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Ibrahim & Ahmaed

المجلة العراقية لبحوث السوق وحماية المستهلك

OPTIMAL CONDITIONS OF WEISSELLICIN PRODUCTION FROM A LOCAL ISOLATE OF *WEISSELLA CIBARIA*

***Ali Abdul Hussein Ibrahim*[1], *Asmaa Sabah Ahmaed*[2]**
[1]Department of Food Science, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq. ali.abd2202m@coagri.uobaghdad.edu.iq
[2]Professor PhD, Department of Food Science, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq. asmaa.s@coagri.uobaghdad.edu.iq

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ABSTRACT

Optimal conditions of weissellicin production from a local isolate of *Weissella cibaria* were studied. The sources included carbon sources, nitrogen sources, salts, temperature, pH, incubation time, and inoculum size. The best conditions for producing weissellicin from bacteria were fructose 15%, peptone 0.5%, yeast extract 0.5%, acidic di potassium phosphate 0.15%, incubation temperature of 37°C, pH 5, incubation duration of 48 hours, and initial inoculum volume 1 x 10^9 cells/ml. Peptone was studied at different concentrations. The best concentration was 1.5%. The yeast extract concentration was also investigated. the best concentration was 1%. The concentration of fructose 2.5% was the best. These concentrations achieved obtaining weissellicin of about 0.848 mg/ml.

Key words: Bacteriocin, weissellicin, *Weissella cibaria*, peptone.

الظروف المثلى لانتاج الويسليسين weissellicin من عزلة محلية من بكتريا *Weissella cibaria*

***علي عبدالحسين ابراهيم*[1]، *أسماء صباح أحميد*[2]**
[1] قسم علوم الاغذية، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق ali.abd2202m@coagri.uobaghdad.edu.iq
[2] الاستاذ الدكتورة ،قسم علوم الاغذية، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق asmaa.s@coagri.uobaghdad.edu.iq

الخلاصة

تمت دراسة الظروف المثلى لانتاج الويسليسين من عزلة محلية لبكتريا*Weissella cibaria* وقد شملت مصادر الكاربون ،مصادر النتروجين,الاملاح,درجة الحرارة,الرقم الهيدروجيني ,مدة الحضن وحجم اللقاح, وقد كانت افضل الظروف لانتاج الويسليسين من البكتريا كما يأتي : فركتوز 10%,الببتون 0.5%,خلاصة الخميرة 0.5%, فوسفات ثنائي البوتاسيوم الحامضي 0.15%,درجة حرارة الحضن 37م°,الرقم الهيدروجيني 5,مدة الحضن 48 ساعة وحجم لقاح ابتدائي 1×10^9خلية /مل . درس الببتون بتراكيز مختلفة وكان افضل تركيز هو 1.5%,كما درس تركيز خلاصة الخميرة وكان افضل تركيز هو 1%ودرس تركيز سكر الفركتوز ليكون 2.5% هو افضل تركيز وقد حققت هذه التراكيز الحصول على الويسليسين بحوالي 0.848 ملغم/مل.

الكلمات المفتاحية: بكتريوسين، الويسليسين، *Weissella cibaria*، بيبتون.

INTRODUCTION

Bacteriocins are generally produced by bacteria and they have the ability to kill or inhibit other bacteria, especially pathogenic ones. They are antimicrobial peptides that are manufactured by ribosomes **(Egan *et al*., 2016; Martinez *et al*., 2013)**. Bacteriocins are usually produced by lactic acid bacteria and are safe to use **(Castellano *et al*., 2008; Balciunas *et al*., 2013; Alvarez-Sieiro *et al*., 2016)**. The bacteriocins have been used as an alternative to chemical preservatives to ensure food safety and increase the shelf life of the product **(Zimina *et al.*, 2020; Kumariya *et al*., 2019; Kaveh *et al*., 2023)**. weissellicin is one of the bacteriocins produced by Wissella bacteria from various isolates such as *W.hellenica*. **(Masuda *et al*., 2012)** and *W.cibaria* **(Srionnual *et al*., 2007; Lan *et al*., 2009; Wu *et al*., 2015).**

Bacteriocins can be produced in large quantities in the laboratory under ideal chemical and physical conditions in the absence of limiting factors **(Leroy & Vuyst, 2000; Verluyten *et al.*, 2004; Van den Berghe *et al*., 2006)**. Bacteriocins are highly effective depending on the carbon and nitrogen sources, temperature, and pH available to the producing microorganism (**Todorov & Dicks, 2004; Furtado *et al*., 2009**). The source of carbon is an essential element for the growth of microscopic cells. It works to build and reproduce cells and is a source of energy **(Bhat *et al*., 1987; Akinyosoye *et al*., 1995)**. **Drosinos *et al*. (2005)** has been indicated that the best carbon source for biomass production is glucose, but fructose is more suitable for bacteriocin production. However, the nitrogen source plays an important role in the fermentation process or is involved in the synthesis of amino acids as they are the basic structural unit of proteins **(Abbasiliasi *et al*., 2017)**. **Upendra *et al*. (2016)** indicated that the best nitrogen source for producing bacteriocins from LAB is yeast extract.

Temperature plays an important role in the production of bacteriocins. It ranged between 30°C **(Casla *et al*., 1996)** and 37°C **(Balasubramanyam & Varadaraji, 1998; Gilbreth & Somkuti, 2005)**. **Daba *et al*. (1991)** have explained that the number of biomass cells used to produce bacteriocins from *Leuconostec mesenteroides* is 8 x 10^8. **Han *et al*. (2011)** also have indicated that the number of *L. plantarum* biomass cells reaches 10^9, which are necessary to produce bacteriocin **(Tawfeeq & Ahmaed, 2023)**. Thus, the study aimed to select optimal conditions of weissellicin that production from a local isolate of *W. cibaria.*

MATERIALS AND METHODS

Effect of optimal conditions for the production of weissellicin

A number of factors that influencing the production of weissellicin produced from the isolate *W.cibaria*, that isolated from a previous study**(Tawfeeq &Ahmaed, 2023)**. It was studied using synthetic media according to **(Suheil & Ahmaed 2023)**. Weissellicin was purified according to the method **(Mezher & Al-Bayyar**, **2018).**

Effect of carbon sources on production

Carbon sources were used including xylose, lactose, glucose, maltose, fructose, sucrose, and table sugar, each separately and at a concentration of 10%. The initial pH was adjusted to 6.5 and sterilized with milipore filter 0.45 µm the media were inoculated with a stimulant culture of the bacterial isolate. The inoculated media were incubated at 30°C for 48 hrs, then subjected to centrifugation × 4000 for 20 mins at 4°C and filtered through microbial filters with a porosity of 0.22 µm. The absorbance of the filtrate containing weissellicin was measured at a wavelength of 600 nm, which was taken as an indication of the amount of weissellicin produced **(Mahdy& Kalaichelvan, 2013; Mezher & Al-Bayyar, 2018).**

Effect of the nitrogen source on production

The carbon source and its percentage were determined. The nitrogenous sources including peptone, meat extract, malt extract, ammonium sulfate, ammonium phosphate, sodium nitrate, and ammonium chloride at a rate of 0.5% were used separately, and the same previous steps were followed. Then, the absorbance of the filtrate was measured at a wavelength of 600 nm, which was taken as an indication of the amount of viscosity produced **(Mahdy& Kalaichelvan, 2013; Mezher & Al-Bayyar, 2018).**

Effect of salts on production

The liquid synthetic medium was prepared using acidic di-potassium phosphate at different concentrations (0.75, 0.5, 0.25, 0.15, 0.1)% respectively. Same previous steps were followed, and then the absorbance of the filtrate was measured at a wavelength of 600 nm, which was taken as an indication of the amount of lysate produced **(Mahdy& Kalaichelvan, 2013; Mezher & Al-Bayyar, 2018).**

Effect of temperature

To determine the optimum fermentation temperature for the production of weissellicin from *W.cibaria* bacteria, temperatures were tested (40, 37, 30, 25, 10)°C. The same previous steps were followed, then the absorbance of the filtrate was measured at a wavelength of 600 nm, which was taken as an indication of the amount of lysate produced **(Mahdy& Kalaichelvan, 2013; Mezher & Al-Bayyar, 2018).**

The effect of pH

To determine the optimal pH for the production of weissellicin, effect of different values of pH was tested (8, 7.5, 7, 6.5, 6, 5.5, 5, 4.5, 4) and the same previous steps were followed. Then the absorbance of the filtrate was measured at a wavelength of 600 nm, which was taken evidence of the value of weissellicin produced **(Mahdy& Kalaichelvan, 2013; Mezher & Al-Bayyar, 2018).**

Effect of incubation duration

The optimal incubation period for the production of weissellicin was determined by studying the time periods (96, 72, 48, 24) mins and the same previous steps were followed. Then, the absorbance of the filtrate was measured at a wavelength of 600 nm, which was taken as an indication of the value of weissellicin produced **(Mahdy& Kalaichelvan, 2013; Mezher & Al-Bayyar, 2018).**

Effect of inoculum size

Gradient inoculum levels of *W. cibaria* were used in amounts of 1×10^6, 1×10^7, 1×10^8, 1×10^9, 1×10^{10}, and 1×10^{11} cells/ml of fermentation medium. The same previous steps were followed, and then the absorbance of the filtrate was measured at wavelength 600 nm, which was taken as an indication of the value of weissellicin produced **(Mahdy& Kalaichelvan, 2013; Mezher & Al-Bayyar, 2018).**

RESULTS AND DISCUSSION

Best conditions for the production of weissellicin from *W. cibaria*

Optimal production achieved through studying the various criteria that affecting the production and fermentation process **(Al Douree, 2014).** An indicator has been adopted to determine the best conditions.

Effect of carbon sources

It can be found that the effect of carbon sources, xylose, lactose, glucose, maltose, fructose, and sucrose, and sugar on the production of weissellicin from *W.cibaria*. The results showed that fructose was superior to fructose by 10%, as the concentration of weissellicin was 0.576 mg/ml compared to other carbon sources (Figure 1).

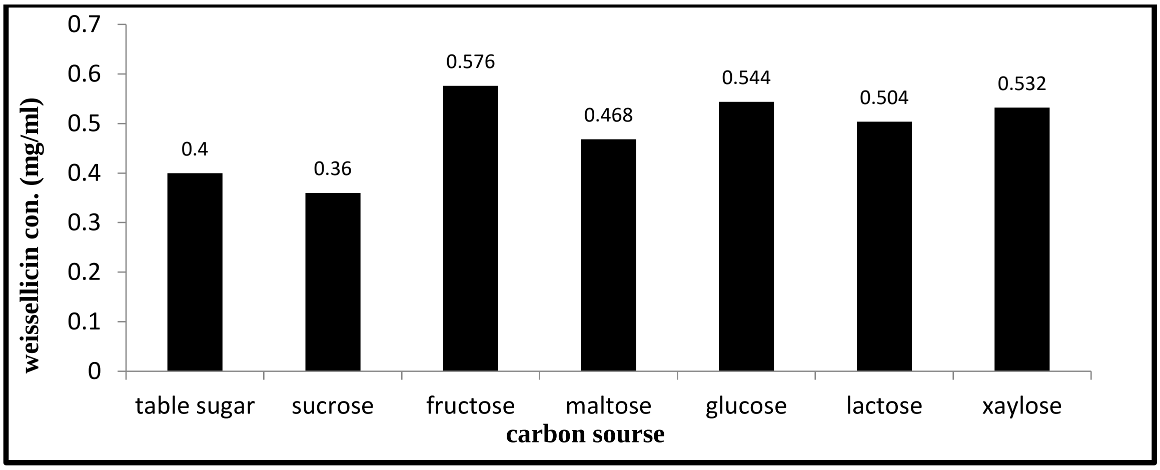

Figure (1): effect of carbon sources on the concentration of weissellicin produced from *W. cibaria*.

Different concentrations of fructose sugar were used. The results showed an increase in the amount of weissellicin produced using fructose by 2.5% to 0.612 mg/ml compared to other concentrations (1,5,10,15)%, respectively (Figure 2). Studies indicate the superiority of carbon sources from LAB vary, as **(Delgado *et al.*, 2007)** noted that glucose is the best source for producing bacteriocins, while the high production of bacteriocins in some LAB strains is linked to the inclusion of other sugars in the growth medium instead of using glucose alone. The bacteria may have an enzymatic system that allows them to use other sugars **(Al-Wendawi & Al-Saady, 2012)**.

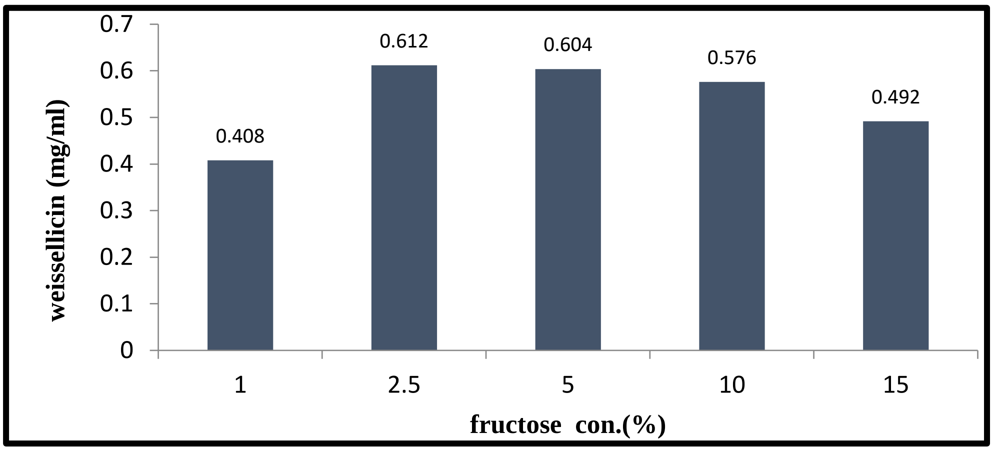

Figure (2): effect of fructose percentage on the production of weissellicin from *W. cibaria*
Effect of nitrogen sources.

Seven nitrogen sources were created at a rate of 0.5%, individually. It is noted from that the best nitrogen source in the production of weissellicin was peptone (Figure 3). The concentration of weissellicin was 0.636 mg/ml, while **(Mezher, 2017)** confirmed that the concentration of 1% peptone gave the highest concentration of weissellicin at 0.263 mg/ml.

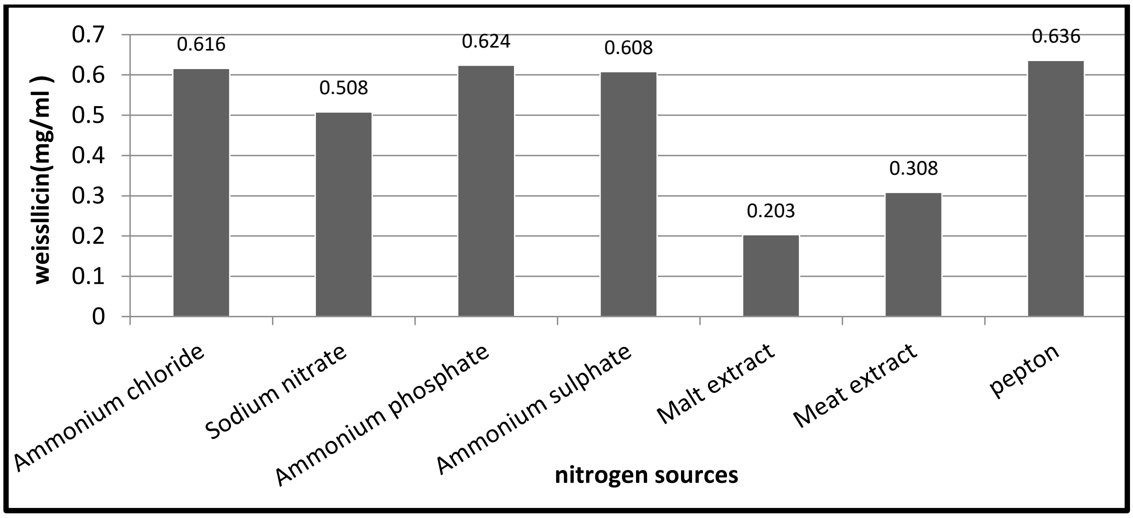

Figure (3): effect of nitrogen sources on the concentration of weissellicin produced by *W. cibaria*.

Figure (4) shows the use of different concentrations of yeast extract in the production of weissellicin, and the best concentration was 1%, reaching 0.776 mg/ml. Yeast extract works to increase the production of bacteriocins **(Riley *et al.*, 2003)**. It stimulates the growth of microbial cells because it contains a large number of growth factors compared to any other protein **(Cheigh *et al*., 2002; Pandey *et al*., 2016)**.

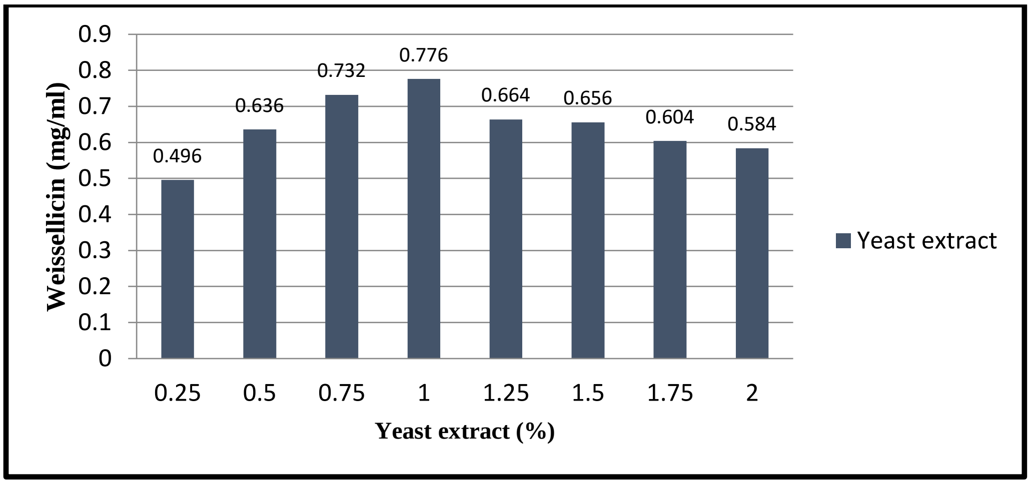

Figure (4): effect of yeast extract on the concentration of weissellicin produced from *W. cibaria.*

The results show that the effect of different concentrations of acidic di-potassium phosphate on the concentration of weissellicin produced from *W.cibaria*. The highest concentration was 0.832 mg/ml at a concentration of 0.15% acidic di-potassium phosphate (Figure 5).

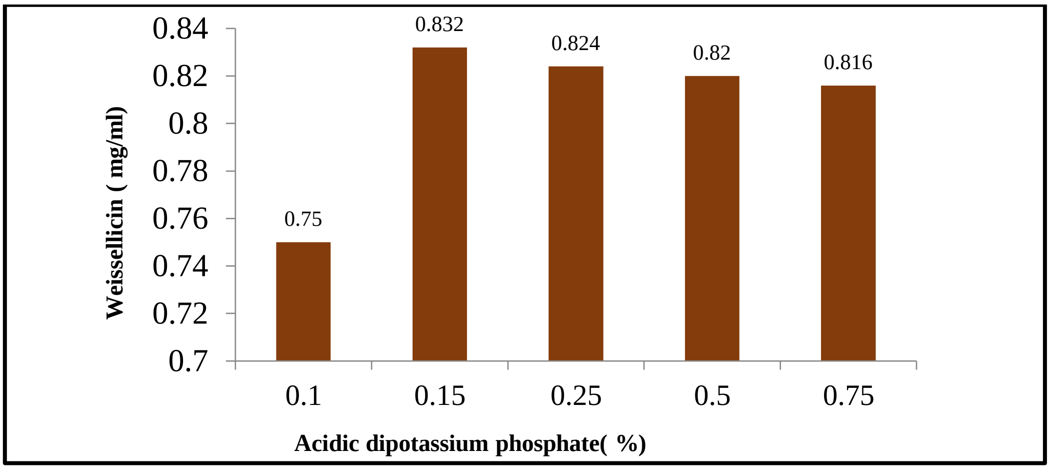

Figure (5): effect of acidic di-potassium phosphate on the concentration of weissellicin produced by *W. cibaria.*

Effect of temperature

Figure (6) shows that the best temperature for the production of weissellicin from *W.cibaria* was 37°C, followed by 30°C, while the production of weissellicin decreased significantly at 10 and 40°C, reaching 0.644 and 0.612 mg/ml, respectively. These results agreed with a number of studies, which indicate that the optimal temperature for bacteriocin production in most isolated strains is between 30°C and 37°C **(Abdul, 2004; Rasheed *et al*., 2020; Dyaee & Luti, 2019; Tom *et al*., 2002)**.

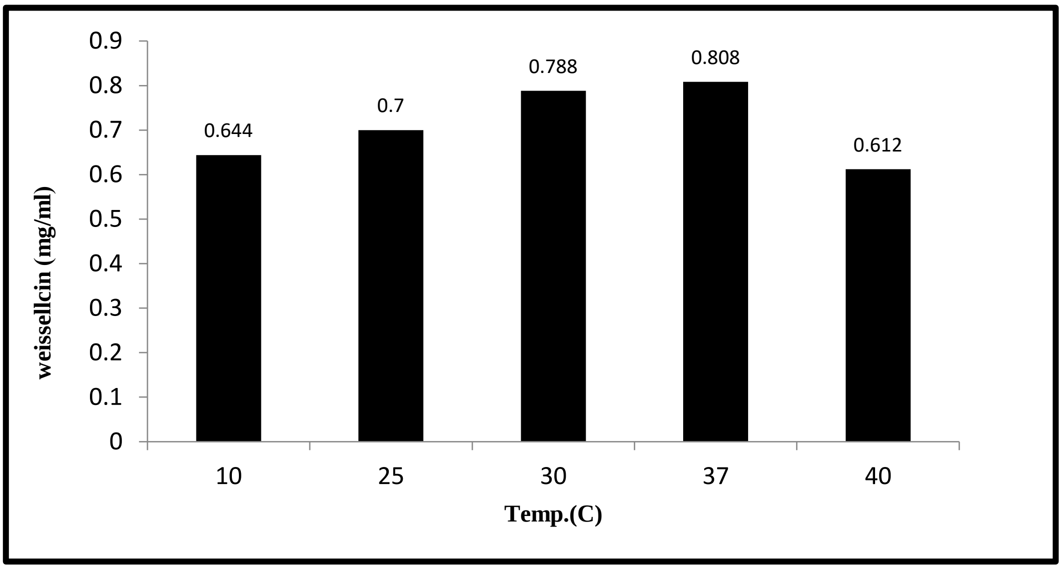

Figure (6): Effect of temperature on the concentration of weissellicin produced from *W.cibaria*.

Effect of pH

The results indicate that the optimum pH for the production of weissellicin from the bacteria *W.cibaria* was 5 (Figure 7). An estimate of about 0.808 mg/ml of weissellicin was obtained, while the production decreased as the pH rose or fell below this value. The pH has a direct effect on the activity of external enzymes and the metabolic process of the microscopic organism and its effect on the ionic state of the base material and its readiness for the developing microorganism **(Jawan *et al*., 2020; Abbasiliasi *et a*l., 2017).**

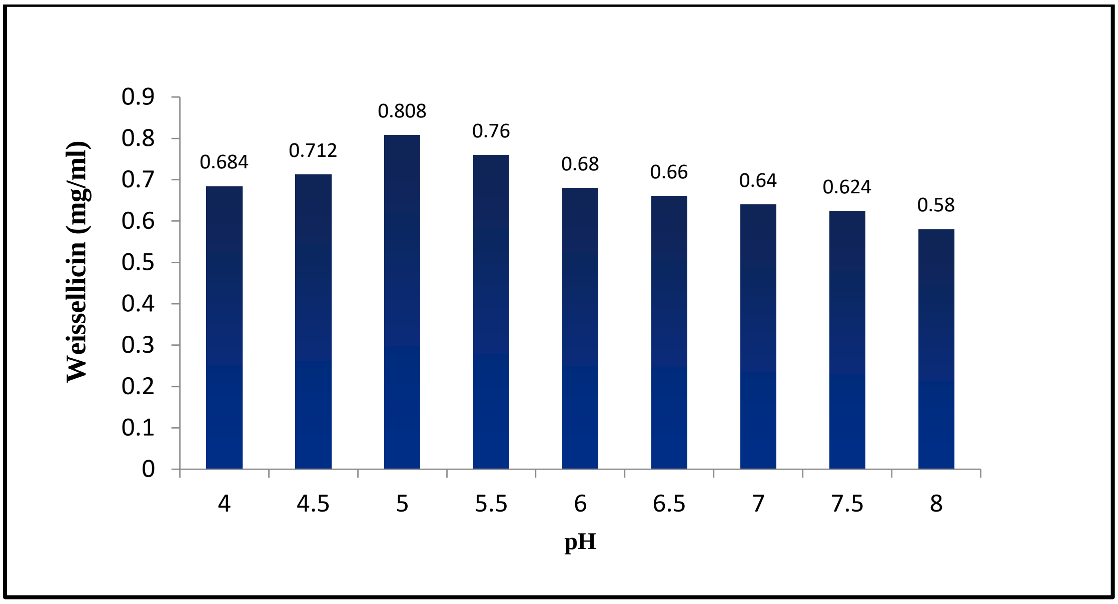

Figure (7): effect of pH on the concentration of weissellicin produced from *W.cibaria* Effect of incubation duration.

The results reported that the best incubation period for producing weissellicin was 48 hours, as the amount of weissellicin reached 0.864 mg/ml, while production decreased with increasing incubation duration (Figure 8). Some study explained on the effect of culture conditions that the production of bacteriocins from bacteria is affected by the type of isolate produced, incubation temperature, pH, and inoculum size **(Sarika *et al*., 2010)**.

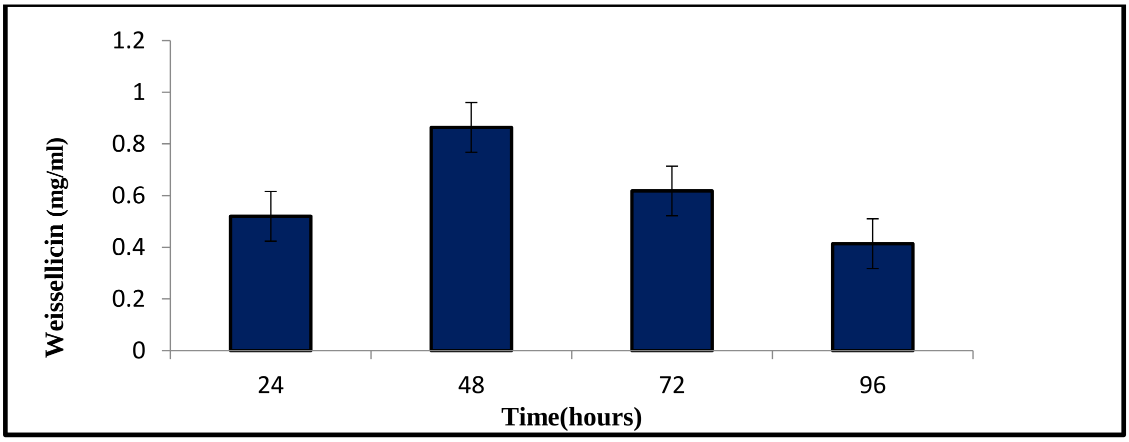

Figure (8): effect of incubation period on the concentration of weissllicin produced from *W.cibaria*.

Effect of inoculum size

Figure (9) shows the use of different levels of inoculum size in the production of weissellicin produced from *W.cibaria*. The increase in this production with the increase in the size of the added inoculum, reaching 1 x 10^9 cells/ml. The amount of weissellicin was 0.848 mg/ml, the optimal inoculum size for production may vary depending on the type of isolate used, the size of the medium used, and the nutrients contained in it **(Lajis, 2020; López-González *et al*., 2018)**. The decrease in production with increasing inoculum size may be due to intense competition for nutrients in the medium, which makes conditions unsuitable for production and growth **(Ramachandran *et al*., 2012; Lechiancole *et al*., 2002; Raji *et al*., 2022).**

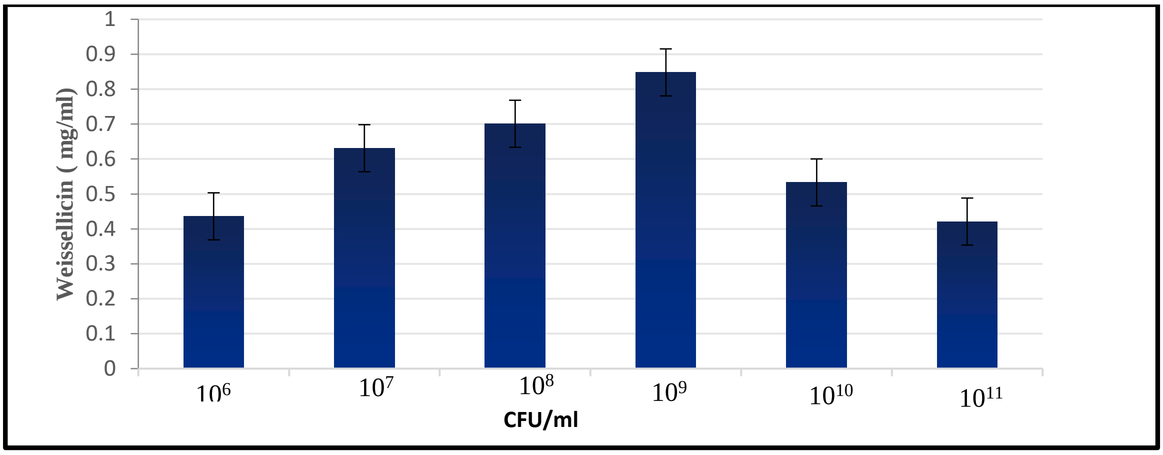

Figure (9): effect of using the inoculum size on the concentration of the weissellicin produced from *W.cibaria.*

CONCLUSION

The study concludes that local bacterial *Weissella cibaria* has shown the ability to produce a type of bacteriocin called weissellicin. The production increase can be controlled by providing appropriate conditions, including carbon and nitrogen sources, temperature, incubation time, and appropriate pH, which can be used as a food preservative.

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