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Incorporation of Spirulina Extract in Pectin Edible Coating for Use as Antimicrobial Agent for Some Fruits

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THIS work aims to investigate the incorporation of spirulina extract (extracted by different solvents) in edible pectin solution to produce eco-friendly edible coatingmaterials. EthanolicSpirulina extract (SE) showed the highest yield (17.5%), phenolic content (19.55 mg GAE/g), total carotenoids (5.54 mg/g) and antioxidant activity of 78.26%. The dried SE showed the highest inhibition zone for all tested microbial strains at concentration of 350 and 500 mg/100 mL. Incorporation of dried SE in pectin coating solution at concentration of 200 mg/mL led to inhibition of the bacterial growth and increased the inhibition rate of *E. coli* 0517-H7; and *S. aureus*. Guava and Apple slices samples coated with pectin solution containing 1%SE showed lower total bacterial count.Also, weight loss was reduced from 11.5 to 0.4% and from 7.8 to 2.5% respectively for Guava and apple fruits during 24 days of cold storage. Infected fruits were decreased over the period of storage. Rate of firmness loss was reduced from (-13.9 to -5.05 g_f/d) for Guava fruit and from (-9.1 to -1.05 g_f/d) for apple slices coated with 1% SE with pectin film solution during storage.

Keywords: Spirulina, Pectin coating, Guava, Apple, Microbial quality, Sensory attributes.

Introduction

Spirulina are multicellular and helical shaped filamentous blue-green microalgae, belongs to cyanophycean family, containing c-phycocyanin pigments and consists of about 15 species (Singh and Parwani, 2018). It grows in fresh water, brackish and seawater. It can be harvested and processed easily and has significantly high macro-and micronutrient contents. Dry spirulina contains 10-20% carbohydrates, 3-10% lipids and 50-70 % proteins. Its amino acids content is estimated to be 71% based on dry matters, which represented 15 and 20 times more than that of meat and soya, respectively and providing all the essential amino acids as well as a whole host of minerals such as calcium, magnesium, zinc, selenium, and iron. It is also rich in vitamin (A, C, E, K, H and B), and covers 4,11 and 15% of the required daily amount (RDA) from vitamins B_3, B_1 and B_2, respectively (Seyidoglu et al., 2021 and Kini et al., 2020). Moreover, spirulina is rich in antioxidant including B-carotene, Zeaxanthin, phycocyanin and much of polyphenols. It is generally recognized as safe food "GRAS" and can be used as immune system enhancement, nutritional supplement, food source and as a coloring agent beside its ability to inhibit viral replication (Al-Zahrani et al., 2017). Cultivation of spirulina algae is possible in alkaline water, where it is difficult for other microorganisms to survive, with optimal growth at temperature between 35 ° and 40 ° with a productivity of 20-30 g/m^2/day. Closed photobioreactors and open raceway ponds are the two major technologies being considered for the cultivation of spirulina (Habib et al., 2008; Sow and Ranjan,2021).

Besides its high nutritional value in form of spirulina paste or dry powder, spirulina extracts

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become attractive to produce bioplastics with improved physical properties (Cinar et al., 2020). On other side, Nakamoto et al. (2023) considered spirulina as a promising raw material to produce multifunctional packaging materials, because of its rich composition and bioactive properties, which could be used in the development of active and smart packaging systems to extend and monitor the shelf life of packaged foods. However, there are limited evidence for use of spirulina tissues in food preservation and food packaging despite its extremely high content in polyphenolic compounds and its high antioxidants activity, which could be used to protect foods from oxidation and deterioration (Singleton et al., 1999). Edible coatings could be incorporated with natural antioxidant from algae, such as spirulina, which has been described to exceed antioxidant compounds of those obtained from plants and herbs (Beyrer et al., 2020). Bioactive compounds from spirulina tissues should be extracted to apply in coating and packaging purposes to reduce water vapor transfer, gaseous exchange, oxidation, and microbial deterioration of fresh products (Lian et al., 2021 and Jaeschke et al., 2021) mentioned that it is essential to choose an appropriate extraction method since the release of the bioactive compounds from spirulina tissues depends on the cell membrane disintegration and the extracted compounds should be protected from fast degradation through using stabilizing agents such as sugars, citric acid, and natural polymers. The factors to be optimized for extraction are temperature, solvent type, pH, biomass / solvent ratio, and biomass form to enhance yield and quality. They suggested the application of some cell disruption methods such as freezing, thawing, homogenization, mixing, bead milling, ultrasound, microwave, and pulsed electric field as pretreatments for solvent extraction, where ethanolic extraction had the widest range of activity. Pyne (2020) and Stunda-Zujeva et al. (2023) confirmed the antioxidant and antibacterial activity of spirulina extract. They referred this effect to the presence of active sites such as -OH, thiol, Carboxylate (active groups in phenolic compounds), which have strong antioxidative and antibacterial effects. They emphasized the use of spirulina extracts as an alternative of chemical preservative and mentioned also the healthier effect of spirulina compared to the harmful chemical preservative, which distribute in kidney, liver, spleen and brain, while spirulina active compounds are easily digested. Application of spirulina dry extract in coating purpose of food has been reported by Stejskal et al. (2020). They incorporated dry extract of spirulina in gelatin – based coating film to enhance the quality of refrigerated Atlantic mackerel (*Scomber scombrus*) for 11 days and found that the applied coatings worked as antimicrobial and antioxidant agent due to the high phenolic and bioactive compounds of spirulina extract and mentioned that this may constitute a novel and promising strategy to enhance the quality of refrigerated fatty fish.

Based on the aforementioned information, the purpose of this research is to investigate the antioxidant and antimicrobial potential of spirulina dry extract, obtained with different solvents, against some common pathogenic bacteria and to evaluate the efficacy of pectin-based edible coatings containing dry spirulina extract in enhancing quality and shelf life of some minimally processed perishable fruits. Pectin, as a plant source – based edible coating, could provide strong, flexible, and thermally stable films with good mechanical properties and excellent barrier characteristics (Youssef and El-Kady, 2016) and as a polymer, it could work as protecting agent for extracted spirulina bioactive compounds.

Materials and Methods

Materials

Spirulina algae *S. platensis* was obtained from a farm, in El-Khatatba town, El-Menoufia Governorate, Egypt. Fruits were collected from Hort. Res. Inst. Plantation, Cairo, Egypt. All of solvents were of analytical grade. The DPPH and Folin- Ciocalteu's phenol reagents, dimethyl sulfoxide (DMSO) and high methoxy pectin were purchased from Merck Co. (Darmstadt, Germany).

Microbial strains and growth media

Bacillus cereus B-3711**,** *Bacillus Subtilis* 3357 and *saccharomyces cerevisiae* Y-2223 its primary source is from the Northern Regional Res. Labs Illinois, USA. and obtained from Microbiological Lab., Agric., college Menoufia Univ., *E. coli* 0157:H7 and *S. aureus* were obtained from dairy microbiological Lab., National research Center, Egypt. The strains were activated in tryptone soya broth, incubated at 37^{o}C for 24hr. All microbiological media (Muller Hinton agar, Tryptone soya media, peptone water and plate count agar) were obtained from Al- Gomhoria Company for Medicines and Medical Supplies -Al Sawah, Cairo -Egypt.

Methods

Preparation of dry spirulina tissues

Fresh spirulina algae harvested from the Sower were put in an aluminum tray and then dried at 45°C under vacuum of 67 kPa using a vacuum oven (Thermolins, Merrick Ville, NSW, Australia). The samples were dried until constant weight is obtained, then milled into fine powder and stored in polyethylene pouches under cooling.

Preparation of spirulina extracts (SE)

For each run,75 g of spirulina dry tissues was extracted with 750 mL each of 4 different solvents (methanol, ethanol, acetone, and chloroform), respectively, at room temperature with stirring for 72 hr. Each extract was filtered using Whatman No. 1 filter paper and evaporated under reduced pressure to dryness below 40°C. The resultant dried extracts were analyzed directly for phenolic, carotenoids contents as well as antioxidant and antimicrobial activities to choose the best extract, which gives the highest antimicrobial and antioxidant potent. All dried extracts were kept in amber glass at -20°C.

Preparation of coating solution and samples coating

Four different pectin solutions were prepared as follows: Five grams of high methoxy pectin were mixed with 100 mL distilled water, polyvinyl alcohol 1.25% (w/v) as a cross linking reagent and 2.5% glycerol (w/v) as plasticizer for 5 min at 25°C, then the suspension was transferred to a water bath at 90°C for 30 min, and agitated for another 30 min. by magnetic stirrer (500 rpm), then pectin-Spirulina emulsions in different concentrations (200,300,500 mg/100 mL) were prepared by mixing appropriate amounts of 5% spirulina- ethanolic extract to the corresponding pectin solution. The final solution was sonicated about 1 hr to remove air bubbles or dissolved air. Surface coatings were applied by dipping of samples in solutions for 10 seconds at room temperature, then drying for 30 sec. This dipping procedure was repeated 3 times. Coated fruit samples were shaken 2 min., for removing the excessive solution and then they were dried for 2h in a laminar flow cabinet at 25°C. The uncoated and the three coated samples of each run were stored in refrigerator at (4°C) for 24 days. The subjective measurements (every 6 days) were weight loss, percentage of surface decay and injury, texture firmness, microbial examination, and sensory properties (texture, color, flavor, taste and over all acceptability).

Microbiological Analysis

Antimicrobial activity of SE

One gram of dried spirulina tissue extract, obtained from different solvent, was dissolved individually in 5.0 mL DMSO (dimethyl sulfoxide) to get 200 mg/mL solution, after that different dilution were prepared in DMSO to get 50, 100, 350 and 500 mg/100 mL solution. The antimicrobial assay for different concentration of SE was performed by agar disc diffusion method using Muller Hinton agar medium according to Singh and Sharma (2012). The medium was poured into the petri dishes and allowed to be solidified. The strains (0.1 ml of approximately 10^9 cells / mL) of the tested microorganisms were spread on the surface of the agar found in Petri dish using a sterile swab. The plates were rested for 2 hr at 37°C to allow the agar to be saturated with strains. For the agar disc diffusion method, 20 µL of each Spirulina concentration was impregnated on different sterile paper discs (Whatman, 106 mm) and placed on the surface of Muller Hinton agar in petri dishes gently. According to this procedure, each disc carried a load of different Spirulina extract equals to 1, 2, 4, 7 and 10 mg extract/L, which represent (0.1, 0.2, 0.4, 0.7 and 1.0% respectively). The plates were incubated at 37°C for 24 hr. and the inhibition zones around each disc were measured in millimeters (Zahra et al., 2020).

Anti-bacterial activity of Spirulina containing pectin films

Antibacterial activity of three pectin films prepared with varied concentration of ethanolicspirulina dry extract (200, 350 and 500 mg/100 mL) were examined for their inhibitory effect on the growth of gram-negative bacteria (*E-coli* 0157:H7) and gram-positive bacteria (*S. aureus*). Test microorganisms were aseptically inoculated in 20 mLof tryptone soya media broth (TSB) and subsequently incubated at 37°C for 24 hr. Each cultured broth was centrifuged at 2000 rpm for 10 min and the cell pellets were suspended in 100 mL of sterile TSB and diluted 10 times with sterile distilled water. Twenty mLof diluted broth (10^6-10^7 CFU/mL) were taken into 100 mLconical flask containing 100 mg of film sample and subsequently incubated at 37°C for 24 hr under mild shaking. The same diluted broth without film sample was used as the control. The cell viability of each microorganism was calculated by counting bacterial colonies on the plates at 0, 4, 8, 12, 16 and 20 hr. Antibacterial tests were performed in triplicate with individually prepared film (Brugger et al.,2012).

Microbiological analysis of coated fruits

At each sampling interval, a package from each treatment was aseptically peeled. 10 g of each sample were homogenized for 150 sec. in sterile stomacher bags containing 90 mL peptone water (1% w/v peptone). Serial dilutions were then made. Total mesophilic bacteria count of samples were enumerated using Plate Count Agar and plates were incubated at 37°C for 48-72hr. The count methods were carried out as proposed by Downes and Ito (2001).

Functional characteristics of SE

Total phenolics content of SE

The total phenolics content of SE was analyzed by the F-C method (Singleton et al., 1999). Briefly, 0.75 mL of Folin - Ciocalteu reagent was added to 100 µL of different dilutions of SE in the test tubes and final volume was made 10 times with distilled water. After 5 min, 0.75 mL of a sodium carbonate solution (7.5%) was added to each tube. The tubes were kept in dark for 90 min. and absorbance was measured against a blank at 725 nm by the spectrophotometer (U-28000 spectrophotometer, Hitachi, Tokyo, Japan). A standard curve was plotted using different concentrations of gallic acid and the amount of total phenolics content was calculated and expressed as milligram gallic acid equivalent per gram dried extract (mg GAE/g dry extract).

Total carotenoids of SE

The HPLC method for analysis of carotenes was carried out using the chromatography system (Agilent 1260 HPLC) as modified by Rodriguez-Amaya (2001). The volume of sample was 20 µL, and the temperature was 45°C. Column effluents were monitored at 285 nm. B-carotene standard (Sigma-Aldrich, USA) was measured at concentration of 0.1 g/L. The amount of total carotenoids content was expressed as mg carotenes per gram dried extract.

Antioxidant activity of SE

The antioxidant activity in terms of radical scavenging activity (RSA) of SE was carried out following the method of Martinez-Avila et al. (2014). Briefly, 2.9 mL of 60 µM DPPH radical were added to 100 µL of each extract. Then, samples were placed in a dark space, and after 30 min. the absorbance was measured at 517 nm using a Spectrophotometer U-28000 Hitachi, Tokyo. Butyl hydroxyl toluene (BHT, sigma) was used as positive control (standard) while the negative control contained the entire reaction reagent except the extracts.

Inhibition % was calculated by the following equation:

$$\text{DPPH scavenging effect (DSE\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Physical characteristics of coated fruits

Weight loss

Three replicates of 20 Guava fruits and/or apple slices were used for each treatment. Every 6 days fruits were weighted regularly to determine weight loss. Results were expressed as the percent loss of the initial total weight (AOAC, 2000).

Extent of decay

For assessment of the coated samples during cold storage, the fruits were examined regularly (every 6 days), and percentage of surface decay and injury were estimated and regarded until detect mold, which, estimated as infected if a visible lesion was observed. Results were expressed as percentage of fruit infected.

Texture firmness

Firmness of coated samples were measured with a universal testing instrument (Brookfield CT3 10K Texture Analyzer, USA) fitted with a compression anvil 12 mm in diameter. Resistance to pressure was recorded after a compression of 3 mm, defined as the maximum force (gram force) "g_f" and equipped with 10 Kilo Newton head load (Jimenez et al., 2010).

Sensory properties

The sensory panel consisted of eight experienced personnel of the Food Packaging Unit. The panelists were familiarized with the samples during the storage and were asked to evaluate the samples for color, flavor, taste, texture, and general acceptance. The analysis was performed at the Food Eng. Res. Lab, A.R.C, Gizeh, Egypt under white fluorescent lights according to the specifications of the int. standards, ISO 6658 (2017). Samples were given scores out of five points, where one represents the most disliked and five represents the most liked (Molla et al., 2020).

Statistical analysis

Results obtained from the replications were evaluated by the ANOVA- GLM procedure of the SAS Statistical Analysis Program according to the Completely Randomized Design (SAS, 2001)($P<0.05$). The rate of change in dependent variables (Y–axis) per unit change of experimental values of independent variables (X-axis) was obtained according to the first – order equation:

$(Y = A \pm B * X)$,

where B is the change in amount of variable Y per each unit change in variable X. Duncan analysis was applied on the results found statistically significant.

Results and Discussions

Yield and functional characteristics of dried spirulina extract (SE)

Figure 1 shows the dry extract yield and some of its functional properties obtained by the different applied extraction methods. As seen, use of ethanol alcohol as a solvent gave the highest yield of dry extract, being 17.5% compared with the other solvents (Methanol, acetone, chloroform), which yielded only 13.92% to 15.06% indicating the suitability of ethanol alcohol as efficient solvent for spirulina extraction. Ethanol extract also gave the highest contents for phenolics and carotenoids, being 19.55 mg GAE/g and 5.54 mg/g for phenolics and carotenoids, respectively. The other solvents gave dry extracts with lower contents in phenolics and carotenoids. The corresponding amounts of phenolics and carotenoids were in the range of 12.09 to 15.8 mg GAE/g and 3.47 to 4.48 mg/g total carotenoids, respectively. The worst solvents used were chloroform and acetone, which significantly gave the lowest yield and contents in phenolics and carotenoids correspondingly. Dried ethanolic extract gave the highest antioxidant activity 78.26% compared with the extracts of the other solvents, which gave significantly lower antioxidant values, being in the range of 48.74% to 63.65%. The antioxidant activity of the ethanolic spirulina extract was close to that of Butyl hydroxy toluene–BHT (88.31%), which proves that dried ethanolic extract can replace BHT as antioxidant agent. The obtained results agree with those of Shah et al. (2015), Mohamed et al. (2018) and IIMSAM (2008). They confirmed the efficiency of ethanol as a solvent for spirulina extraction due to its high polarity and ability to pullout more carotenoids and poly phenolic compounds and revealed the importance of the high amount of vitamins A, C, E, B, B-carotene, phycocyanin, selenium and polyphenols in spirulina tissues, which acts as a potent antioxidant.

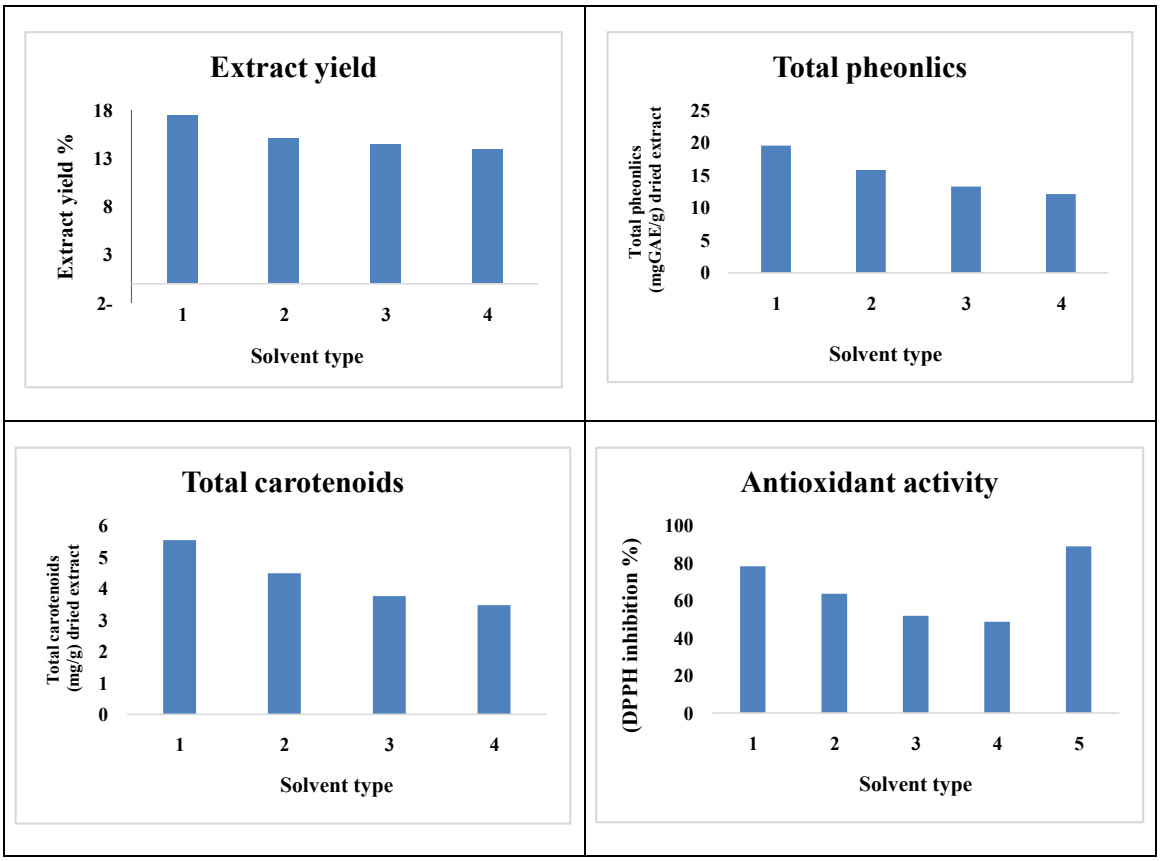

Fig. 1. Total phenolics, carotenoids content and antioxidant activity of different spirulina extracts .

1: Ethanol **2:**Methanol **3:** Acetone **4:** Chloroform **5:**BHT

Antimicrobial activity of the obtained dry spirulina extracts

The antimicrobial activity of the four different spirulina dry extracts at varied solution concentrations (50, 100, 200, 350 and 500 mg/100 mL) against different microbial strains was carried out as described in methods (Section 2.2.4.1) and the results are given in Fig. 2 (A, B, C and D). As seen, the four different dry extracts were tested against five different microbial strains and the highest inhibition zones ever were recorded for the ethanolic dry extract for all tested microbial strains and at all applied concentrations. The other dry extracts showed lower inhibiting effect, especially those obtained by acetone and chloroform as solvents. *Saccharomyces cerevisiae* was the most sensitive microbial strains for inhibition by spirulina dry extracts compared with the other tested bacterial strains. As seen, the inhibition zone of *Saccharomyces cerevisiae* was 14.68 mm with 50 mg/mL dry ethanolic spirulina extract followed by *Staphylococcus aures* (10.25 mm), *E. coli* 0157:H7 and *B. subtilis* (9.00 mm and 8.7 mm, respectively), while *B. cereus* showed the lowest inhibition zone (7.46 mm). Similar trend was found also for the dry spirulina extracts obtained by methanol, acetone, and chloroform (as solvents) but with lower inhibition zones compared with those achieved by the ethanolic extract. The effectiveness of ethanolic and methanolic spirulina against pathogenic bacteria and yeasts was also confirmed by the research carried out by El-Baz et al. (2013), Usharani et al. (2015) and Abdel-Moniem et al. (2022). The anti-microbial activity of spirulina was increased by increasing the concentration of dry spirulina extract from 50 to 500 mg/100 mL (Fig 2- A, B, C and D). However, the increase in inhibition zones was not increased in proportion with the increase in concentration of the applied spirulina extract. For example, by increasing the extract concentration from 50 mg/100 mL to 500 mg/100 mL (10 -folds increase), the inhibition zones against the tested microbial strains were increased by 1.48-folds to 1.93-folds only, which indicate the need to optimize the effective dose necessary to achieve the required inhibition without excessive costs. The relationship between the concentration of spirulina extract and diameter of inhibition zone proved to be linear, since the obtained R^2-values were higher than 0.9 in all cases. *E. coli* 0157:H7 showed, in all treatments, the lowest increase in inhibition diameter (0.0163 to 0.0095 cm/mg/mL) according to the applied extraction solvent, which expresses the resistance of Gram-negative bacteria for inhibition. On other side, Gram-positive *S. aureus* showed corresponding higher rate of inhibition being 0.0208 to 0.0109 cm/mg/mL. Similar trend was found for *saccharomyces cerevisiae*, while both *B. subtilis* and *B. cereus* showed moderate inhibition rates. Nayyef and Thalij (2020) resumed, from their study, that Spirulina could be compared with chitosan nanoparticles as antibacterial agent, while Awadalla et al. (2021) concluded that *S. platensis* should be considered as an economic antibacterial agent than using medicalantibacterial against pathogenic bacteria.

Antibacterial activity of pectin films enriched with dried spirulina extract

The antibacterial activity of pectin films enriched with dried spirulina extract was carried out for extracts obtained by ethanol as solvent and for two pathogenic bacterial strains: *E. coli* 0157:H7 and *Staphylococcus aureus* and the results are presented in Fig. 3 (A and B). As seen, spirulina free pectin films (control sample) showed a linear increase in the growth of colony forming units (CFU) during the entire incubation period of 20 hr. For *E. coli*, the log number of CFU/g was increased from 2.95 at zero time and reached 7.64 log cycles after 20 hr with an increase rate of 0.245 log cycle per hour, while the corresponding growth rate for the control film of *S. aureus* was slightly lower and recorded 0.164 log cycle per hour (Fig. 3 B). Incorporation of spirulina extracts in the pectin films at concentration of 200 mg/100 mL led to curb the bacterial growth after 12 hr for *E. coli* and after 8 hr for *S. aureus* (Fig. 3). However, the overall growth rate was positive for *E. coli* and reached 0.012 log cycle/hr and negative for *S. aureus* and recorded inhibition of -0.043 log cycle/hr. Increasing the concentration of spirulina dry extract in the pectin film to 350 and 500 mg/ 100 mL has reduced the growth rate during the first 12 hr and increased the inhibition rate thereafter. The overall growth rate was negative and reached (- 0.0026 and -0.043) log cycle /hr for *E. coli* and (-0.042 to - 0.086) log cycle /hr for *S. aureus*, respectively for 350 and 500 mg/100 mL incorporated spirulina extract. The results revealed that the Gram-negative bacteria (*E. coli*) was more resistant to the inhibitory effect of spirulina extract than did the Gram-positive bacteria (*S. aureus*) due to the differences in cell wall composition of both strains and to presence of a thick peptidoglycan layer in the cell wall of Gram-negative bacteria strains as well as the presence of lipoprotein layer in the outer membrane of Gram – negative *E. coli* (Breijyeh et al., 2020 and Abdel-Moniem et al., 2022). The strong antibacterial effect of spirulina extract has been referred to its content of phenolic compounds, especially chlorogenic acid (Balti et

al.,2017), which can bind and permeabilize the cell membrane of microorganisms, causing cells to loosen the ability to maintain membrane potential. The obtained results evident the suitability of spirulina extract as antimicrobial additive to food packaging material for maintaining the safety and extending the shelf life of packaged foods.

The obtained results agree with those reported by Ali and Doumandji (2017); Ben Hlima et al. (2019) and Alshuniaber et al. (2021). They confirmed the antimicrobial and antifungal activity of spirulina ethanolic extract against a wide spectrum of bacteria strains and mold species.

Change in nicrobial load of coated fruits during storage

Table 1 shows the change in total bacterial count of coated guava fruits and apple slices during 24 days of storage.The initial total bacterial count of uncoated guava fruits was 1.24 log CFU/g and it continued to increase linearly during the storage time to reach 3.15 log CFU/g after 24 days of storage making an increase in the log CFU by 154%. Fruit samples coated with pectin solution containing spirulina extract showed significantly lower bacterial count during storage period and this trend was increased by increasing spirulina concentration in the coating solution from 0.4 to 1% for coated guava samples. The change in bacterial count by applying 1% Spirulina concentration was reduced to the level of only 32.26%. On other side, apple slices showed lower initial bacterial load, 1.19 log CFU/g, may be due to the acidic nature of apple, which restrict the bacterial growth. Also, the rate of bacterial growth was lower than that recorded for guava samples and the increase (change %) in CFU at end of storage period was in the range of 80.67% (for control) to 22.69% (for 1% Spirulina treatment). These results revealed thatspirulina extract has a broad spectrum of activity on the tested bacteria.The relationship between storage period and log CFU/g was found to be linear with R^2 – values between 0.935 and 0.978. The slope of the obtained relationship was used to compute D-value of growth (Time in days required to increase the CFU/count by 10- folds or one log cycle) and the results are also included in Table (2). As seen, D-value was increased from 12.048 and 33.17 days (d) for control samples to 58.14 and 85.47 (d) for 1% Spirulina treated samples, respectively for Guava fruits and Apple slices.

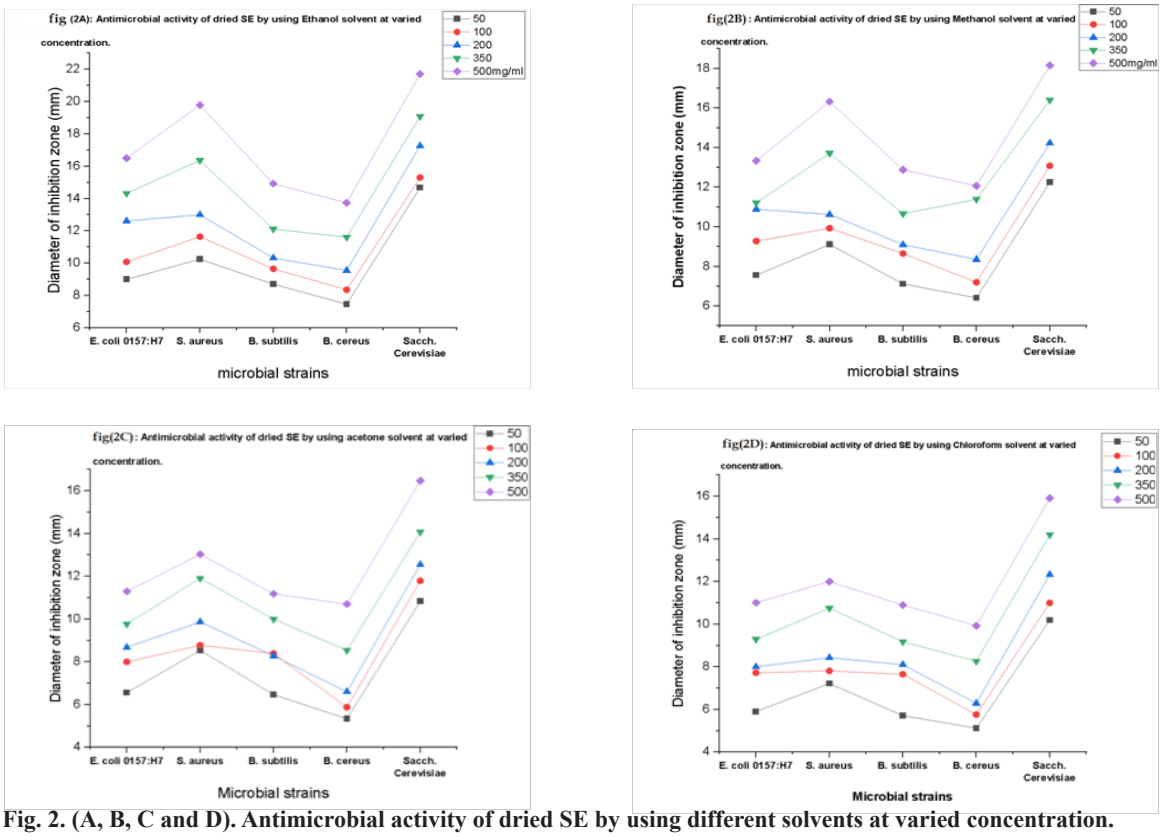

Fig. 2. (A, B, C and D). Antimicrobial activity of dried SE by using different solvents at varied concentration.

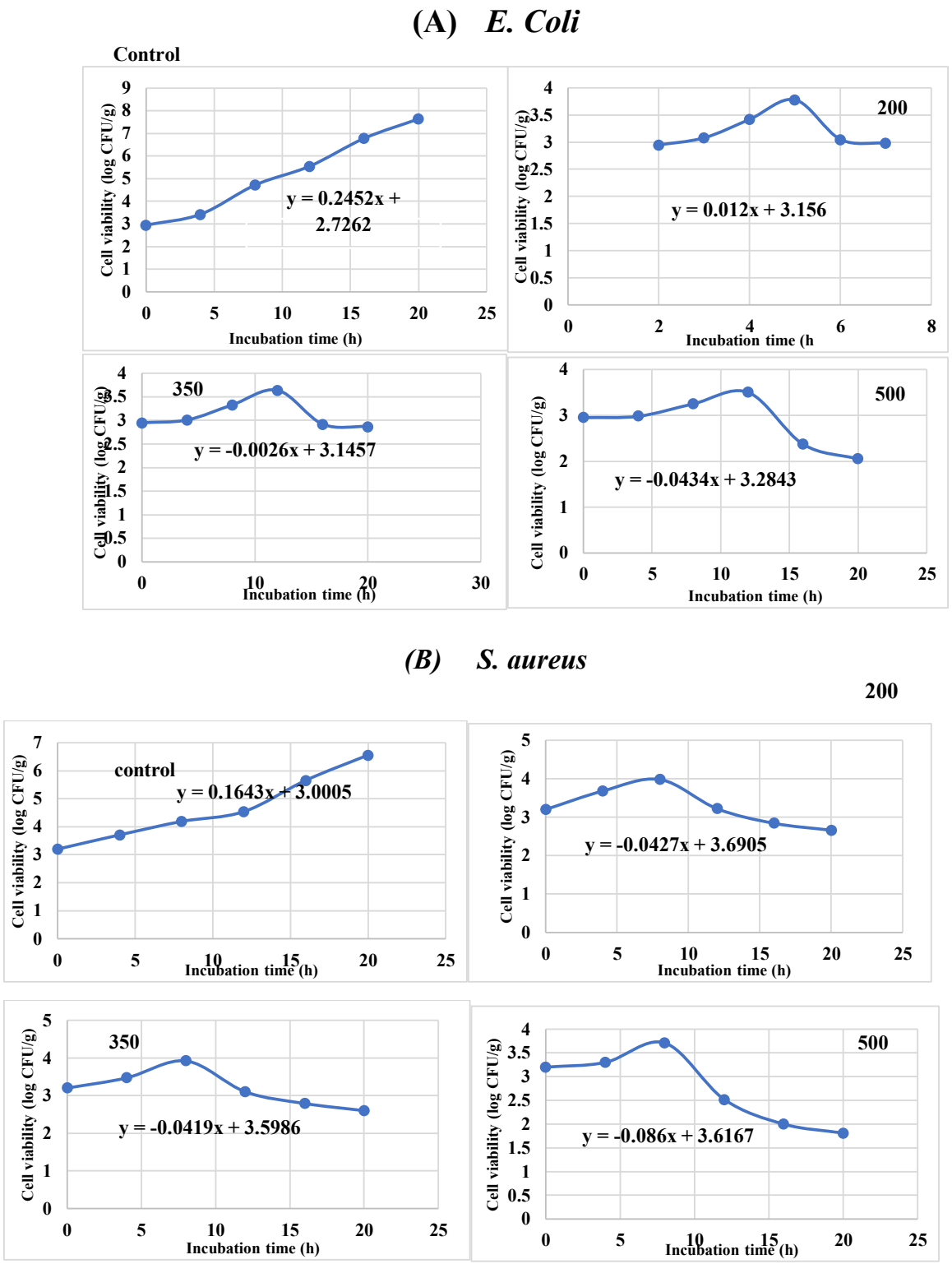

Fig. 3. Relationship between applied spirulina dose (200-500mg/100 mL) in pectin coating solution and cell viability (log CFU/g) for two bacterail strains during 20 h incubation time.

TABLE 1. Change in log number of total bacterial count of coated guava fruits and apple slices during storage at 4℃.

Sample	Concentrate of applied spirulina extract	Storage period (days)					Change (%)	* D-Value (Days)
		0	6	12	18	24		
Guava	0	1.24^{aD}	1.40^{abD}	1.99^{aC}	2.56^{aB}	3.15^{aA}	154.03	12.048
	0.4	1.24^{aB}	1.34^{abB}	1.74^{abcA}	1.80^{bcA}	1.95^{bcA}	57.26	31.948
	0.7	1.24^{aB}	1.36^{abB}	1.60^{bcdA}	1.68^{cdA}	1.81^{cdA}	45.97	41.152
	1.0	1.24^{aB}	1.27^{abB}	1.45^{deAB}	1.50^{deA}	1.64^{deA}	32.26	58.139
Apple slice	0	1.19^{aD}	1.54^{aC}	1.86^{abB}	2.00^{bAB}	2.15^{bA}	80.67	25.188
	0.4	1.19^{aC}	1.38^{abBC}	1.50^{cdeB}	1.77^{bcdA}	1.90^{cdA}	59.66	33.113
	0.7	1.19^{aD}	1.26^{abCD}	1.45^{deBC}	1.66^{cdeAB}	1.72^{cdeA}	44.54	41.152
	1.0	1.19^{aC}	1.23^{bBC}	1.31^{eABC}	1.39^{eAB}	1.46^{eA}	22.69	85.470

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter;

A, B & C: There is no significant difference (P>0.05) between any two means, within the same row have the same superscript letter.

*D-Value: Time (days) required for microbial increase by one logarithmic cycle.

The superior antibacterial effect of Spirulina extract compared to chitosan for extending shelf life of fruits was confirmed byAzhar et al. (2023), where fruits treated with spirulina containing coatings showed the lowest deterioration parameters compared with those containing chitosan.

Quality characteristics of coated fruit samples during storage

Weight loss, decay and texture characteristics of guava fruits and apple slices coated with pectin edible coating containing spirulina extract were assessed during 24 days of storage and the results are given in Table 2.

Weight loss

As seen, in Table 2, weight loss of guava fruit and apple slices reached 11.5% and 7.8%, respectively for uncoated (control samples), while pectin coated samples showed significantly lower weight loss during the storage period. Increasing the spirulina extract content in the pectin coatings to 1% drastically reduced the weight losses to a very low level, being 4.0% for guava fruits and only 2.5% for apple slices after 24 days of cold storage. The incorporation of 1% spirulina extract in pectin coating reduced the weight losses of guava fruits and apple slices by 65% and 68%, respectively after 24 days of storage, which would be economically feasible to apply spirulina coating in fruit packaging. The rate of weight loss g/day for both tested fruits was also included in Table 2. The reduction in weight losses could be referred to the less water vapor permeability and good barriers characteristics of spirulina coated surface. The results agree with the findings of Rastegar and Atrash (2021), who found that incorporation of spirulina extract in alginate coating reduced the weight losses of mango fruits by 44% after 4 weeks of storage, despite the existing vapor pressure differences inside and outside the fruits.

Incidence of decay

The ratio of infected and decayed guava fruits reached 22.7% for the uncoated (control) fruits Table 2. Coating of the fruits with pectin emulsion containing up to 1% dry spirulina extract reduced the ratio of decayed fruits by almost 50% to reach 12.8% of the tested fruits. On contrary, uncoated (control) apple slices showed a decay of only 4.9% at the end of storage period at 4℃ for 24 days, while coating the slices with pectin emulsions containing up to 1% spirulina extract resulted in only 1.9% decayed samples after 24 days storage. Table 2 include the rate of decay for both fruits (%/day). These results confirm the findings of BenHalima et al. (2019), who proved the strong inhibitory effect of spirulina containing coatings on a wide spectrum of fungi, which are the main causes for fruit decay during storage. On other side, the acidic nature of apple slices could assist the protection against mold infection.

Texture properties of coated fruits

Results of firmness measurements for guava fruits and apple slices samples during storage are included in Table 2. As seen, guava fruit samples showed firmness values between 790 and 799.2-gram force (g_f) at 0 time of storage. Control uncoated samples showed a continuous deterioration in firmness values during storage period and recorded a firmness value of 452.5 g_f after 24 days of storage making about 43% loss in firmness during this period. Coating of guava fruits with pectin emulsions containing up to 1% spirulina extract maintained the texture of the fruits so that the recorded firmness reached a level of (676 g_f) after 24 days for samples coated with 1% spirulina extract respectively making a loss of only 15.5% in firmness. The partial maintenance of coated guava fruits firmness could be referred to the ability of spirulina extract to reduce the respiration rate and the activity of pectolytic enzymes, which results in keeping the firmness of the fruits during storage.The rate of loss in firmness of Guava fruits was changed from 13.94 g_f /day for control sample to only 5.05 g_f/day for 1% spirulina coated samples. On other side, apple slices showed firmness values in the range of 910 to 916.4 g_f, which are slightly higher than their corresponding values of guava fruits may be due to the higher pectin content in the walls of apple cells compared with that of guava fruit. During storage, the control apple slices loosed 25% of its firmness (reaching 689 g_f), while the coated slices loosed only 5% to 3% of their initial firmness value, which emphasizes the role of spirulina extracts in suppressing the activity of pectolytic enzymes. Values of rates in firmness loss for apple slices are also included in Table 2. The obtained results agree with those reported by Ebrahimi&Rastiger (2020) for mango fruits as well as Chavan et al. (2023) for different fruits (Apple, Persimmon, avocado, mango and papaya).

TABLE 2. Change in weight loss (%), decay fruit (%) and structure firmness for spirulina pectin coated fruits .

Experiment	Samples	Spirulina extract (%)	Storage periods (days)					Rate (g/day)
			0	6	12	18	24	
Weight loss (%)	Guava	Control	0.0	5.5	6.0	8.3	11.5	0.43
		0.4	0.0	3.0	4.5	6.6	7.0	0.293
		0.7	0.0	2.1	4.2	5.0	6.5	0.265
		1.0	0.0	1.5	2.0	2.9	4.0	0.1567
	Apple slices	Control	0.0	3.8	4.9	6.6	7.8	0.3067
		0.4	0.0	2.5	2.7	3.4	4.0	0.1483
		0.7	0.0	1.9	2.0	3.6	3.9	0.1583
		1.0	0.0	1.2	1.5	3.9	2.5	0.0967
Infected Fruit (%)	Guava	Control	0.0	8.0	13.1	18.4	22.7	0.93
		0.4	0.0	7.3	11.3	15.0	19.2	0.7683
		0.7	0.0	6.7	10.5	12.9	17.5	0.6867
		1.0	0.0	5.4	7.2	9.0	12.8	0.4867
	Apple slices	Control	0.0	2.4	3.6	4.3	4.9	0.195
		0.4	0.0	--	2.0	3.1	3.6	0.1548
		0.7	0.0	--	--	2.0	2.8	0.1154
		1.0	0.0	--	--	--	1.9	0.079
Firmness (g_f)	Guava	Control	790.0	732.0	662.0	570.4	452.5	-13.943
		0.4	792.8	750.5	700.5	622.2	510.0	-11.565
		0.7	797.0	763.2	724.3	676.3	653.9	-6.2183
		1.0	799.2	780.7	763.1	724.0	676.0	-5.0517
	Apple slices	Control	910.0	874.1	812.6	767.0	689.0	-9.1517
		0.4	910.0	892.3	883.8	872.4	860.3	-1.9883
		0.7	914.2	900.5	890.9	883.2	878.9	-1.465
		1.0	916.4	905.3	897.5	894.0	890.4	-1.055

The aforementioned results emphasized the economic importance of use spirulina extract in fruit coating. Extending shelf life and keeping quality of fresh fruits for a long period is essential in marketing fruits and vegetables.

Sensory evaluation of coated guava fruits and apple slices

Change in sensory analysis scores of whole guava fruits and apple slices samples are presented in Fig 4 (a and b). Samples were evaluated in terms of color, flavor, taste, texture and general acceptance. According to sensory and statistical results, there were significant changes observed in all scores of uncoated samples and, especially in taste and texture ($P<0.05$). On the other hand, there were no significant changes reported in all scores of both 0.7 and 1.0% SE treatment for guava and/or apple slices samples. As seen in Fig (4) 0.7 and 1.0% samples received higher color, flavor, taste and texture scores when compared with control sample, and they were the most preferred treatments in terms of general acceptance scores, which at the end of storage period received four points out of five. This evidenced that spirulina-pectin based edible coating can form stable layers on the surface of fruits and it has been proved, in emulsion-based films, that the potency of SE at the layer of fruits inhibits the growth of microorganisms which corrupt the natural physical properties of coated fruits during the storage.Samples of 0.4% SE received middle acceptance scores; they received three points out of five ($P<0.05$). The obtained results agree with those of Molla et al. (2020).

Conclusion

It can be concluded from the results obtained during the present investigation that ethanolic SE had higher antioxidant and antimicrobial activities compared to methanolic, acetone, and chloroform extracts. Also, it may be successfully incorporate 0.4-1.0% of concentrated spirulina extract to an edible pectin film to extend the shelf life and maintain the quality of its packaged food sufficiently.Results affirmed that 0.7 – 1.0% of concentrated SE seemed to be more promising for controlling the visual quality of guava and apple slices samples for 24 days storage at 4°C and maintained the microbiological quality and its firmness value.Based on these results, ethanolic SE can potentially be used as an alternative to popular plant and herbs antioxidants to secure the food safety.This investigation shed the light on the potential use of Spirulina extract in fruit coating, which gains wide acceptability by consumer. Further researches applying other economical fruits and combined coatings with spirulina need to implement spirulina as an effective bio-preservative.

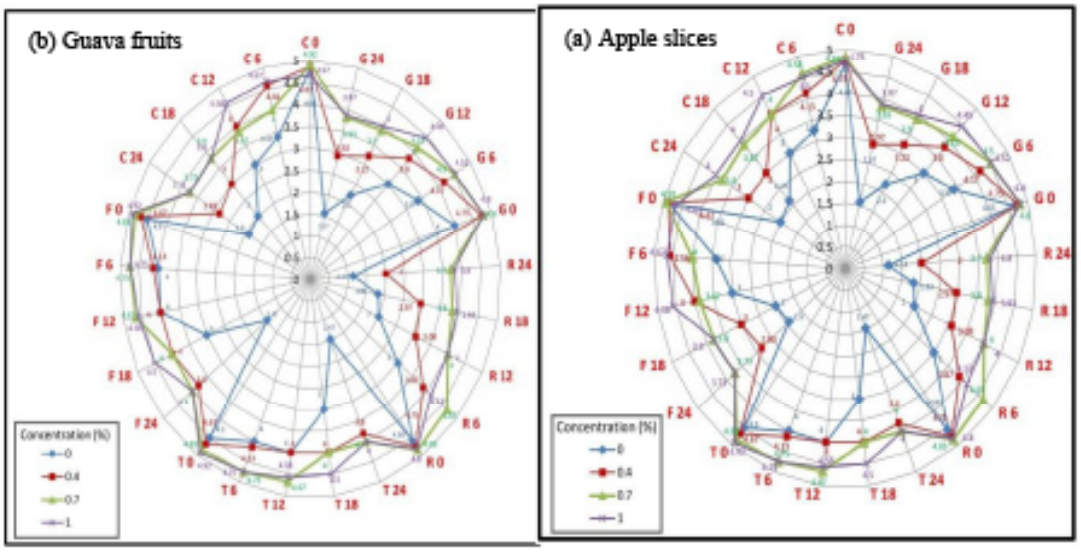

Fig. 4. Sensory evaluation of Guava fruits and Apple slices in relation to Spirulina concentration in coating (%) and storage time.

C= Color ، F= Flavor ، T= Taste ،R = Texture ، G = General acceptance – 0- 6- 12- 18- 24 = Storage days -0 to 5 = Scale of sensory scores.

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