examined ice cream samples, moreover, El-Khawaga (2006) could isolate *H.pylori* from ice cream samples, while Saad and El-Zamkan (2017) isolated *H.pylori* from 3.3% of the examined ice cream samples.

Several studies have cleared the favorable broad-spectrum of antibacterial efficiency of curcumin as it showed a significant effect against 65 isolated strains of *H.pylori* (De *et al.,* 2009). On the other hand, (Baker (2020) demonstrated that curcumin has anti-*H.pylori* potentials. Ice cream has been considered an ideal medium to deliver curcumin, due to its neutral pH, it is highly accepted by different populations (Xiao *et al.,* 2014) and it could provide extra nutritional value. Nevertheless, only a few studies have cleared the application of curcumin in this product (Sunwaterhouse *et al.,* 2013 and Kumar *et al.,* 2016).

It is clear from Table 2 that the results of the sensory evaluation of ice cream were highly satisfactory, as the grades of overall acceptability (OAA) remained between 9 given to ice cream with 6 % curcumin and 8 given to the ice cream with 5 % and 7% added curcumin. Nearly all ice cream received the same grades of appearance, body and texture, flavour and mouth feel as the control ice cream. Kumar *et al*. (2016) found that there was no major difference between the sensory properties of curcumin added and the control ice cream. The improvement in sensory acceptance and quality of ice cream suggested that curcumin a natural product derived from plant, had a good potential to be applied in ice cream as a natural coloring and an alternative preservative because it has good antibacterial effects against *H.pylori* in vitro and vivo with health benefits for consumers.

In conclusion, this study showed the significant antibacterial activity of curcumin against *H.pylori*. Thus, it an interesting to be addressed in future studies on this subject to assure the consumers with a safe food supply.

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التأثير المضاد للكركمين على نشاط جرثومة المعدة (الهيلكوباكتر بيلوري) أثناء تصنيع وتخزين الآيس كريم المصنع معملياً

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تم عمل هذه الدراسة للوقوف على فاعلية استخدام الكركمين فى مقاومة ميكروب الهيلوباكتر بيلوري, المسبب لالتهاب المعدة والأمعاء والذى فى النهاية يؤدى إلى السرطان، من هذا المنطلق تم اختيار الآيس كريم كنموذج لمنتجات الألبان. وقد أظهرت الدراسة المبدئية أن الحد الادنى للتركيز المثبط للكركمين على نمو الميكروب تحت الدراسة هو ٦,٢٥% .

أما عن استخدام الكركمين بتركيزات ٥ ، ٦، ٧ % على حيوية الهيلوباكتر بيلورى أثناء تصنيع وتخزين الآيس كريم فى درجة حرارة الفريزر (- ٥° م) ، تم انخفاض عدد الميكروب تدريجياً (log/g) حتى الأسبوع الرابع والثانى وذلك عند اضافة تركيزى ٥ ، ٦% على التوالى ، كما لوحظ انخفاض كبير فى عدد هذا الميكروب حتى اختفى عند الأسبوع الثانى من التخزين حيث لم يتم عزله بعد استخدام تركيز ٧% من الكركمين. وقد أسفرت الملاحظات على أن اضافة الكركمين لم تؤثر على الخواص الحسية للآيس كريم بالسلب. هذا وتم مناقشة النتائج مع ذكر الاجراءات الصحية الواجب اتباعها للحد من تلوث الآيس كريم بميكروب الهيلوباكتر بيلورى حفاظا على صحة المستهلك.