Valorization of some fermented products as antioxidants

تعظيم الإستفادة من بعض المنتجات المتخمرة كمضادات للأكسدة

BY

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Abstract:

Fermented products produced by lactic acid bacteria (LAB) improve intestinal function. Lately, there has been a growing focus on probiotics as an alternative to antibiotics. Medicinal and aromatic plants, which are rich sources of bioactive compounds acting as antioxidants and antimicrobial agents, improve health. In this study, extracts of 3 commonly used natural herbs were fermented using Lactobacillus acidophilus bacteria to evaluate their antioxidant and antimicrobial properties. Roselle's findings indicated a reduction in pH levels across all fermented extracts, with the most notable decrease observed in the Roselle extract which dropped from 7.64 to 2.22. The total phenolic and antioxidant content of fermented extracts was higher than that of non-fermented extracts. Roselle's total phenols increased from 66.12 to 85.42 ppm. Furthermore, Roselle and fennel have the highest antioxidant activity growth rates, at around 90.12% and 45.41%, respectively. The effect of various fermented extracts on pathogens' growth was investigated, and it was discovered that the fermented extracts had the most potent effect on both Escherichia coli and Salmonella enteritidis. In conclusion, the high concentration of antioxidants and phenols as well as the majority of them having the ability to act as antimicrobials increase the health benefits of plant extracts that can be valorized by the fermentation process.

Keywords: Fermented Products, Natural Herbal Drinks, Antioxidant, Antimicrobial.

المستخلص:

التي تنتجها بكتيريا حمض اللاكتيك (LAB) على تحسين وظيفة الأمعاء. في الآونة الأخيرة، كان هناك اهتمام متزايد على استخدام البروبيوتيك كبديل

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للمضادات الحيوية. تعمل النباتات الطبية والعطرية، وهي مصادر غنية بالمركبات النشطة بيولوجيًا التي تعمل كمضادات للأكسدة ومضادات للميكروبات، على تحسين الصحة. في هذه الدراسة، تم تخمير مستخلصات ثلاثة أعشاب طبيعية شائعة الاستخدام باستخدام بكتيريا Lactobacillus acidophilus لتقييم خصائصها المضادة للأكسدة والمضادة للميكروبات. أشارت النتائج التي توصلت إليها الدراسة ان الكركديه سجل انخفاض في مستويات الرقم الهيدروجيني في جميع المستخلصات المتخمرة من ٧,٦٤ إلى ٢,٢٢. كان إجمالي محتوى الفينول ومضادات الأكسدة في المستخلصات المتخمرة أعلى من المستخلصات غير المتخمرة. ارتفع إجمالي الفينولات في الكركديه من ٦٦,١٢ إلى ٨٥,٤٢ جزء في المليون. علاوة على ذلك، يتمتع الكركديه والشمر بأعلى معدلات نشاط مضادات للأكسدة، بحوالي ٩٠,١٢% و٤٥,٤١% على التوالي. تم دراسة تأثير المستخلصات المتخمرة المختلفة على نمو مسببات الأمراض، واكتشف أن المستخلصات المتخمرة لها التأثير الأقوى على كل من الإشريكية القولونية والسالمونيلا المعوية. في الختام، فإن التركيز العالي لمضادات الأكسدة والفينولات بالإضافة إلى قدرة غالبيتها على العمل كمضادات للميكروبات يزيد من الفوائد الصحية للمستخلصات النباتية التي يمكن تثمينها من خلال عملية التخمير.

Introduction

For centuries, medicinal plants have been valued for their metabolic properties and have been used to promote human health. Even today, they still play an important role in the production of food, medicine, and energy. Advanced fermentation technologies have made it possible to transform complex substrates and modify the bioactive compounds, thus enhancing the biological properties of plants, vegetables, and herbs. Additionally, the fermentation process improves the nutritional and sensory qualities of the final products, while also preserving the taste and texture of food and beverages. (Das et al., 2012).

Fermented foods and beverages are made using a wide variety of microbes. The main organisms with advantageous technological applications for which a wide range of knowledge of use is available are yeasts, lactic acid bacteria (LAB), and

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acetic acid bacteria (AAB). The flavor of fermented foods is primarily influenced by the volatile compounds, active amino acids, peptides, and sugars produced by LAB strains in particular. Additionally, some LABs have probiotic activity, so they can enhance the health benefits of fermented foods and drinks (Gullo et al., 2016).

Plants can serve as a substrate for growing LAB, with aromatic and medicinal varieties proving especially beneficial for selective bioprocesses. These plants naturally contain a variety of bioactive compounds, including phenolic compounds, carotenoids, anthocyanins, and tocopherols. Furthermore, the fermentation process involving microorganisms can even enhance the levels of these compounds. Studies have demonstrated that during lactic acid fermentation, there exists a link between phenolic compound metabolism and heightened antioxidant activity (Gadhoumi et al., 2021).

Food-borne illnesses such as gastritis, enteritis, peptic ulcers, and ulcerative colitis are caused when pathogens from contaminated food enter the gastrointestinal tract. Lactic acid bacteria (LAB) with high antimicrobial activity against pathogenic and saprophytic microorganisms are being added to foods to ensure microbial food safety and public health. LAB are naturally occurring in the human gastrointestinal tract. For many years, LAB strains have been widely utilized in both industrial and artisanal fermentation processes for plants, meat, and dairy products. Certain strains have even been scientifically demonstrated to offer health advantages and are sold as probiotics. These bacteria have a critical function in sustaining ecological equilibrium by producing lactic acid, which results in a low pH level in the gastrointestinal tract. Moreover, they generate compounds that prevent harmful substances such as hydrogen peroxide, bacteriocins, and organic acids. They also

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compete for nutrients, prevent pathogen adherence to surfaces, and stimulate the immune system (Al-Madboly et al., 2015).

The purpose of this research is to investigate the use of LAB, previously known as probiotics, to produce functional extracts from aromatic plants and sucrose as raw materials. Furthermore, our research aimed to assess the level of potent antioxidant and antibacterial activity of fermentation products as promising products against a variety of common food-associated pathogens.

2. Materials & methods

2.1. Plant Preparation and Fermentation

The plant samples used for the formulation of the fermented products are fennel, roselle, and ginger. Tested plants were purchased from the commercial central market in Egypt. 10 g from each selected plant sample was mixed with 70 g of sucrose solubilized in 1 L of distilled water, and each sample was divided into 2 groups: the control group (without inoculation with the selected strains of Lactobacillus acidophilus) and the fermented group (with inoculation by the selected strains of Lactobacillus acidophilus). The fermented group solution was sterilized by pasteurization (80^o C in 15 minutes) after the pH was adjusted to 7.8. Equivalent batches of Lactobacillus acidophilus ATCC 43121 were prepared and fermented using strains obtained from the Egyptian Microbial Culture Collection at the Cairo Microbiological Resources Centre (MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. The LAB culture was previously grown for 24 hours on de Man Rogosa Sharpe (MRS broth, Oxoid, UK) at 37^o C. Each sample batch was then given 10 mL of prepared culture inoculum. After 10 days of room temperature fermentation, the fermented samples were filtered and stored at $+4^o$ C until analysis.

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2.2. Fermentative Parameters

2.2.1. pH value

The pH of the fermented products was measured with an electronic pH meter (Hanna).

2.2.2. Total polyphenols content

The Folin-Ciocalteau method was used to determine the total polyphenol content of fermented extracts (Tlili et al., 2013). Approximately 0.1 ml of the tested samples were transferred to a 100-ml Erlenmeyer flask, and the final volume was adjusted to 46 ml by adding distilled water and 1.0 ml of Folin-Ciocalteau reagent. After that, 1 ml of Folin-Ciocalteau reactive solution was added and incubated for 3 minutes at room temperature. The above solution was mixed with 3 ml of 2% sodium carbonate. After 30 minutes, the absorbance at 760 nm was measured. The total phenol was calculated using the calibration curve and expressed as gallic acid equivalents.

2.2.3. Antioxidant activity

According to Burda and Oleszek, (2001), the antioxidant activity of tested samples was determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH). 2 ml of tested samples solutions was mixed with 2 ml of 0.1 mmol/L DPPH (Diphenyl-2-picrylhydrazyl) methanolic solution. After vigorously shaking the mixture and allowing it to stand in the dark for 20 minutes, the absorbance at 517 nm was measured with a spectrophotometer.

2.2.4. Antimicrobial activity

Staphylococcus aureus ATCC3536 and Escherichia coli ATCC8739 were the pathogenic bacteria strains used in this study. They were obtained from the Egyptian Microbial Culture Collection at the Cairo Microbiological Resources

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Centre (MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt, and were grown on nutrient agar (Cockerill et al., 1996) or dextrose agar (Domesle et al., 2021) slants at 4°C. An agar-well diffusion assay was used to evaluate the tested samples' antimicrobial activity against pathogenic strains (Nalawade et al., 2016). In Petri dishes, Müller-Hinton agar (MHA) medium was added. For the wells, a sterile cork borer was used to create 7 mm-diameter holes out of suspensions (100 μL) of the target strain that had previously been incubated for 24 hours on the plates (Gangoué-Piéboji et al., 2009). Supernatant samples (100 μL) from the tested samples were then added to the wells in the agar plates that had already been inoculated with the target strain after being centrifuged at 10,000 rpm for 10 min to remove cell fragments. After all of the test samples had been absorbed, the plates were left to stand before being incubated at 37°C for an overnight period. The growth inhibition was apparent after 24 hours of incubation, and the diameter of the inhibition zone (DIZ) was measured with a ruler. Measurements were made of the growth inhibition zone surrounding the wells to assess the antimicrobial activity.

2.3. Statistical analysis

ANOVA was used with IBM® SPSS® Statistics Server Version 23.0 to analyze the differences between the parameters measured before and after fermentation (Ashton, 2015). The $p<0.05$ level for significant differences between means. The multiple range tests by Duncan were applied.

3. Results Discussion

After obtaining the fermented products, the results of the analysis performed on the fermented samples (fennel, Roselle, and ginger) were compared to examine how the physio-chemical and microbiological characteristics interacted.

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3.1. pH value

The pH of a solution reveals how basic or acidic it is. The range is 0 to 14, with 7 representing neutrality. A pH value above 7 indicates an alkaline state, while one below 7 indicates acidity. The pH scale actually measures the concentration of free hydrogen and hydroxyl ions in a solution. If there are more free hydrogen ions than free hydroxyl ions, the solution is said to be acidic, and vice versa. The pH value is a key indicator of a chemically changing solution because chemicals in the solution can affect pH. For reporting pH, "logarithmic units" are employed. Each number represents the acidity or basicity of the solution as a 10-fold change. Ten times as acidic is a solution with a pH of five than one with a pH of six. (Schultze et al., 2021).

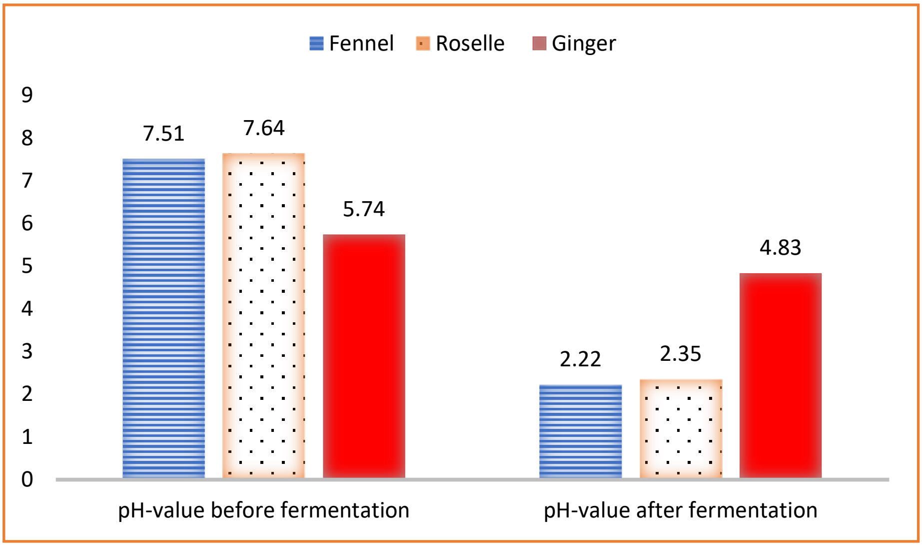

Figure 1. pH-values of plant extracts before and after fermentation

There is a significant difference in pH in all fermented samples before and after fermentation. At the beginning of

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fermentation, the lowest pH value was recorded for the ginger sample (5.74), while the roselle had the highest pH value (7.64). At the end of the fermentation period, the pH values of all samples decreased. Fennel had the lowest pH value of 2.22, while ginger had the highest pH value of 4.83 (Figure 1). In the fermented samples, the pH value decreased at the end time of the experiment. This decrease can be attributed to the higher composition of organic acids produced by fermentation (Loncar et al., 2006; Kallel et al., 2012).

3.2. Total polyphenols content

Total phenolic compounds were determined as data presented in Figure (2), show that roselle conducted the highest amount of total phenolic production after fermentation which upgraded from 66.12 up to 85.42 ppm in total phenolic compound content, this high phenolic content affected the antioxidant activity of fermented roselle extract which registered as the highest antioxidant activity.

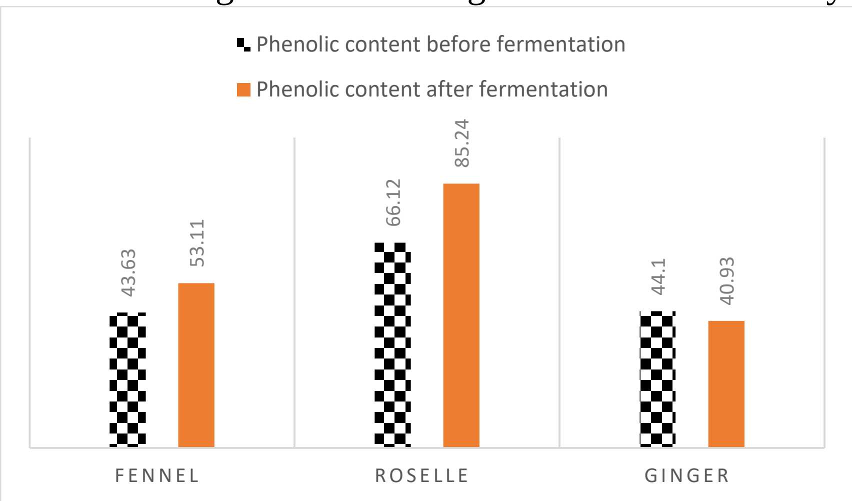

Figure 2. Phenolic content of plant extracts before and after fermentation

Herbal antioxidants are generated by phenolic compounds, according to Marzanti et al, (2006). The hydroxyl group in phenolic compounds acts as a reducing agent by donating a hydrogen proton. Before and after fermentation, the total amount of phenolic content in fermented extract samples differed significantly. According to Abdel-Aty et al. (2023), the increase in total phenolic bioactive compounds and other secondary metabolites with antioxidant activity in fermented herbal extracts may be due to an increase in acid production such as acetic and lactic acid.

3.3. Antioxidant activity

As shown in Figure (3), antioxidant levels in plant extracts were measured both before and after fermentation. In order of increasing antioxidant activity, ginger, fennel, and rosella who is the top-ranking fermented plant extract, with antioxidant activity ranging from 30.05 to 90.12%. Additionally, roselle and fennel exhibit the highest rates of antioxidant activity growth, at about 90.12% and 45.41%, respectively.

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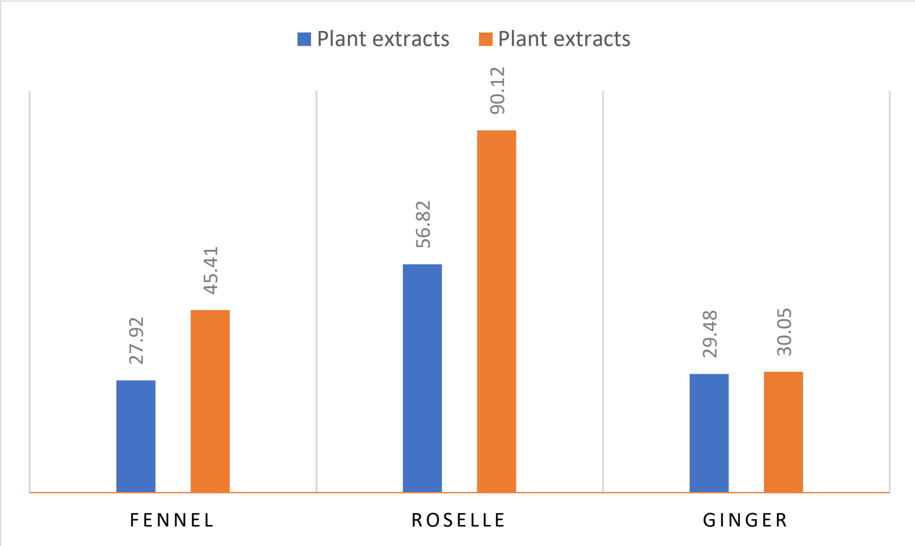

Figure (3): Antioxidant activity of fermented plants extracts

Indeed, it is well understood that the presence of phenolic and bioactive compounds contributes significantly to total antioxidant capacity. Several studies found that polyphenols found in fermented beverages have high scavenging activity against oxidative stress. Fermentation has recently been used to boost the antioxidant activity of food and medical compounds, as well as for food preservation. Furthermore, many bioactive compounds produced during fermentation have been shown to scavenge free radicals and reactive oxygen species (Uchida et al., 2017).

3.4. Antimicrobial activity

The antibacterial properties of fermented herbal extracts are shown in Table (1). The obtained results demonstrate that after the fermentation period, all tested samples' antibacterial activity efficiency is increased by the

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fermentation process of fermented herbal extracts. Escherichia coli growth was impeded by ginger, roselle, and fennel-fermented extracts with diameters of 16, 20, and 13 mm, respectively. Numerous studies have discovered that a variety of Gram-positive and Gram-negative bacteria are inhibited by polyphenols extracted from aromatic plants (Sreeramuluetal, 2000).

Furthermore, antibacterial efficiency improves following herbal fermentation. This may be explained by the capacity of bacteria found in fermented products to reproduce antimicrobial products such as ethanol, acetic acid, and phenolic products, as reported by Sabel et al., (2017).

Table 1. **Antibacterial activity of fermented samples**

Plant extracts	Zone of inhibition (mm)			
	S. enteritidis	S. aureus	E. coli	B. cereus
Fennel	4	6	16	3
Roselle	11	10	20	7
Ginger	6	5	13	5

Good probiotics should target the pathogens in the gastrointestinal tract specifically with their antimicrobial effects. The probiotic bacteria that are taken orally also need to protect themselves from the bile salt that is present in the digestive tract in addition to the stomach's strong acidity. Therefore, bile tolerance is one of the most important characteristics of probiotic bacteria since it affects both their capacity to function as a probiotic and their ability to survive in the small intestine (Al-Madboly et al., 2015). Finally, the ability of probiotic microorganisms included in fermented products of aromatic plants to prevent the growth

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of some enteric and diarrheal pathogens and even to kill them suggests the potential health benefits of consuming such products. Protection from diarrhea, food poisoning, and even systemic and enteric infections may be among these advantages.

4. Conclusion

In this study, medicinal plants and sucrose were tested as raw materials for lactic fermentation to produce functional extracts. In our study, the six fermented extracts had higher levels of polyphenols, which increased antioxidant and antimicrobial activity. This novel approach, which is based on lactic fermentation performed by LAB, can be used in general for secondary metabolite production and extraction from plant materials, and it can be used to develop promising products to boost immunity and gastrointestinal integrity.

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