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The Effect of Active Ingredient of Safflower on The Bacterial Inhibition in Croissants and Studying Its Sensory Properties

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ABSTRACT

This study aims to assess the impact of incorporating hot aqueous extracts derived from the safflower plant *Carthamus tinctorius* L. at concentrations of 100, 200, and 300 mg/kg, compared to a control treatment. The evaluation focuses on the chemical, microbiological, and sensory properties of laboratory-produced croissants stored for over 10 days at room temperature. Four components of the hot aqueous safflower extract were identified using HPLC. Quercetin and Kaempferol achieved the highest concentrations, reaching 97.4 and 88.6 ppm, respectively. Rutin decreased to 49.8 ppm. Peroxides were estimated in the laboratory-produced croissants, with treatment T2 showing the greatest decrease in peroxides, reaching 5.365 on the tenth day of storage, compared to the control treatment C, whose values increased from 3.08 to 12.466. There was a significant decrease in the TBA values of treatments A2 and A3, reaching 0.663 and 0.0816, respectively. The identified chemically active compounds played a key role in the development of microbial growth in laboratory-produced croissants at varying doses. The concentration of 300 mg/kg in the manufactured croissants achieved the highest inhibition of bacteria until the sixth day of storage, reaching 2×10^5 CFU, which is considered one of the most effective concentrations. This indicates the effect of the active compounds and phenolic compounds present in the safflower extract in inhibiting bacterial colonies. The extract did not show any inhibitory activity against bacteria at a concentration of 100 mg/kg. The bacterial counts were recorded from the beginning of storage, reaching 1×10^5 CFU. The product was evaluated sensorily. The results showed significant differences ($p \leq 0.05$) between treatment A2 and the other treatments in terms of appearance. Treatment A2 at a concentration of (200 mg/kg) recorded the highest value of 7.0, followed by treatment A3 (300 mg/kg), which reached 6.8. As for the properties of texture and softness, treatment A3 recorded the highest values of 7 for both, respectively. While the control treatment had the lowest values of 5.4 and 5.0, respectively, for both characteristics, treatment A2 outperformed the control in terms of flavour, flaky texture, and colour, with values of 6.8, 7, and 7, respectively.

تاثير المركبات الفعالة للعصفر في تثبيط البكتريا في الكرواسون ودراسة صفاتها الحسية

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الخلاصة :

يهدف البحث الى تقييم تأثير اضافة المستخلص المائي الساخن لنبات العصفر *Carthamus tinctorius L* في الصفات الكيميائيةوالميكروبية والحسية بتراكيز مختلفة 200,100, 300ملغم /كغم على التوالي اضافة الى معاملة السيطرة Control في تحضير الكرواسون المصنع مختبريا والمخزن لمدة 10 ايام على درجة حرارة الغرفة.

تم التعرف على اربع مكونات من مستخلص العصفرالمائي الساخن باستخدام HPLC . حيث حقق كورستين والكيمفيرول أعلى التركيزات، وصلت إلى 97.4 و 88.6 جزء في المليون على التوالي وقد انخفض تركيز روتين إلى 49.8 جزء في المليون . وقدرت قيمة البيروكسيدات في الكرواسون المصنع مختبريا حيث حصلت المعاملة T2 اعلى نقصان في البيروكسيدات حيث بلغت 5.365 في اليوم العاشر من الخزن مقارنة بمعاملة السيطرة C التي ازدادت قيمتها من 3.08الى 12.466 وتم انخفاض ملحوظ في قيم TBA للمعاملتين A2,A3 حيث بلغت قيمتهما0.663 ,0.0816 على التوالي.وكان للمركبات الفعالة الكيميائية المشخصة دورا اساسيا من تطور النموالميكروبي في الكرواسون المصنع مختبريا بجرعات متفاوتة. حيث حقق التركيز300ملغم /كغم في الكرواسون المصنع اعلى تثبيطا للبكتريا حتى اليوم السادس من الخزن،حيث بلغت 2×10^5cfu وتعتبرمن افضل التراكيز مما يدل على تاثير المركبات الفعالة و المركبات الفينولية الموجودة في مستخلص العصفر في تثبيط مستعمرات البكتريا في حين لم يُظهر المستخلص أي نشاط مثبط ضد البكتيريا عند تركيز 100 ملغم/كغم، حيث سجلت أعداد البكتيريا منذ بداية التخزين، حيث بلغت 1×10^5cfu .وقد تم تقييم المنتوج حسيا حيث اظهرت النتائج فروقات ذات دلالة معنوية $p<0.05$ بين معاملة A2 وبين باقي المعاملات الاخرى من حيث المظهر حيث سجلت المعاملة A2 بتركيز (200ملغم /كغم) اعلى قيمة بلغت 7.0 ثم تلتها المعاملةA3 (300ملغم /كغم) حيث بلغت 6.8.اما بالنسبة لخاصيتي النسجة والطراوة فقد سجلت المعاملة A3 اعلى قيمة بلغتا 7 لكلا منهما على التوالي .في حين حصلت معاملة السيطرة على اقل قيمة بلغت 5.4,5.0 على التوالي. في كلا الخاصيتين في حين تفوقت المعاملة A2 بالنكهة والرقائقية واللون حيث بلغت قيمهم 6.8,7,7على التوالي.

الكلمات المفتاحية : نبات العصفر، المستخلصات، مضادات الاكسدة، مضادات البكتيريا، الكرواسون.

INTRODUCTION

Pastries (baked products) have long been produced in a wide variety of forms and types across different societies. This industry has continued to evolve globally due to the nutritional and health value of these products. Bakery items such as biscuits, cookies, pies, and bread, among others, are often fortified to enhance their unique flavor and distinctive taste (Patel, 2013). The croissant is considered one of the most important baked products, widely favored by people in all countries. It belongs to the category of pastries or pies and is characterized by its crescent shape with several rings. Croissants are made from leavened pastry dough, distinguished by their thin layers, delicate and airy texture, golden colour, and distinctive flavour. In some varieties, emulsifying agents and various fillings are incorporated, which are blended with the stuffed product. Although its name is French, the croissant actually traces its origins back to 17th-century Vienna, specifically to the year 1683. There are many types of croissants available, including those filled with cheese, thyme, or chocolate, as well as plain varieties (Sutoof, 2013**).**

Natural extracts**,** on the other hand, are compounds derived from natural sources such as plants and are used in various food and industrial applications. These extracts may be in the form of liquids, powders, or oils and typically contain bioactive components. Safflower, *Carthamus*

tinctorius, is considered one such natural plant.
The safflower plant, *Carthamus tinctorius*, belongs to the genus *Carthamus A.* and the compositae family Asteraceae. It is considered a winter oil crop. It has been established that safflower, a perennial herbaceous plant of the Asteraceae family, existed as early as 4500 BC (Jawad et al., 2015). Not only is it well-known for its use in food colouring, but it also has several medicinal advantages; for example, the linoleic and linolenic acids it contains make it ideal for making natural cream, which helps keep the skin smooth (Matthaus et al., 2015). There are 600 different types of safflowers, and they all help the immune system (Ullah M.F., 2019). Originating in South Asia, it has now spread to many European countries, as well as to China, India, Egypt, Italy, and France. Luteolin, safflor yellow *A, safflamin C, Carthamidin*, and isocarthamidin are plant-essential components found *in C. tinctorius*.

Turgumbayeva et al.,(2020) explained that the seeds are a good source of oil, protein, and crude fiber. They also include a lot of vitamins and minerals, including thiamine, beta-carotene, and tocopherol. C. tinctorius is grown for its carthamin pigment, which colours rice, bread, and cloth red, orange, or yellow. Its dye was mostly synthetic aniline dyes in the 19th century (Shirwaikar et al., 2010 When used in cooking, it does not produce smoke or cause allergic reactions. It has a distinctive flavour similar to that of sunflower oil (Han et al., 2009). It contains antioxidant, anti-inflammatory, and antibacterial polyunsaturated fatty acids, including oleic acid (70%) and linoleic acid (10%), as well as numerous phenols, saturated fatty acids, polyphenols, and gallic acid (Qaragholi et al., 2022). According to Khalid et al. (2017), it finds use in baked goods, drinks, and the cosmetics and food sectors. Turkeymbayeva et al. (2020) found that safflower water extract inhibits the growth of many bacteria, including *Bacillus subtilis, Bacillus cereus*, and *Bacillus mycoides*. The oil is edible and used in cooking as a fever reducer and detoxifier for conditions such as difficult menstrual cycles, postpartum depression, and osteoporosis (Khalid et al., 2017and Hagr et al., 2021). The plant contains copper, manganese, zinc, antioxidants, analgesic, and anti-inflammatory properties, and is anti-diabetic (Qaragholi et al., 2022). It is used in a wide range of applications, including pharmaceutical botany, medical and clinical microbiology (Qaragholi et al., 2022). The use of herbal medicines has gained increasing importance in recent years due to their potential as antioxidants and their antimicrobial effects against a wide range of pathogens (Qaragholi et al., 2022). Safflower Carthamus holds significant industrial importance. In the tanning industry, safflower is used to remove excess leather tanning agents that accumulate from dyes. The root extract of safflower is also employed as an environmentally friendly insecticide, effective against pests that threaten major crops. Additionally, safflower oil is recognized globally as a high-quality lipid, used for energy generation with lower emissions compared to conventional petroleum-based products. Microbiological testing of products is essential for assessing the impact of such extracts on inhibiting microbial growth and extending the shelf life of products compared to control treatments (Aameed,et.al., 2021). Several types of safflower extract: alcoholic and cold aqueous given the potential adverse effects of synthetic preservatives on public health, and the limited research concerning the use of safflower extract as a natural preservative, this study aims to: 1-Identify the bioactive compounds present in safflower,2-Evaluate the effectiveness of natural safflower extract in inhibiting microbial growth and extending the shelf life of laboratory-produced croissants, and its impact on microbial inhibition..3-Investigate the effect of the bioactive compounds in safflower extract on the

physical, chemical, and sensory properties of croissants produced and stored at room temperature.

MATERIALS AND METHODS

The dry safflower leaves were taken from *Carthamus tinctorius*. was obtained from local markets in Baghdad. The plant material was identified as *Carthamus tinctorius L*. It was authenticated by the College of Science for Women. The safflower was ground into a powder using a sterilised electric mill to obtain the safflower powder, which was then stored in airtight glass containers until further use (Qaragholi et al., 2022).

Preparation of the extract

Hot water extract:

Thirty grams of safflower powder and 300 millilitres of heated distilled water were combined to make a hot water extract. Then, for the next 24 hours at 37°C, the mixture was stirred using a magnetic stirrer. After passing through a Buchner funnel, the sample was filtered and concentrated at 40 °C using a rotary evaporator. A refrigeration unit was used to maintain the temperature of the dried extract. Ravovanovic et al. (2015) reports.

Extraction of The Active Compounds of Safflower

Ultrasonic Extraction of Phenols

Ethanol solvents separated phenolics from a 10.0 g homogenized plant sample. The ultrasonic bath (USA) extraction was performed for one hour at ambient temperature (Radovanovic et al., 2015). The extraction yield after filtration was estimated using 5 mL of liquid extract. Slovenia used a vacuum-operated revolving evaporator to remove solvent. Next, it was dried at 60°C until a consistent mass was achieved. In glass vials at 4°C, dried extracts were preserved from oxidative degradation before analysis (Ajanal et al., 2018).

Quantification of individual phenolic compounds.

The Environmental, Water, and Renewable Energy Research and Technology Centre, located within the Scientific Research Authority Laboratories, conducted the research. The Food Contamination Research Department uses a SYKAMN HPLC system with a UV detector for reversed-phase HPLC. Data processing software was Chemstation. Used Zorbax Eclipse Plus-C18-OSD stationary phase. The column is 25 cm tall and 4.6 mm wide. A temperature of 30 °C was recorded for the column. This experiment used methanol and 1% formic acid in water (v/v) as eluents. This was done using gradient elution. Elution was performed in two stages: 0–4 minutes with 80% B and 5–10 minutes with 60% B. Experiment flow was 1.1 mL/min. This process was automated using an autosampler, injecting 100 µL of samples and standards. Spectra were taken at 280 nm (Radovanovic et al., 2015).

Chemistry Revealed the Concentrations of Aggregates and Totals of Active Compounds

Determination of total phenols

Ethanolic extract phenolics were evaluated using a standardized Folin-Ciocalteu reagent. The mixture consisted of 20% sodium carbonate, 500 µL of Folin-Ciocalteu reagent from Merck, Germany, and 100 µL of extract. After agitating the sample using a vortex mixer, 10 mL of pure water was added. After the 2-hour reaction time had elapsed, the phenolic content was determined by measuring the absorbance at 765 nm. The German company Sigma-Aldrich supplied the gallic acid used to build the calibration curve upon which this estimate was based.

Per gram of dry weight, the total quantity of phenolic compounds was expressed as milligrams of gallic acid equivalent (GAE) (Zare et al., 2014).

Total flavonoid content

The flavonoids in the raw extract were measured using aluminium chloride colourimetry. (Chemically free extract) Methanol was used to dilute 50 µL of crude extract (1 mg/mL ethanol) to 1 mL. This solution was mixed with 4 mL of purified water and 0.3 mL of 5% sodium nitrite (NaNO2). The mixture was incubated for 5 minutes before 0.3 mL of 10% AlCl3 was added. It was left alone for 6 minutes. Two millilitres of 1 mole/L sodium hydroxide (NaOH) solution were then added. The mixture was then diluted with 10 mL double-distilled water. The solution was left alone for 15 minutes before measuring 510 nm light absorption. The total flavonoid content was calculated as milligrams of rutin equivalent per gram of dry weight using a calibration curve. Subtracting "3--3-2" gives the alkaloids' concentration (Tohidi et al., 2017).

Extraction: Twenty grams of each plant material were powdered and subjected to a twenty-four-hour methanol extraction in a Soxhlet apparatus. See Table 3 for details on how the sample was dried to a completely dry state using a rotary evaporator set at 45°C with decreasing pressure until all the methanol had evaporated as shown in Table 3.

Qualitative estimation (Test for alkaloids)

Alkaloids were found using Dragendroff's method. After dissolving various extracts, two drops of Dragon Drops' reagent were added to mild HCl. Orange crystalline precipitate implies an alkaloid. Alkaloid-positive samples were submitted for quantitative analysis, as described by Turgumbayeva et al. (2018).

Separation of Alkaloid

Part of the extracted residue was treated with 2N HCl and filtered. Transferring 1 M/L of solution using a separatory funnel. Then, 10 M/L of chloroform was added to wash. 0.1 N NaOH neutralized the solution pH. Next, 5 M/L of phosphate buffer and 5 M/L of BCG solution were added to the mixture.

Atropine standard solution was portioned out and poured into individual disposable funnels at exact intervals of 0.4, 0.6, 0.8, 1.0, and 1.2 mL to create the standard curve. The next stage involved combining 5 millilitres of BCG solution with 0.1 milligrams of phosphate buffer (pH 4.7) and the extract. Chloroform at varying concentrations (1, 2, 3, and 4 ml) was then added to the mixture and stirred. To obtain the correct concentration of the solution, the extracts were first collected in a 10 mL volumetric flask and then diluted with chloroform. A UV-spectrophotometer (Shimadzu UV-1800) operating at 470 nm was used to determine the absorbance of the complex in chloroform. The comparative blank was prepared according to the method above, except that Atropine was omitted (Ajanal et al., 2018).

Total Saponins content

The samples were subjected to natural air drying within the laboratory environment at a temperature consistent with room conditions for one day. Subsequently, the desiccated specimens were subjected to crushing using a mortar and pestle, followed by further pulverisation into a fine powder using a manual grinder.

Qualitative Determination of Saponins:

Here is a brief description of the qualitative tests conducted: Half a gram of the extract was mixed with water and shaken in a test tube. It was determined that saponins were present because steady foaming occurred.

Quantitative Determination of Saponin:
The sample saponin was measured via a twofold extraction using a gravimetric method. In a flask, 50 mL of 20% ethanol and 5 grams of powder were mixed. Three hours were spent heating the mixture to 55°C in a water bath. Next, Whatman filter paper (No. 42) was employed to filter. The residue was extracted with 50 cc of 20% ethanol. The extracts were combined and concentrated to 40 mL at 90 °C. After transferring the concentrated solution to a separating funnel, 40 mL of diethyl ether was added. It was well-mixed. Repeated partitioning extraction made the water layer see-through. Saponins were extracted with 60 mL of standard butanol. The combined extracts were rinsed with 5% NaCl in water. After that, they dried on a pre-weighed evaporation plate. The product was weighed again after being dried at 60 °C in the oven and then cooled in a desiccator. The process was repeated twice to get an average. The saponin content was calculated by removing the original amount and computing the percentage.

sample thus:“ Percentage of saponins = (W2 – W1 / Wt. of the sample) X 100
W1= weight of evaporating dish / W2 = weight of evaporating dish + sample.” (Ezeabara et al.,2014)

Total glycosides
Maceration in an 80% methanol solution was performed at room temperature on the 10 g of dried seeds from the sample. At 48-hour intervals, the solvent was refilled a total of three times, with each refill adding 1 liter. Vacuum concentration was applied to the whole extract. Mixing 10 mL of Baljet's reagent (a 95% solution of 1% picric acid and 5% 10% NaOH) with a solution that included 10% extract from each generation and a full seed extract allowed us to evaluate the presence of glycosides. After the mixture was diluted for one hour, twenty millilitres of distilled water were added to it. The absorbance of the diluted mixture was then measured at a wavelength of 495 nm using a Shimadzu UV/VIS spectrophotometer model 1600A, which was produced in Kyoto, Japan. To create the standard curve, a series of solutions containing 10 mL of securidaside with different concentrations (ranging from 12.5 to 100 mg/L) was prepared. The material that was extracted included the active ingredient securidaside. Using milligrams of securidase per gram of dried extract, the total glycosides were measured in three duplicate samples (Trease & Evans, 2002).

Determine the percentage of terpenes.
To determine the percentage of terpenoids in the sample, the procedure described by Radovanovic (2015) was followed. The first step was to extract 7 mL of a combination of the sample's methanol and acetonitrile. Shaking the sample for half an hour was the next procedure. After shaking, the sample was left in the dark for 24 hours. The next step was to spin the sample in a centrifuge at 6,000 rpm for a few minutes. The yielded liquid was then collected in 5 milliliters.
The resulting supernatant was then mixed with 1.5 ml of chloroform and 0.5 ml of sodium hydroxide solution. For one minute, the ingredients were stirred constantly. Placing the tube on ice for no more than 5 minutes will cool it down in the event of an exothermic reaction that produces heat. To achieve a total volume of 10 ml, methanol was then added to the sample.
Spectrophotometer readings were taken at 538 nm from a linalool standard solution prepared

at various concentrations. The results were documented. The next steps included reading the sample, recording its absorbance, and then calculating the amount of terpenoids:
Determination of the percentage of terpenes:
The proportion of terpenes was calculated using the technique described by Radovanovic et al. (2015). We added 1.5 g of terpenes to a sample we had collected. Step one of the process involves combining 7 millilitres of the sample with a solution of methanol and acetonitrile; however, the exact amount of this solution is not specified in the text. The next step is to shake the sample for 30 minutes. After that, it was left alone for an entire day. The next step is to spin the sample in a centrifuge at 6,000 rpm for 30 minutes. Once 5 mL of extraction has been added, 1.5 mL of chloroform and 0.5 mL of concentrated sulfuric acid are blended in. Everything is mixed and whisked for one whole minute. Exothermic reactions may only be cooled for no more than five minutes by placing the tube on ice.
To achieve a final volume of 10 ml, methanol is added to the sample after it has cooled. Spectrophotometer readings taken at 538 nm reveal the concentration of linalool in a standard solution prepared at varying concentrations. The absorbance of the model is then measured and reported once the data are obtained. These absorbance data are used to quantify the proportion of terpenes. (Ezeabara et al.,2014)

Laboratory Production of Croissants
The croissants were made in a lab using a conventional recipe (Labensky et al., 2024). They were manufactured to ingredient weights and utilized these quantities: making croissants Ingredients: 1 ½ cups butter or margarine, ⅓ cup all-purpose flour, 2 active dry yeast packets, ½ cup warm water (110-115^{0}), ¾ cup milk, ¼ cup sugar, 1 teaspoon salt, 1 egg, 3-4 ¼ cups all-purpose flour, 1 egg yolk, 1 tablespoon milk, Oven 375. In addition to the control treatment, the hot aqueous extract of safflower was added to laboratory croissants at concentrations of 100, 200, and 300 mg/kg in the croissant dough to examine its effects on sensory and microbiological properties.
The croissants were made according to Labensky et al. (2024). After cooling, the croissants were kept in sterile polyethene bags at 28–37°C for 10 days. Chemical, microbiological, and sensory tests were done every three days till storage ended.
Preparing the sample for counting bacteria :*Total count of bacteria*
Two duplicates of each dilution were used to count bacteria on nutrient agar pour plates. After grinding 50 grams of croissants, 450 cc of sterile distilled water was added. Using a sterilized electric blender, shake the mixture for 2 minutes. This suspension was diluted 1/10 with 9 cc of distilled water. Bacterial colonies were counted using decimal dilutions from 10^{-1} to 10^{-8}. A sterile dish was filled with 1 millilitre of each dilution, and the nutritional medium was gently stirred in. The dish was then incubated at 37 °C for 24-48 hours. The same dilution was used on 30-300 colony plates (Asgary et al., 2012).
Bacterial diagnosis :The types of bacteria that appeared during the colony count were identified and diagnosed. The bacteria were diagnosed. A study of the phenotypic characteristics of *Escherichia coli* bacteria growing on McConkey agar medium, along with biochemical tests for the bacteria, was conducted according to the methods stated by Brown and Smith (2017), Sharmin et al. (2010), and Tofighi and Saeidi (2016). 1- Oxidase test, 2- Catalase test, 3- Indol test, and 4- Methyl red reaction test. 5- The Fox-Proskouer test, 6- The

citrate Utilization test, and 7- The Urea Hydrolysis test.

Estimation of Peroxide Value

The estimate came from (Anwar & Baker, 2022). 2 grams of extracted fat were weighed using a saxolite apparatus, and 30 ml of a combination of 3 parts glacial acetic acid and 2 parts chloroform, 0.5 ml saturated potassium iodide, 30 ml distilled water, and 1 ml starch indicator (1%) was added. The mixture was washed with 0.01% sodium thiosulfate until the blue hue faded. Equation used to estimate it:

"Peroxide number (mEq) = number of milliliters of sodium thiosulfate x 0.01 x 1000 / weight of sample"

Lipid Oxidation Indicator in Croissant Measurement of Thiobarbituric Acid (TBA)

Witte et al. (1970) used the thiobarbituric acid method to quantify fat oxidation in the sample. This approach is summarised: A homogeniser was used to homogenise 1 g of the material for 2 minutes with 25 mL of a cold solution containing 20% trichloroacetic acid (TCA) dissolved in 2 M phosphoric acid. In a 50 mL volumetric flask, distilled water was added to the mark. Shake the mixture, take 25 ml, and centrifuge at 30,000 rpm for 30 minutes. The mixture was filtered using filter paper No. 1, and 5 ml was placed in a test tube. Five mL of 0.005 M TBA reagent solution in distilled water was added. Mixing all components except the sample to be measured created the blank. The materials were combined, sealed in test tubes, and kept at room temperature for 15-16 hours in the dark. The contents were cooked in a water bath for 30 minutes (Gheiene, 2011). The absorbance of the resulting color A was measured at a wavelength of 530 nm using a spectrophotometer. The TBA value was calculated by multiplying the absorbance value by a factor of 5.2. The value was expressed as mg malondialdehyde (MDA) per kg of seeds according to the following equation: TBA value (mg/MDA kg) = A530 x 5.2.

Free Fatty Acids (FFA)

Free fatty acids were calculated using the approach described by Mani et al. (2020) and Ebeed (2020). The process involves cold fat extraction. Then, 25 mL of 95% neutral ether and ethanol were added to 10 grams of fat. One ml of phenonaphthalene reagent was treated with 0.1 N sodium hydroxide to become pink. Free fatty acid% was estimated from oleic acid concentration.

Free fatty acids (FFA) were used to determine the percentage according to the following formula: FFA% = 0.282 × ml NaOH × 0.1N NaOH / Weight of sample (g).

Sensory Evaluation

The University of Baghdad Department of Home Economics provided Croissants samples for sensory examination. The department-approved assessment form included 10 assessors and utilised the test to analyse the data statistically (Eshraq, 2024).

Table 1. Sensory evaluation scores for laboratory-made croissants.

Sensory Evaluation	degree
appearance	7
texture	7
freshness	7
flavor	7
thickness	7
color	7

general acceptance	7
total	49

7 (excellent) represents the highest rating, 6 (very good), 5 (good), 4 (high average), 3 (average), 2 (acceptable), and 1 (very poor) represent the lowest rating

Statistical Analysis

To examine the impact of various characteristics on the traits under investigation, using CRD as a factor, the statistical application SAS (SPSS, 2019) was used for data analysis. The Least Significant Difference (LSD) test was used to compare the means and identify any significant differences.

RESULTS AND DISCUSSION

Table 2 shows the presence of four distinct compounds in the hot aqueous safflower extract. The retention times were 3.82, 4.14, 4.96, and 7.02 seconds for the compounds quercetin, rutin, catechin, and Kaempferol, respectively. The Table shows the concentrations of the four compounds identified using the HPLC liquid chromatography technique, where Kaempferol and quercetin recorded high values of 88.6 and 97.4, respectively, compared to the other compounds such as phenylethanoid glycosides, flavonoids, coumarins, and fatty acids , which reached 49.8 and 66.4, respectively.

Table 2 indicates the retention times of the hot aqueous safflower extract compounds when analyzed using an (HPLC) device, as in Figures 1, 2, 3، 4 and 5. These results are similar to those of Ullah (2019). According to (Jiang et al., 2005), the flowers also include the following compounds: quercetin, Kaempferol, 6-hydroxy Kaempferol and its glycosides, safflomin-A, safflamin C, safflamin A, hydroxysafflor yellow A, safflor yellow A, and chalcones. (Onodera, 2006)(Wu.et.al.,2021).

Table 2. Retention time and concentrations of compounds separated by HPLC technology.

Diagnostic compounds	p**pm**	**Retention time [min]**
Rutin	49.8	3.82
Kaempferol	88.6	4.96
Catechine	66.4	6.14
qurcetine	97.4	7.02

Result Table (Uncal - F:\qurcetin 20 ppm - Detector 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	7.017	453.350	69.706	100.0	100.0	0.08	
	Total	453.350	69.706	100.0	100.0		

Result Table (Uncal - F:\catechine 15 ppm - Detector 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	6.137	1123.624	185.661	100.0	100.0	0.10	
	Total	1123.624	185.661	100.0	100.0		

Figure 1. HPLC Chromatograph of quercetin at Rt.7.02.

Figure 2. HPLC Chromatograph of catechin at Rt. 6.34.

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.820	1104.651	187.480	100.0	100.0	0.10	
	Total	1104.651	187.480	100.0	100.0		

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.960	23451.002	1224.157	100.0	100.0	0.28	
	Total	23451.002	1224.157	100.0	100.0		

Figure 3. HPLC Chromatograph of rutin Rt.3.82.

Figure 4. HPLC Chromatograph of Kaempferol at Rt.4.96.

	Reten Tim[min]	Area [mAU.s]	Height[mAU]	Area[%]	Height	WO5[min]	Compound Name
1	3.837	1809.644	100.985	18.6	18.9	0.34	
2	4.990	334.329	13.890	3.4	2.6	0.42	
3	6.337	159.550	11.369	1.6	2.1	0.27	
4	6.930	7408.902	408.844	76.3	76.4	0.31	
	Total	9712.425	535.087	100.0	100.0		

Figure 5. HPLC Chromatograph of the analyzed samples.

Table 3 presents a preliminary chemical analysis of the concentrations of active compounds and groups in safflower extracts using hot distilled water. The concentrations of

phenols were the highest, reaching 63.2 mg/kg^{-1}, followed by flavonoids, whose percentage was 41.0 mg/kg^{-1}. In comparison, terpenes appeared at a rate of 12.2%, followed by glycosides. As for saponins, their values were 8.9 and 4.1%, respectively. As found(Moumen et al,2014; Aziz et al,2022; Ferreira et al., 2015), *C. tinctorius*, with a concentration of 37% and 58%, respectively.Where the majority of the types were beta-carotene and sterols, and their concentrations were for safflower plants and both sexes.
C. tinctorius and C.oxacantha were 36.4 and 46.5, respectively.

Table 3. Preliminary detection of the active compounds in the safflower plant.

Concentration	Name the concentration analysis
63.2	**Total content of phenols (mg/kg^{-1})**
41.0	**Total flavonoid content (mg/kg^{-1})**
30.7	**Total alkaloids content %**
4.1	**Total saponin content %**
8.9	**Total glycoside content %**
12.2	**Total terpene content %**

Bacterial isolates from croissants, which contain safflower extract, form small bacillary cells that do not form spores and grow in both aerobic and anaerobic conditions. Bright pink colonies appeared on the MacConkey differential medium, exhibiting all the characteristic structures of the bacterium *E. coli* (Brown & Smith, 2017; Sharmin et al., 2010; Tofighi & Saeidi, 2016). It did not appear on blood agar medium, and it was positive for catalase, indole, and methyl red tests, and negative for oxidase, urea, Fox-Proskouer, and citrate tests. (Abuova et., 2022) Found that flavonoid and safflower hot water extracts had a good effect on the bacteria *Staphylococcus*. Aureus, more inhibiting than the negative bacteria, which are more resistant due to the thick murein layer, which prevents the inhibitors from penetrating the cell membrane, and also because their membrane consists of several layers of lipids compared to the membrane of the favorable bacteria (Abdel Moneim et al., 2018). The presence of polyphenolic chemicals is further linked to the antibacterial action of plant extracts. Bacterial cell wall and membrane destruction are two primary ways in which these chemicals inhibit bacterial growth. When tested against several bacteria, including *S. aureus, K. pneumoniae, E. coli,* and *A. baumannii*, safflower extracts were found to have an inhibitory effect on these bacteria.

Table 4 presents the effect of adding safflower aqueous extract to croissants in the control treatment prior to storage. It reached 1×10^5CFU. The 100mg/Kg concentration did not show any effectiveness in inhibiting the bacterial population. Its effect was similar to that of the control treatment. In comparison, the 200mg/Kg concentration had an effect that inhibited bacterial growth until the second day, when the bacterial population reached 1.5 x 10^{5} cells/g and gradually increased until it reached 6.8×10^5 cells/g. On the ninth day, the concentration of 300mg /Kg inhibited the number of bacteria. It delayed the appearance of bacterial growth until the sixth day, when it reached 2×10^{5} cells/g. It was found that a concentration of 300mg/Kg is the most effective in delaying the growth of bacteria. The antimicrobial properties of plant extracts are attributed to the presence of phenolic compounds. The antimicrobial effect of the safflower plant is attributed to its ability to disrupt membranes. The volatile oil has a vital role in inhibiting microorganisms, as it

reduces the effectiveness of primary metabolism and stops the processes of oxidative phosphorylation and the electron transfer chain inside the cell due to the active groups of the oil interacting with the protein structures of the enzymes, which lead to stopping their work (Rakaa & Obaid, 2020). The resistance shown by gram-negative bacteria to volatile oils, unlike that of gram-positive bacteria, is due to the differences in the cell walls of both types. Harmful bacteria have an outer membrane composed of a chain of polysaccharides, which acts as a barrier and prevents affinity for volatile oils, as this study agrees with (Hassan, 2024), as these compounds were found. The research revealed that yeasts are more susceptible to oil extracts than bacteria, and that Gram-negative bacteria are less sensitive to these extracts. Similar studies have also found this. At the same time, the aqueous turmeric (Hussein, 2012) The results showed that "the ethanolic turmeric extracts can affect the growth of S. aureus & B. subtilis more than the growth of *E. coli* & *Shigella sp*" And as found (Juma,2011) '' The test results show the growth of Bacteria in the treatment which is Staphylococcus spp Appositive Bacteria''. and found as(Hafud,, 2017)" that asreducing microbial load more higher than 150 & 300 mg/ml. All of these samples conformed".

Table 4. Effect of extracts on bacterial numbers (cfu/10^5)

Transactions	**Concentrations %**	**Period**		**day**								
	Mg/kg	**0**	1	2	3	4	5	6	7	8	9	10
A1	Control	1	1.6	2.4	3	4.8	5.2	6	7.5	8.6	9.4	10
A2	100	2	3	3.6	4	5.6	6.8	7	7.6	8	8.9	9
A3	200	0	0	1.5	2	3.4	4.6	5	5.8	6	6.8	7
A4	300	0	0	0	0	0	0	2	2.6	2.8	3.8	4

4. Estimation of peroxide value

It is used as an indicator of peroxide values in croissant products stored at 37°C for 10 days (Anwar &Baker, 2022). The control treatments and T3, T2, and T1 at the start of storage did not exhibit any significant changes in peroxide value ($P < 0.05$). Nevertheless, by the time the storage period was over, the PV value of the control treatment had risen from 3.08 to 12.46. On the tenth day of storage, treatments T2 and T3 had significantly lower values, coming in at 5.565 and 6.486, respectively, compared to treatment T1, whose value had risen to 6.745 by day 10. The presence of phenols in the essential oils of safflower extract gives them antioxidant properties, as shown in Table 5.

Table 5. Effect of safflower extract on changes at 37°C for 10 days (.meq O2/kg fat)

Treatment	**Period day**			
	1	**3**	**6**	**10**
C	3.08 ±0.17	5.08 ±0.32 a	7.745 ±0.36 a	12.466 ±0.52 a
T1	3.005 ±0.14	4.01 ±0.19 b	5.19 ±0.23 b	6.745 ±0.35 b
T2	3.06 ±0.14	4.01 ±0.19 b	5.095 ±0.25 b	5.365 ±0.27 b

T3	3.05 ±0.12	3.90 ±0.16 b	5.365 ±0.23 b	6.476 ±0.31 b
LSD value	0.164NS	0.827 *	0.483 *	1.366 *

"Means having with the different letters in the same column differed significantly", * (P≤0.05).
C=control T1=100, T2=200, T3=300.

5. TBA: The results show that while stored at 37°C, the treated samples had considerably reduced TBA levels (P<0.05) compared to the control sample, according to the TBA value. From 0.295 mg MDA/kg croissant on the first day of storage to 1.0745 mg MDA/kg croissant on the tenth day, lipid oxidation in the control sample started to rise. Simultaneously, oxidative rancidity was shown to decrease with on the tenth day, treatments T3and T2. storage period, their values were 0.063 and 0.0816, respectively, compared to treatment T1, which reached 0.827.as shone on Tabel 6.

Table 6. Effect of safflower extracts (SLE) on changes at 37° for 10 days. Milligrams of MDA per kilogram **(**mg.MDA/Kg)

	Period day			
Treatment	**1**	**3**	**6**	**10**
C	0.0295 ±0.007	0.033 ±0.004	0.067 ±0.009	1.0745 ±0.21 a
T1	0.0295 ±0.007	0.032 ±0.004	0.047 ±0.007	0.0827 ±0.18 a
T2	0.0275 ±0.005	0.031 ±0.002	0.0405 ±0.007	0.0816 ±0.02 b
T3	0.0275 ±0.005	0.042 ±0.005	0.052 ±0.005	0.063 ±0.02 b
LSD value	0.0084 NS	0.0187 NS	0.0278 NS	0.198 *

"Means having with the different letters in the same column differed significantly", * (P≤0.05), C=control. T1=100, T2=200, T3=300.

6. **Free fatty acids (FFA)**

As a consequence of fat breakdown, croissants contain free fatty acids (Mani et al., 2020). All treatment and control samples in this investigation began with FFA values ranging from 0.23 to 0.27. At the conclusion of 10 days of storage at 37°C, the control treatment reached maximum values of 1.354, respectively, indicating a substantial increase ($p < 0.05$) over time (Table 7). The unsaturated fats are oxidized so quickly that it leads to an increase in fat decomposition and the breakdown of free fatty acids (FFA). Additionally, the findings demonstrated that, particularly after 10 days of storage, the addition of safflower extract considerably decreased ($p<0.05$) the FFA content in comparison to the control treatment that was not treated, as shown in Table 7

Table 7. Safflower extract's impact on croissants' free fatty acid (FFA) % at 37°C for 10 days.

	Period day			
Treatment	**1**	**3**	**6**	**10**
C	0 .27 ±0.06	0.45 ±0.08	0.64 ±0.09 a	1.354 ±0.26 a
T1	0.26 ±0.04	0.32 ±0.05	0.46 ±0.07 b	0.48 ±0.06 b

T2	0. 23 ±0.04	0.30 ±0.05	0.41 ±0.07 b	0.47 ±0.05 b
T3	0.25 ±0.05	0.42 ±0.07	0.515 ±0.08 b	0.615 ±0.06 b
LSD value	0.038 NS	0.172 NS	0.115 *	0.277 *

"Means having the different letters in the same column differed significantly, * (P≤0.05). C=control, T1=100, T2=200, T3=300"

Compare the sensory evaluation results of the 10-day-old control treatment form included 10 assessors and utilised the test to analyse the data statistically (Eshraq, 2024). Table 8 compares croissants to lab-made croissants with different quantities of hot safflower extract. The statistical analysis indicates no significant difference in color features between the control treatment (A) and transactions A1,A2,A3 respectively. (P < 0.05).

Table 8. Effect of the safflower extracts on sensory properties of laboratory-made croissants.

Sensory qualities								
Concentration	**concentrations**	**Appearance**	**Texture**	**Freshness**	**flavor**	**Thickness**	**Color**	**General Acceptance**
Control(A)	0	6.4± 0.37ab	5.0±0.24b	5.4±o.31b	5.1 b	5.7 ±0.28b	6.0 ±0.33a	5.4 ±0.29ab
A1	100	5.4± 0.25b	6.1 ±0.32ab	6.0±0.39ab	6.2 ± 0.28ab	6.5 ±0.38ab	6.8 ±0.42a	6.4 ±0.41a
A2	200	7.0 ±0.44a	6.5±0.38ab	5.9±0.28ab	6.8 ±0.41a	7.0±0.42a	7.0±0.45a	5.6±0.36b
A3	300	6.8 ±0.38ab	7.0 ±0.43a	7.0±.0.40a	5.0±0.26b	5.0 ±0.31b	6. 4±0.32a	6.0 ±0.30ab
LSD value		1.485*	1.502*	1.477*	1.494*	1.811*	1.078NS	1.335*

*(P<0.05.) Different-letter averages in the same column vary dramatically. Excellent 7, very good 6, good 5, average 4, acceptable 3, bad 2, extremely poor 1.

Although treatment A2 obtained the highest value of 7.0, it was followed by the other transactions, A3, A2, and A1, which had values of 6.0, 6.4, and 6.8, respectively. Significant differences were found between treatment A2 and transaction A1 in appearance, where treatment A2 excelled, obtaining a value of 7.0 in appearance. Treatment A1 obtained the highest value of 5.0. At the same time, significant differences appeared at the level of P< 0.05 between control treatment A and treatment A3. The A control treatment obtained the lowest value. At the same time, the percentages of quenching reached 5.4 and 5.0, respectively, while the A3 treatment obtained the highest value, reaching 7.0 for both. As for the flavour characteristic, treatment A2 showed a significant difference from treatments A3 and A, as it obtained values of 5 and 5.1, respectively, whereas treatment A2 obtained a value of 6.8. As for the control treatment, treatment A2 obtained the highest value, reaching 7.0, while the control treatment and treatment A3 did not differ significantly, as they obtained values of 5.0 and 5, respectively. As for general acceptance, treatment A1 obtained the highest value, amounting to 6.4, and it differed significantly from treatment A2, which

obtained the lowest value, amounting to 5.0

"The smoothness attribute, treatment A2 (0.25%) received the highest score of 5.70, while treatment A4 (0.75%)"(Eshraq,2024). According to AL-Timim (2011), "there were no notable variations in organoleptic traits among the different treatments, except for flavour value."

CONCLUSIONS

The research aims to prepare an aqueous extract of safflower *Carthamus tinctorius L*. in the laboratory and then detect the active compounds in it. The most effective compounds were selected following an investigation using HPLC analysis. Compounds were selected for the quantitative determination of phenolic compounds. A double extraction process confirmed the saponin content in the samples, and secoisolaricyrizinol was isolated from the extract. The total glycosides and the percentage of terpenoids were determined. The new active compounds were evaluated for their biological activity, which demonstrated good antibacterial and antifungal properties.

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COMPETING INTERESTS

The authors declare that they have no conflicts of interest.

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