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Original Article

Investigating the antimicrobial effect of Maillard Products on pathogens microorganisms

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ABSTRACT

Background. The Maillard product was prepared from four model systems (aminoacids-glucose) at pH 10 and 110°C for 120 mn.

Aim. This study aims to investigate the antimicrobial potential of different Maillard products.

Methods. Maillard products were prepared by four models (Gly-glu), (Val-glu), (Try-glu) and (Asp-glu). The prepared Maillard product antimicrobial activity

was tested at concentration of 0.24, 0.48, 0.97, 1.95 and 3.90 mg/ml against *Escherichia coli* ATCC 25923, *Staphylococcus aureus* ATCC 25922, *Candida albicans* and *Fusarium graminearum*).

Results. The inhibition diameter of the test isolate by-products from 4 models of Maillard reaction (Gly-glu), (Val-glu), (Try-glu), and (Asp-glu) ranged from 15±0mm to 28.3±0.4mm. The minimum inhibitory concentration (MIC) ranged from 0.97 mg/ml to 1.95 mg/ml for only two test isolates. Maillard reaction products also showed a similar effect of growth inhibition compared with antibiotics frequently used for treating bacterial infections.

Conclusion. The Maillard products tested had vigorous antimicrobial activity against some pathogenic microorganisms. The Maillard reaction product can be used as a narrow-spectrum antibiotic.

Keywords: Maillard products, Antimicrobial activity, Microorganisms. Antibiotics

INTRODUCTION

Many patients have been spared potentially fatal bacterial infections thanks to antimicrobial medicines, which have been essential components of clinical medicine since the latter part of the 20th century. However, antibiotic resistance in pathogenic bacteria has emerged and expanded globally over the last ten years of the 20th century and the first decade of the 21 st, leading to the failure of antibiotic therapy [1]. The food sector now uses organic acids and their salts, such as acetic, lactic, sorbic, or benzoic acid, as antimicrobial agents; these are preferred due to their great efficacy and comparatively low cost [2]. Recently, there has been a lot of interest in using natural and potent antimicrobial agents in place of chemical ones. When food is processed and stored, reducing sugars and free amino acid groups react to produce the Maillard reaction, also known as non-enzymatic browning [3]. The primary response transforms precursors into colorants and flavor compounds during food processing and preservation [4]. This reaction produces a variety of early, intermediate, and advanced compounds [5]. In the advanced stage of

the Maillard reaction, melanoidins are formed [6]. Food scientists are consequently very interested in clarifying the function and traits of AR. Maillard reaction products (MRPs) have been linked to a number of advantageous properties, including antioxidative [7-10], antimicrobial [11,12], anticarcinogenic and antimutagenic properties [13-17]. In the search for alternatives to antibiotics and to further describe the functional properties of Maillard products, this study investigated the effects of Maillard products melanoidins synthesized from four model systems against *E. coli* ATCC 25923, *S. aureus* ATCC 25922, *C. albicans* and *Fusarium graminearum*.

MATERIALS AND METHODS

Preparation of Maillard products

Purified Maillard products were prepared according to Trang et al. and Pischetsrieder et al. [18,19] with slight modifications. Soluble Maillard products were synthesized from four model systems of amino acid and glucose: (glycine-glucose), (valine-glucose), (tryptophan-glucose) and (aspartic-glucose) dissolved in a tampon (pH 10). After being heated to 110 °C for 120 minutes, the sample solution was cooled on ice and extracted using twice as much ethyl acetate. Under lower pressure, the ethyl acetate layer evaporated until it was completely dry. 10 cc of 20% methanol was used to dissolve the residue before filtering it. The pure Maillard product was obtained by evaporating and freeze-drying the clear elute under lowered pressure.

Culture and Maintenance of microorganisms

The Maillard reaction products were tested against a panel of clinical isolates: *Escherichia coli* ATCC 25923, *Staphylococcus aureus* ATCC 25922, *Candida albicans*, and *Fusarium graminearum*. Pure cultures of all experimental bacteria and fungi were obtained from the Hospital of Mascara City (Algeria). The resistance patterns of these isolates were investigated according to the National Committee for Clinical Laboratory Standards (NCCLS, 2002) criteria

and the Manual of Antimicrobial Susceptibility Testing guidelines [20]. The pure bacterial cultures were maintained on a nutrient agar medium, and the fungal culture was on a potato dextrose agar (PDA) medium. Each bacterial and fungal culture was further maintained by subculturing on the same medium and storing at 4 ºC.

Disc Diffusion Susceptibility Methods

Antimicrobial activity was evaluated using the agar diffusion technique in Petri dishes. Briefly, 25 µl of each extract model of Maillard product was loaded on sterile filter paper discs 6 mm in diameter and air-dried. Indicator microorganisms were spread on Mueller- Hinton agar plates (bacteria strains) on Sabouraud's dextrose agar plates (fungi and Yeats) with sterile effusion, and the discs were placed on plates, after incubation for 24 h at 37°C for bacteria and the incubated for three days at 30°C for (fungi and Yeats), in an aerobic environment [21]. Next, an average diameter of two repeats was computed by measuring and recording the inhibition zones in millimeters. This assay also makes use of nine common commercially available antibiotic discs, including AM (ampicillin), AMC (amoxicillin), AMX (amoxicillin), NI (nitroxollin), CRO (ceftriaxone), and ketoconazole.

Determination of Minimum Inhibitory Concentrations (MIC)

The minimal inhibitory concentration (MIC) was determined for each model of Maillard reaction products by a serial tube dilution technique described by Reiner [22] to give a concentration ranging from 0.24 to 3.90 mg/ml. Each tube was inoculated with 50 µl of three sensitive tested bacterial strains adjusted to Mc Ferland standard equivalent to 2x 10^5 (CFU/ml). The minimal concentration (MIC) that demonstrates complete inhibition of a tested strain was determined. Each microorganism under evaluation had at least two copies of each test run, and the arithmetic average of the results was used to report the findings.

Statistical Analysis

All analyses were carried out in triplicate. Data were recorded as means ± standard deviations and analyzed using the software package Statgraphics Plus. Analysis of variance (ANOVA) was carried out to test for any significant differences at ($p<0.05$).

RESULTS AND DISCUSSION

The inhibitory effects of Maillard products from four models of Maillard reaction were studied against *S. aureus* ATCC 25922, *E. coli* ATCC 25923, *C. albicans*, *and Fusarium graminearum* by the standard disc diffusion and serial dilution methods, which are widely used to study the bioactivity of chemical compounds.

The results revealed that the tested models Maillard products showed antibacterial activity with varying values summarized in (Figure1 and 2). Several investigators showed antimicrobial activity of Maillard products against different strains [23,24]. Three models of Maillard products such as (Gly—glu), (Val—glu), and (Try—glu), showed significant inhibitory effects against *S. aureus* ATCC 25922 similar findings were reported by Stecchini [25], while that of (Asp—glu*)* model was utterly inactive. The model (Gly—glu)was found to be highly inhibitory against *S. aureus*, followed by (Try—glu) and (Val—glu) with zones of inhibition 22, 20, and 13 mm, respectively. The results also showed that (Gly—glu), (Try—glu), and(Val—glu)models had similar effect on growth inhibition in comparison with antibiotics frequently used for treating infectious bacteria, such as ampicillin, amoxicillin, amoxicillin, and nitroxillin.

Table 1 indicates that the MIC results of the three models of Maillard products indicated that antibacterial activities in lower concentrations ranging from (0.97mg/ml) for the (Val—glu) model and (0.97 mg/ml) for (Try—glu) model to (1.95 mg/ml) for (Gly—glu) model on *S. aureus*. The model system demonstrated significantly greater efficacy against Gram-positive strains than against Gram-negative stains, according to the results. This suggests that the bacterial cell wall thickness and membrane permeability are responsible for the Maillard product's potent antibacterial activity [12].

The broad-spectrum antibiotics were used to compare the inhibition activity of four models of Maillard products with those antibiotics against *S. aureus* ATCC 25922. When comparing the inhibition activity of (Gly—glu)(Try—glu) and (Val—glu) models with the inhibition activity of antibiotics such as ampicilliillinn, amoxicillin, amoxicillin nitroxillin, and ceftriaxone (Figure 2), it showed that there were no differences with ampicillin, amoxicillin, and amoxicillin which exhibited more muscular inhibition activity against *S.aureus* ranged from 18 mm to 22 mm than two models Gly—Glu) and (Try—glu), whereas *S. aureus* showed high resistance to ceftriaxone antibiotic (2mm), as well as to model (Asp-Glu) (1mm).

The four models Maillard products tested were screened for antifungal activity. Agar diffusion assay showed that (Gly—glu), (Try—glu), and (Val—glu)models exhibit antifungal activities against *C. albicans* with zones of inhibition of 13, 11, and 5 mm, respectively. (Gly—Glu) and (Try—glu) models are highly active compared with the (Val—glu) model and have low activity compared with the reference (Ketoconazole). The fungal zone of inhibition values is summarized in (Figures 1 and 3). The MIC results of MRP for (Gly--glu),(Val-glu), and (Try--glu) were 0.97 mg/ml in all models.

C. albicans was tested against one broad-spectrum Antibiotic, Ketoconazole. The result in (Figure 3) revealed the apparent sensitivity of *C. albicans* to antibiotics tested with inhibition zone (20mm), whereas *C. albicans* showed moderate sensitivity against three models of Maillard products (Gly--glu),(Val-glu) and (Try--glu) with inhibition zone recorded between (5mm to 13mm).

Higher antimicrobial activity was observed for Maillard products derived from different models of Maillard reaction, such as (Gly—glu) and (Val—glu), due to the structure of the amino acid present in glucosamine. The types of bacteria, the chemical makeup of the sugar and amino acid reactant, as well as the reaction's temperature, time, and pH, all seem to affect how inhibiting MRP is against pathogens [26].

In conclusion, this investigation reports that the Maillard products of the Maillard reaction formed at (pH 10 and 110°C) for 120 mn possess antibacterial and antifungal activity and could be used as antimicrobial agents.

CONCLUSION

Using various Maillard reaction models, this study assessed the antibacterial activity of Maillard compounds. This study demonstrated that Maillard products are a strong contender to lessen the risk associated with infection by pathogenic microorganisms. Food that contains Maillard products may therefore be employed as a functional food to cure and prevent infectious illnesses.

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Table 1. MIC (mg/ml) of Maillard products.

Model of Maillard products	Concentration of Maillard products (mg/ml)	00	3.90	1.95	0.97	0.48	0.24
Gly-glu	*S. aureus*	+	--	--	+	+	+
	C. albicans	+	--	--	--	+	+
Val-Glu	Concentration of Maillard products	00	3.90	1.95	0.97	0.48	0.24
	S. aureus	+	--	--	--	+	+
	C. albicans	+	--	--	--	+	+
Try-Glu	Concentration of Maillard products	00	3.90	1.9	0.97	0.48	0.24
	S. aureus	+	--	--	--	+	+
	C. albicans	+	--	--	--	+	+

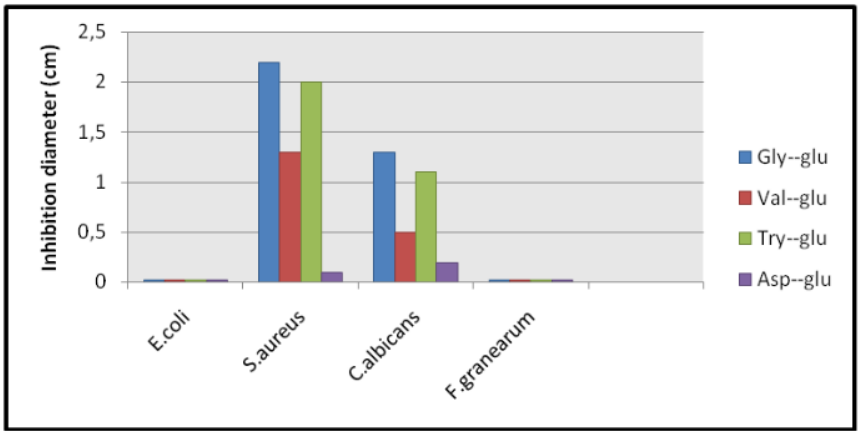

Figure.1. antimicrobial activity of Maillard products from four models of Maillard reaction.

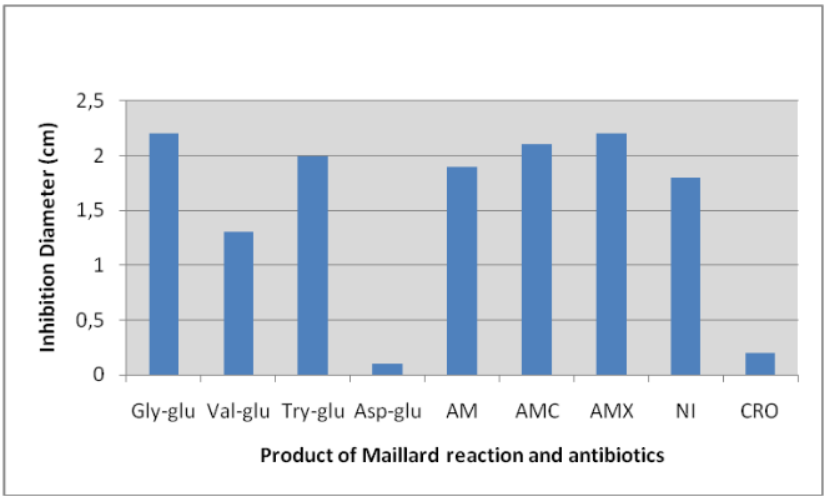

Figure.2. Inhibitory effect of Maillard products and antibiotics against *Staphylococcus aureus*

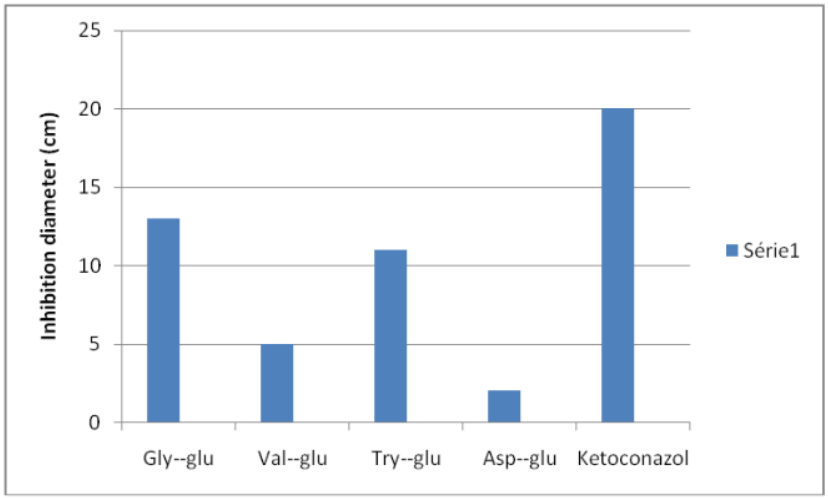

Figure 3. Inhibitory effect of Maillard products and antibiotics against *C.albicans*