



Enhancing Quality Properties of Fresh Orange Juice through the Addition of Freeze-Dried *Cassia javanica* Extracts' Coacervates

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Abstract: This study investigated the impact of freeze-dried coacervates at various concentrations (0.5, 1, and 1.5%) on physicochemical and microbiological properties of orange juice. Either 60% ethanol freeze-dried coacervates (EFC) or absolute ethanol freeze-dried coacervates (AFC) were used. The addition of EFC or AFC to orange juice did not significantly ($p < 0.05$) affect pH, viscosity, or electric conductivity, as pH was unchanged for a 0.5% concentration and slightly increased from 3.99 to 4.01 at 1% and 1.5% concentrations. After adding AFC and EFC, viscosity were 52, 53, and 53 mPas at 0.5, 1, and 1.5% concentrations, respectively. Electric conductivity increased from 0.278 s.m^{-1} (control) to 0.334, 0.347, and 0.375 s.m^{-1} at 0.5, 1, and 1.5% concentrations after adding EFC, while were 0.325, 0.335, and 0.373 s.m^{-1} at the same concentration after adding AFC, respectively. However, after adding EFC, total phenolic content increased to 77.25, 115.96, and 154.95 mg.100mL^{-1} , total flavonoid content (TFC) increased to 34.76, 52.18, and 69.73 mg.100mL^{-1} , and antioxidant activity (AA) enhanced to 70.36, 74.36, and 79.58% at concentrations 0.5, 1, and 1.5%, respectively. Also, after adding AFC, total phenolic content increased to 79.26, 117.78, and 156.25 mg.100mL^{-1} , TFC increased to 35.67, 53.00, and 70.31 mg.100mL^{-1} , and AA enhanced to 71.65, 75.84, and 81.21% at concentrations 0.5, 1, and 1.5%, respectively. At concentrations 0.5, 1, and 1.5%, EFC decreased total plate count (TPC) to 2.12, 2.02, and 1.78 $\log \text{cfu.mL}^{-1}$, respectively and mold and yeast counts decreased to 1.5, 1.35, and 1.1 $\log \text{cfu.mL}^{-1}$, respectively. Also, adding AFC caused TPC to decrease to 2.18, 2.04, and 1.84 $\log \text{cfu.mL}^{-1}$, respectively and mold and yeast count decreased to 1.53, 1.33, and 1.12 $\log \text{cfu.mL}^{-1}$, respectively. Overall, the results indicate that adding EFC or AFC to fresh orange juice can enhance its nutritional and microbiological qualities without degrading its sensory qualities.

Keywords: *Cassia javanica*, Coacervates, Orange juice.

Introduction

Essential oils, often abbreviated as EOs, capture the true essence of a plant's scent, despite the term "oil" being used to describe the plant's oil-soluble components rather than its texture. They also go by other names like aetheroleum, ethereal oils, and volatile oils (Al-Khayri *et al.*, 2023). These oils are the result of secondary compounds produced by oil-producing plants. EOs are composed of a diverse mixture of hydrophobic components, containing as many as several hundred different volatile chemicals, each present in varying amounts. Typically, you'll find around 100 to 200 different chemicals in each essential oil (Napiórkowska & Kurek, 2022). The unique properties of essential oils are largely influenced by two or three major components, which make up a significant portion (20-70%) of the oil's composition. These primary chemical constituents encompass a variety of compounds like terpenes, aldehydes, ketones, phenols, alcohols, and more (Delshadi *et al.*, 2020). It's this intricate chemical makeup that gives essential oils their therapeutic qualities. This complexity also clarifies why different essential oils can have similar effects (Falleh *et al.*, 2019; 2020). Two examples of significant ingredients are carvacrol and thymol, which make about 30% and 27%, respectively, of the oregano essential oil (*Origanum compactum*). Linalool makes up 65% of the chemical makeup of coriander (*Coriandrum sativum*) essential oils. Menthol (59%) and menthone (19%) are contained in peppermint essential oils (*Mentha piperita*) (Veiga *et al.*, 2019). Essential oils have anti-inflammatory, antispasmodic, sedative, analgesic, and digestive support properties among their pharmacological properties. Because essential oils have such a varied and rich chemical makeup, they can have a multitude of positive effects (Vianna *et al.*, 2021). Take rosemary

essential oil, for example; it has the remarkable ability to stimulate your appetite, assist with digestion, and even help alleviate gas and bloating (Rašković *et al.*, 2014). Furthermore, essential oils have demonstrated their ability to effectively combat bacteria, yeast, and mold. Thanks to the intricate blend of compounds within a single essential oil, it can effectively put a stop to the growth of both bacteria and fungi. For instance, the same rosemary essential oil can inhibit the growth of yeasts like *Candida* spp., molds such as *Penicillium* spp., as well as both Gram-positive (*Enterococcus* spp.) and Gram-negative (*Salmonella* spp.) bacteria (Stojanović-Radić *et al.*, 2018; Valková *et al.*, 2021). Using pure essential oils in cooking has its limitations. To begin with, their incredibly strong aroma and flavor can potentially alter the taste and overall sensory experience of a dish in a not-so-great way. Furthermore, environmental factors such as temperature, O₂, and light can greatly affect essential oils. Eos are less effective in culinary applications due to their preference for fats over water which affects their absorption and utilization by the body (Bakry *et al.*, 2016). Microencapsulation as a recent method of keeping the purity of sensitive compounds, can help solve these problems. Microencapsulation can be described as covering or encasing a specific substance or mixture of substances with an outer shell of a specific material or system. The encapsulated material can also be referred to as payload, active, core, or encapsulate comprises about 30-99% of the capsule's total weight. The encapsulation can consist of a single component or a mixture of components in the three stages of matter. The surrounding polymer is referred to as the "shell", "wall", "matrix", or "coating" (Arenas-Jal *et al.*, 2020). Typically, neither the wall material nor the core substance responds when the wall material is exposed to water. The wall can be made from gums, proteins, lipids, and

synthetic polymers. The wall material is frequently applied as a liquid (solution, suspension, or molten substance) to enable enrobing of the core material. The shell must create excellent films and act as a barrier against oxygen, water, pressure, heat, and/or light in order to protect the substance it is encasing (Shishir *et al.*, 2018; Hassan *et al.*, 2022). When essential oils are shielded from potential hazards like light, oxygen, moisture, heat, and mechanical stress, it actually enhances their ability to dissolve and be absorbed in the body. This protection also means they can be released in a controlled manner and prevents unwanted interactions with other ingredients when incorporated into food recipes. Furthermore, this protection allows for alterations in the physical characteristics of the core ingredients and reduces the evaporation of volatile components. This ultimately extends their effectiveness, reduces the reactivity of the core ingredients, and minimizes the loss of liquid through evaporation (Mohammadalinejad & Kurek, 2021). Coacervation stands out as one of the earliest and widely used encapsulation methods. It's a relatively straightforward technique, somewhat akin to a tweaked emulsification process. The term "coacervation" has its roots in the Latin word "acervus" which signifies the gathering or coming together of colloidal particles. Amalgamation of these particles resulted in the addition of the prefix "co" (Napiórkowska & Kurek, 2022). During this process, the hydrocolloids unite together into the distinct liquid phase called coacervate after separated from the main solution. Continuous phase can be defined as the site where coacervates form while equilibrium phase can be defined as the part which maintains equilibrium (Alemzadeh, 2013). Coacervation process comprises of four steps that can be described as: suspension of core material's particles in the liquid phase;

three-phase system production by forming the second liquid phase (coacervate); inserting liquid polymer around the core; gelling; microcapsule wall hardening (Napiórkowska & Kurek, 2022). The two types of coacervation process are simple and complex coacervation. Simple coacervation is defined as the process of heating or cooling, desolvating by water-miscible nonsolvent or salting out by electrolytes of one polymer (Mahanta *et al.*, 2021). The phase separation caused by interaction between two or more biopolymers with opposing charges is known as complex coacervation. Moreover, the process can be affected by various factors such as ionic strength, total biopolymer concentration, protein polysaccharide ratio, core material type, core to wall ratio, pH (coacervates form within a certain pH range below isoelectric point) (Yang *et al.*, 2015; Rojas-Moreno *et al.*, 2018). Despite the fact that the produced microcapsules water- and heat-resistant, higher overall efficiency and possibility of controlled release are offered by complex coacervation as it surrounds the center of the active compound with wall material and protects it (Shaddel *et al.*, 2018).

There has been a noticeable rise in consumer interest for orange juice of superior quality, characterized by its authentic flavor, pleasing textures, limited use of additives, and enhanced health-promoting attributes; the *Cassia javanica* flowers are subjected to a processing method that transforms them into a desiccated, pulverized state, which is afterwards utilized for its advantageous properties (Younis *et al.*, 2022). To the best of our current understanding, there is no existing reports on the efficacy of *Cassia javanica* flower extract in enhancing the quality attributes of orange juice. Acquiring this knowledge would be highly advantageous for consumers. Hence, the present study aimed to

examine the alterations in physicochemical characteristics, free radical activity, total phenolic compounds, sensory attributes, and microbial activity of orange juice enriched with coacervates of *Cassia javanica* flower extracts.

Materials & Methods

Chemical reagents and materials

All chemicals and reagents used in this study were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA).

Preparation and extraction

Fresh *Cassia javanica* flowers were collected in flowering season from local farm at Faculty of Agriculture, Cairo University and transferred to laboratory immediately for hot air drying. Flowers' petals were dried at 40°C in a hot air dryer (Shel-lab, Suite G Naperville, IL, USA) for 48h to reach a consistent weight before being ground in an analytical mill to a size of 1 mm (Cole-Parmer, Vernon Hills, IL, USA), sieved by a 50-mesh screen, and stored at -20 °C in a freezer.

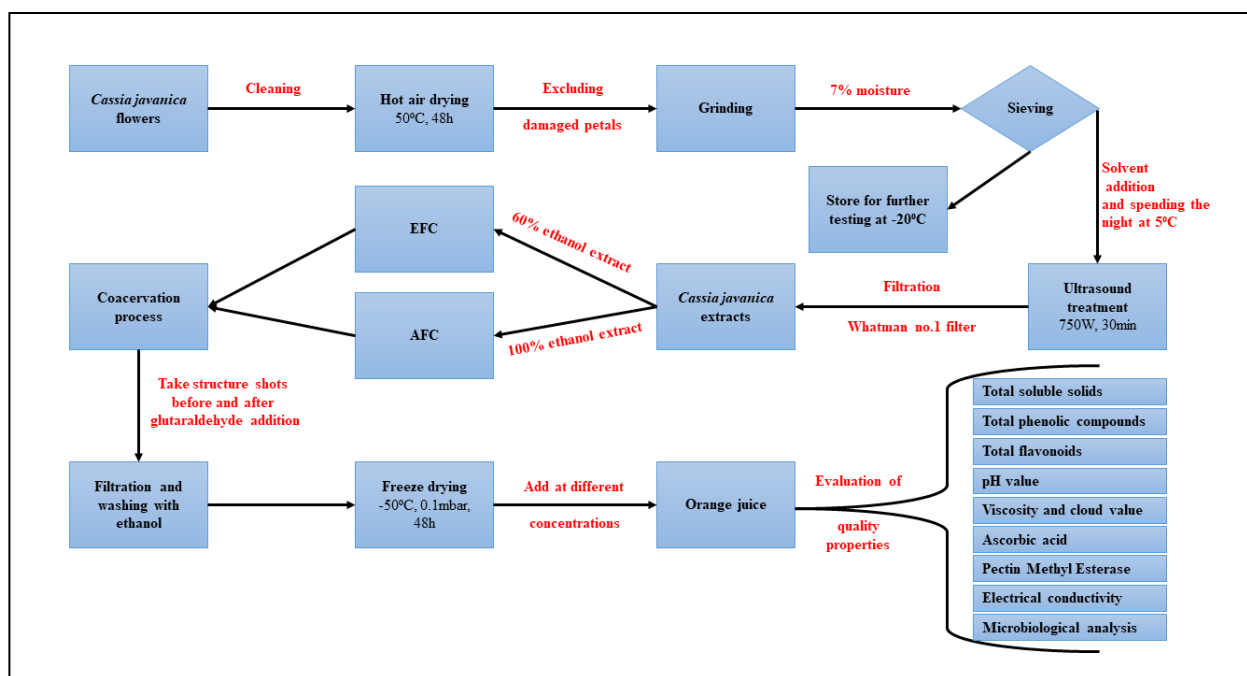


Fig. (1): Preparation, freeze-drying, and addition of coacervates to orange juice.

The method is described as, 25 g of *Cassia javanica* petals powder was soaked in 250 mL of ethanol (60% concentration and absolute) to prepare the extraction. The mixture spent the night in a fridge at 5°C. Before being filtered through Whatman No. 1 filter, the mixture was subjected to ultrasound treatment with power of 750W for 30min (Sharmila *et al.*, 2016).

Coacervation and freeze-drying

The 10% w/w gelatin solution, pH 9, and 50°C were used to emulsify the *Cassia javanica* extract. For 30min, the emulsion was agitated

at 200 rpm. After 30min of stirring, the 10% w/w acacia solution at 50°C was progressively added. The colloidal concentration was diluted to less than 3% by the addition of warm water (50°C) (100g of water per 30g of colloidal solution). The mixture's pH was changed to 4 by adding a drop at a time of a 10% v/v acetic acid solution. The mixture was kept agitated while being cooled to 5°C. Next, glutaraldehyde was added, and mixed for 2h. Sodium hydroxide 20% w/v was added to the solution to bring the pH to 9. The microcapsules were agitated for another 30min, after which they were vacuum-filtered

(0.05 MPa) and washed with ethanol (same concentration used for extraction). The microcapsules were then freeze-dried (Model 77530-30, Labconco Co., USA) for 48h at -50°C, and 0.1mbar to make 60% ethanol freeze-dried coacervates (EFC) and absolute ethanol freeze-dried coacervates (AFC) (Timilsena *et al.*, 2019).

Total soluble solids (TSS)

Total Soluble Solids (TSS) was measured to assess the concentration of dissolved substances in the orange juice following the addition of EFC and AFC. TSS encompasses various soluble components like sugars, salts, organic acids, and other dissolved compounds within a given solution or material. To determine TSS, two drops of the prepared juice samples were placed into a portable refractometer (Model No. p 300003, UK) and measured the TSS in Brix % at room temperature.

The pH value

The acidity or alkalinity of the orange juice samples was evaluated by measuring their pH. The pH value is an important parameter that serves a vital role in various chemical and biological processes. A digital pH-meter (Metrohm 780 Benchtop, Switzerland) was used to measure the pH of the juice samples at room temperature.

Total phenolic content (TPC)

Total phenolic content includes antioxidant and bioactive secondary metabolites such as phenolic acids, flavonoids, and polyphenolic compounds. Based on Singleton & Rossi (1965) and updated by Elsayed *et al.* (2022), Folin-Ciocalteu reagent to measure total phenolic content was used. In 15mL centrifuge tubes (Sarstedt), 5mL of juice was mixed with 5mL of 80% methanol. After that, we

centrifuged the tubes at 2509g for 20 minutes at 4°C using a Sigma 4-16KS (Germany).

The analysis involved mixing 100µL of diluted samples or standard solutions (10-100µg.mL⁻¹) with 100µL of Folin-Ciocalteu reagent and 3000µL of deionized water, then vortexing. After a 10-minute incubation at room temperature, we added 100µL of a 20% sodium carbonate solution, mixed it immediately, and incubated the mixture at room temperature for two hours in the dark. The absorbance of the mixture was then measured at 765nm using a Microplate reader (Biotek Synergy 2 Microplate reader, U.S.A). Gallic acid served as the standard, and we expressed the total phenolic content of the samples as milligrams of gallic acid per 100mL.

Total flavonoids content (TF)

The measurement of total flavonoids was conducted in order to evaluate the concentration of flavonoids present in orange juice samples. Flavonoids are a subgroup of polyphenolic substances that are well acknowledged for their notable antioxidant, anti-inflammatory, and possibly medicinal characteristics.

Total flavonoids content was measured by a colourimetric assay (Mohamed *et al.*, 2021), with some modifications. In a 10mL test tube, 250µL of extract or standard solution of catechin at different concentration (20-260µg.mL⁻¹) and 1mL of distilled water were mixed. The following were successively added: at zero time, 75µL of 5% NaNO₂; at 5min, 75µL of 10 % AlCl₃; and at 6min and 500µl of 1M NaOH. The solution was then immediately diluted by adding 2.5mL of distilled water and mixed thoroughly. The absorbance of the mixture (pink colour) was directly measured by a microplate reader at 510nm against a

blank sample and the results were expressed as catechin equivalents (mg CE.g⁻¹).

Colourimetric analysis

The colour properties of orange juice samples were evaluated using the colourimetric analysis method developed by the United States Department of Agriculture (USDA). This method, endorsed and widely utilized, provides an objective means of quantifying colour attributes in various food and agricultural products.

For colour measurement, we used spectrophotometer (Scilogex SP-UV1000, China). Following the USDA colour scale, we placed 10 mL of the sample into the device, and the colour value for the yellow spectrum ranged from 1 to 6, where 1 represented light yellow and 6 indicated dark yellow (USDA, 1983).

Viscosity

The assessment of orange juice samples' viscosity was conducted in order to evaluate their resistance to flow and examine their fluidic characteristics. Viscosity is a fundamental rheological characteristic that has significant importance in a wide range of scientific and industrial contexts. The viscosity of juice samples (100 mL) was measured at room temperature using a model IKA viscometer (IKA - Viscometer, China), the spindle was RH3 Max with speed 200 rpm, time 30s, viscosity was expressed as centipoise (mPas) (Al-Jammaas *et al.*, 2019).

Cloud value

Cloud value is an essential measure for evaluating the clarity and particulate composition of liquids, including juices. Cloud value was determined by adding five mL of sample and subjected to centrifugation for 10 minutes at 1411g (Sigma 1A, AGB scientific ltd, Dublin, Ireland). The supernatant

absorbance at 650 nm (UV-VIS spectrophotometer, Unicam, UV2), and distilled water as a blank were conducted (Aghajanzadeh *et al.*, 2020).

Ascorbic acid

Ascorbic acid plays a decisive role in this study because it is a water-soluble antioxidant for orange juice (Niu *et al.*, 2008).

The titration method using 2, 6-dichlorophenol-indophenol (DCPIP) was selected to determine ascorbic acid as per the protocol described by Mohsen *et al.* (2013). Five mL of 1% oxalic acid or 3% metaphosphoric acid (HPO₃) was added to 5mL of sample to stop any degradation of ascorbic acid then titration took place with standardized dye solution (250 mg.L⁻¹ DCPIP was dissolved in distilled water). The dye was delivered to the sample using an auto-titrator (Dos Bio-5, 665 Dosimat, Metrohm, Swiss) until reaching pink color as end point of titration (colour should continue for at least 15 seconds). The results obtained were expressed as mg of ascorbic acid per 100mL sample.

Pectin methyl esterase (PME)

The enzymatic activity of pectin methyl esterase (PME) was evaluated in order to ascertain its presence and level of activity in orange juice samples. Pectin methyl esterase (PME) is an enzyme that plays a significant role in the alteration of pectin, a vital constituent of the cell wall in plants. Its primary function involves catalyzing the demethylation process of methyl ester groups. Quantitative measurement of PME was based on the methods reported by Amaro & Tadini, (2021). Juice samples were mixed by inverting the bottle several times and 2mL was transferred into 20mL of a 1% citrus pectin substrate solution in 0.2M sodium chloride. The sample was titrated to pH 7.5 with 0.2N NaOH. An auto-titrator (Dos Bio-5, 665

Dosimat, metrohm, Swiss) was used to deliver 0.05N NaOH to the sample to maintain the pH at 7.5 for 10min during hydrolysis at 30°C. The volume of 0.05N NaOH consumed during this time was recorded. The PME activity expressed as PME units per mL was calculated by using (Eq. 1):

$$PME = \frac{(mL\ of\ NaOH) * (NaOH\ normality)}{[(mL\ of\ sample) * (10)] * 10^4} \quad (1)$$

Electrical conductivity (EC)

The purpose of measuring electrical conductivity was to evaluate the capacity of orange juice samples to conduct electrical current, which acts as an indicator of their ionic content, dissolved substances, and overall solution qualities. Many fields such as chemistry, environmental science, and materials research include this property.

The conductivity meter (WTW82362 Weinheim, LF323 Instrument, serial. n. cond 61485058, Germany) was used to measure electrical conductivity of the juice samples at room temperature, Cell constant = 0.475(1.cm⁻¹), %K=0. The equipment was calibrated before measurements (García-Girón *et al.*, 2019).

DPPH scavenging assay

DPPH (2, 2-diphenyl-1-picrylhydrazyl) method as radical scavenging for antioxidant activity and it is a free radical distinguished by its intense purple hue and it changes to yellow upon reduction by antioxidants.

The radical scavenging ability which is the core principle of antioxidant activity determination was conducted by mixing one gram of sample with 80% of methanol (v/v) at a ratio 1:4, ethanolic extract was prepared (Česonienė *et al.*, 2022). The mixture was then placed in covered conical flask and stirred up at 200 rpm, at 25°C using an orbital shaker (Heidolph Unimax 1010, Schwabach, Germany) for 2h. The mixture was then

filtered through a filter paper (Whatman No. 4) to obtain a clear solution (Ikram *et al.*, 2009). The solution of DPPH in methanol 6 × 10⁻⁵ M (2.3661 mg DPPH (Molecular Weight: 394.35 g.mol⁻¹) in 100 mL methanol) was prepared fresh daily before UV measurements. Then 3.9 mL of a methanol solution of DPPH was added to 0.1 mL of methanolic extract and then allowed to stand in the dark for 30 min at room temperature. The change in colour (from deep violet to light yellow) was measured at 515 nm. The absorbance of the control sample was also determined. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Antioxidant activity was calculated as % of radical scavenging percentage using the following Eq. (2):

$$\% \text{ Radical scavenging} = \frac{A_0 - A_1}{A_0} * 100 \quad (2)$$

Where: A₀= the absorbance of the control reaction (containing all reagents except the test compounds [t= 0 min]). A₁= absorption of test extract solution (t=30 mins).

Microbiological analysis

Total plate count

One mL of each juice sample and mixed it with 9 mL of either 0.1% peptone water or sterile physiological saline solution (0.85%). This mixture was homogenized using a blender for two minutes. From the initial 10⁻¹ dilution, we prepared additional decimal dilutions.

To determine the total plate count and the presence of psychrotrophic bacteria, we used the pour plate method, employing plate count agar as the growth medium. The plates were then incubated at 35 °C for 48±2h to assess the total plate count (Nathaniel, 2020) , while for the psychrotrophic plate count, they were refrigerated at 5±2°C for a duration of 10 days (Baïoumy & Abedelmaksoud, 2021).

Mold and yeast

Mold and yeast were determined using potato dextrose agar medium, plates were incubated in the dark at 22-25°C for 5 days (Nathaniel, 2020).

Results & Discussion

Total soluble solids (TSS)

The total soluble solids (TSS) results for orange juice samples are presented in table (1). TSS levels were quantified prior to and after the addition of EFC and AFC at varying concentrations. The control sample exhibited an initial TSS measurement of 9.78 brix.

After adding EFC, the measurements of 0.5%, 1%, and 1.5% concentrations were 9.44, 10.1, and 10.56 brix, respectively. While after adding AFC with same concentrations, the

measurements were 10.09, 10.44, and 10.84 brix, respectively.

The results prove that the addition of EFC and AFC at different concentrations greatly affected TSS of orange juice. TSS showed direct relationship with concentrations of the coacervates. The increase in total soluble solids (TSS) could be because of the coacervates soluble in the juice (Agulheiro-Santos *et al.*, 2022).

Studies showed that TSS may influence sensory characteristics and shelf life (Robertson & Samaniego-Esguerra, 1990; Muhammad Zahir *et al.*, 2021) and some customers might desire a sweeter taste which can be achieved with higher TSS (Al-Hilphy *et al.*, 2023). However, it could also impact the juice's overall flavor harmony and shorten its freshness and shelf life (Song *et al.*, 2022).

Table (1): Total soluble solids after addition of 60% ethanol freeze-dried coacervates (EFC) and 100% ethanol freeze-dried coacervates (AFC) at different concentrations.

Treatments	Total soluble solids (Brix)		
	Control	EFC	AFC
Control	9.78	-	-
0.5%	-	9.91	10.09
1%	-	10.1	10.44
1.5%	-	10.56	10.84

The pH value

The pH of the control sample was 3.94, as can be seen in fig. (2). The pH of orange juice was significantly changed ($p < 0.05$) by the addition of EFC and AFC in various concentrations. Orange juice pH was not affected significantly by increasing concentration after the addition of EFC and AFC. This increase in pH values of

EFC and AFC in various concentrations may be due to an increase of TSS that led to increase in acidity (Islam *et al.*, 2013). Also, the acidic or basic composition of EFC and AFC may be responsible for the pH variations. The pH of orange juice rises as a result of the weak organic acid EFC partly dissociating in water and releasing H⁺ ions (Lee, 1993).

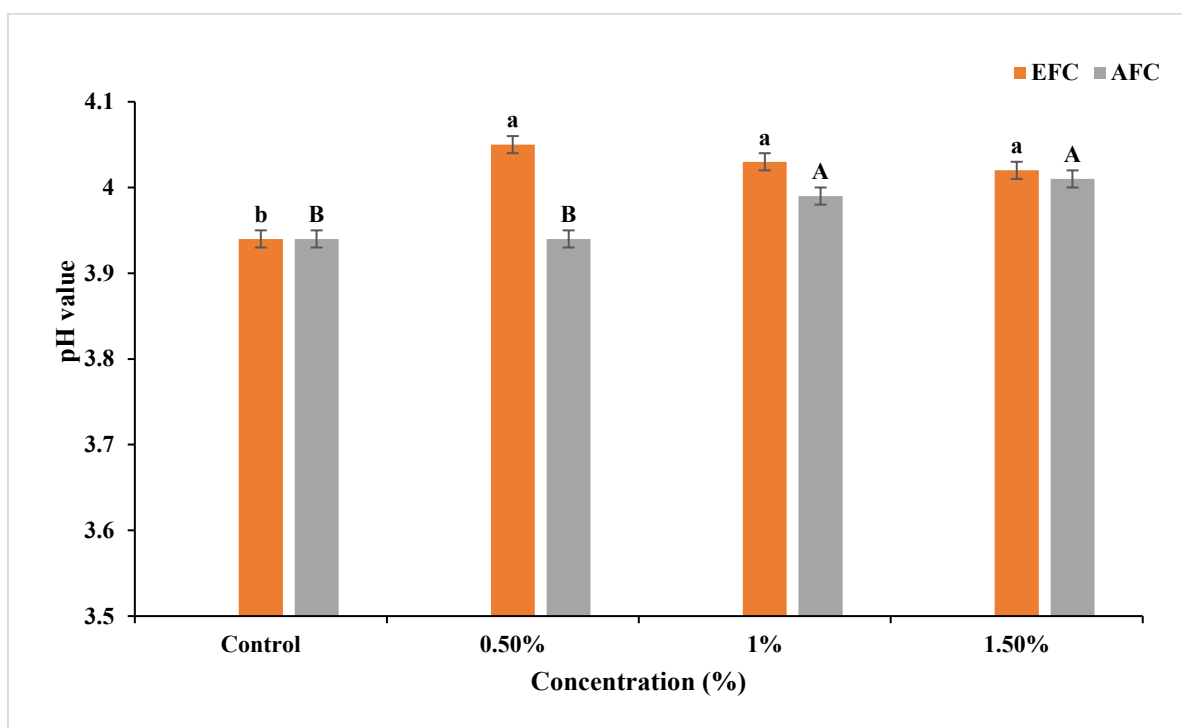


Fig. (2): pH value of orange juice after addition of 60% ethanol freeze-dried coacervates (EFC) and 100% ethanol freeze-dried coacervates (AFC) at different concentrations.

The different in capital letters refer to the significant differences among concentrations of AFC, and the different small letters refer to the significant differences among concentrations in EFC at significant level of $p < 0.05$.

Total phenolic content (TPC) and total flavonoids (TF)

Table (2) clearly demonstrates the significant influence of varying EFC and AFC concentrations on total phenolic content (TPC). Initially, the control sample had a TPC of $39.26 \text{ mg} \cdot 100 \text{ mL}^{-1}$. With the addition of EFC at concentrations of 0.5%, 1%, and 1.5%, the TPC substantially increased to $77.25 \text{ mg} \cdot 100 \text{ mL}^{-1}$, $115.96 \text{ mg} \cdot 100 \text{ mL}^{-1}$, and $154.95 \text{ mg} \cdot 100 \text{ mL}^{-1}$, respectively. Likewise, the introduction of AFC at concentrations of 0.5%, 1%, and 1.5% led to TPC values of $79.26 \text{ mg} \cdot 100 \text{ mL}^{-1}$, $117.78 \text{ mg} \cdot 100 \text{ mL}^{-1}$, and $156.25 \text{ mg} \cdot 100 \text{ mL}^{-1}$, respectively.

These results clearly demonstrate that both EFC and AFC have a positive impact on the TPC of orange juice, leading to a significant increase in phenolic content (Ma *et al.*, 2019).

Using EFC and AFC at different concentrations, TPC was significantly increased. Thus, the results of this study were very exciting for producing functional orange juice with high antioxidant activity. Also, total flavonoid content in the control sample was $22.79 \text{ mg} \cdot 100 \text{ mL}^{-1}$ (Table 2).

After introducing EFC at concentrations of 0.5%, 1%, and 1.5%, the total flavonoid content notably increased to $34.76 \text{ mg} \cdot 100 \text{ mL}^{-1}$, $52.18 \text{ mg} \cdot 100 \text{ mL}^{-1}$, and $69.73 \text{ mg} \cdot 100 \text{ mL}^{-1}$, respectively. Similarly, the addition of AFC resulted in total flavonoid content reaching $35.67 \text{ mg} \cdot 100 \text{ mL}^{-1}$ at 0.5%, $53.00 \text{ mg} \cdot 100 \text{ mL}^{-1}$ at 1%, and $70.31 \text{ mg} \cdot 100 \text{ mL}^{-1}$ at 1.5%. Total flavonoid content in orange juice was raised after adding both EFC and AFC, this supported orange juice products to have more antioxidant activity (Stinco *et al.*, 2020).

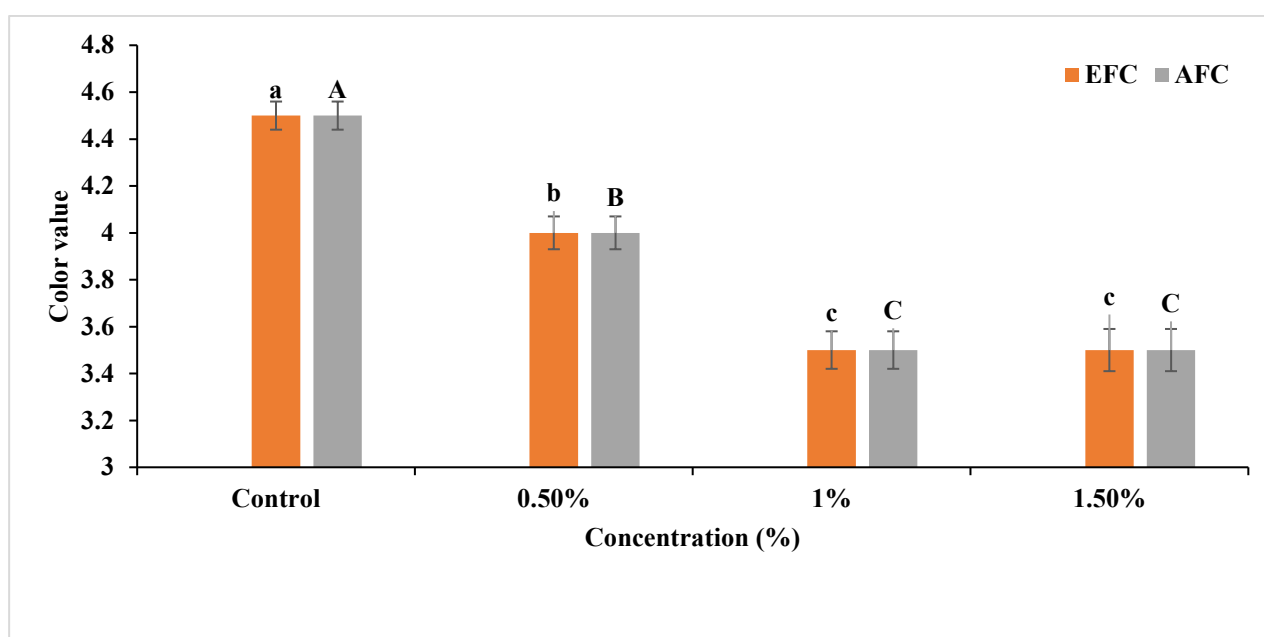
Table (2): Total phenolic and flavonoids contents in orange juice prepared using different concentrations of EFC and AFC.

	Total phenolic content (mg.100mL ⁻¹)			Total flavonoids(mg.100mL ⁻¹)		
	Control	EFC	AFC	Control	EFC	AFC
Control	39.26	-	-	22.79	-	-
0.5%	-	77.25	79.26	-	34.76	35.67
1%	-	115.96	117.78	-	52.18	53
1.5%	-	154.95	156.25	-	69.73	70.31

Colourimetric analysis

Fig. (3) displays orange juice colour values based on colour scale as in the United States Department of Agriculture (USDA), which were at range of 1 to 6 (USDA, 1983), and control value was 4.5. The colour values for sample 2 after the addition of EFC were discovered to be 4, 3.5, and 3.5 at concentrations of 0.5%, 1%, and 1.5%, respectively. The colour values for sample 3 were also found to be 4, 3.5, and 3.5 at concentrations of 0.5%, 1%, and 1.5%,

respectively, when AFC was added to the orange juice. Results illustration that by adding EFC and AFC to orange juice, the colour was greatly changed as the concentration of these coacervates had an inverse relationship with colour values. This could be because of reduced colour intensity caused by interaction between pigments in extracts and juice (Meléndez-Martínez *et al.*, 2011). Details about caliber and freshness of orange juice could be deduced from the colour data which makes it significant (Fernández-Vázquez *et al.*, 2014).

**Fig. (3): Colour values of orange juice at different concentrations of both EFC and AFC.**

The different in capital letters refer to the significant differences among concentrations of AFC, and the different small letters refer to the significant differences among concentrations in EFC at significant level of $p < 0.05$.

Viscosity

As it greatly affects orange juice texture and mouthfeel, viscosity was determined and the results were as shown in fig. (4). The result for the control sample was 52 mPas. After adding EFC at concentrations 0.5, 1, and 1.5%, the results were 52, 53, and 53 mPas, respectively. While after adding AFC, results for

concentrations 0.5, 1, and 1.5% were 52, 53, and 53 mPas, respectively. The addition of coacervates did not cause a change in the viscosity which was similar to previous studies (Katariya *et al.*, 2020). The variation in viscosity was negligible compared to the control sample. These results show that there were no significant differences ($p < 0.05$) after adding the coacervates (Gomes *et al.*, 2022).

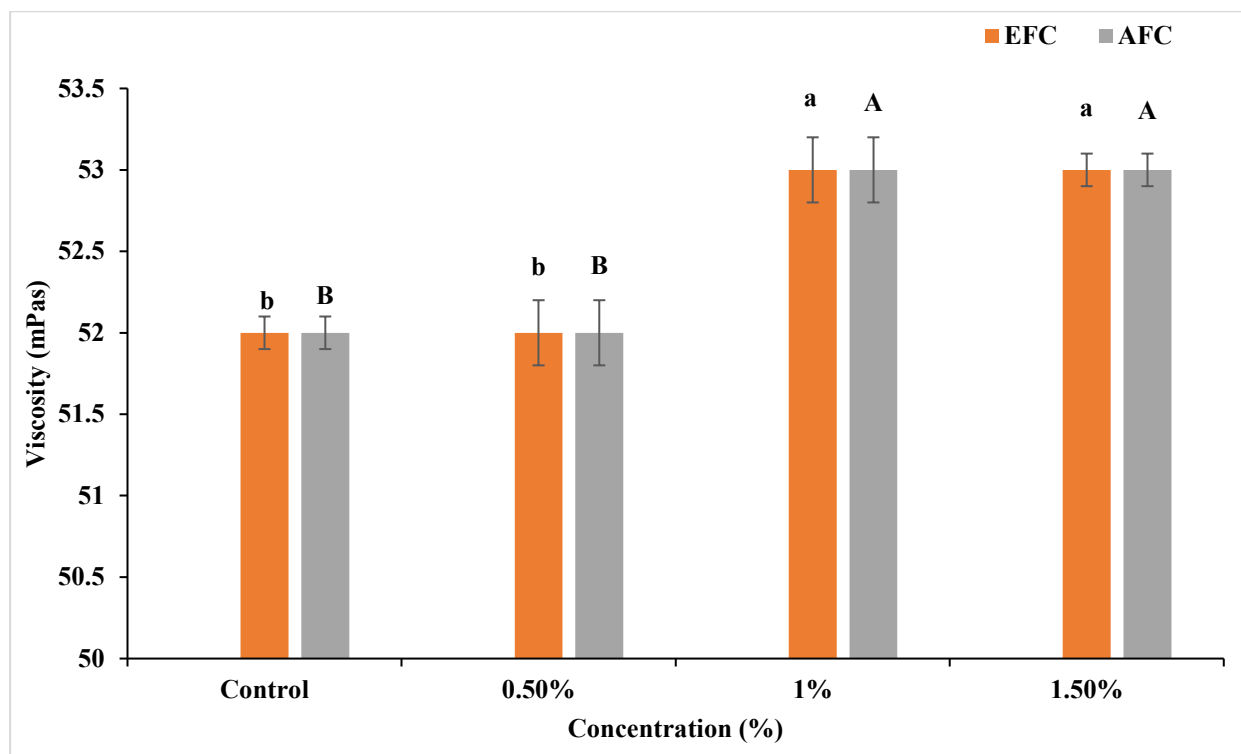


Fig. (4): Viscosity value at different concentrations of both EFC and AFC.

The different in capital letters refer to the significant differences among concentrations of AFC, and the different small letters refer to the significant differences among concentrations in EFC at significant level of $p < 0.05$.

Cloud value

The cloud value of the control sample was 0.247, but as shown in fig. (5), the addition of EFC and AFC increased it. This increase could be attributed to the presence of pectin. It can be concluded that higher concentrations of coacervates can result in increased turbidity (Yu *et al.*, 2021) and affect the sensory properties of the juice.

Ascorbic acid

The results as shown in fig. (6) demonstrated that the amount of ascorbic acid in the control

sample was 55.22 $\text{mg} \cdot 100\text{mL}^{-1}$. After adding EFC, the results for concentrations 0.5, 1, and 1.5% were 55.74, 55.25, and 55.16 $\text{mg} \cdot 100\text{mL}^{-1}$, respectively. After adding AFC, the results for concentrations 0.5, 1, and 1.5% were 55.85, 55.29, and 55.58 $\text{mg} \cdot 100\text{mL}^{-1}$, respectively. Following the addition of the coacervates, factors such as pH (fig. 7), temperature, and processing conditions can cause variations in ascorbic acid content (Gomes *et al.*, 2022). AFC as a way of increasing ascorbic acid content may be superior to EFC.

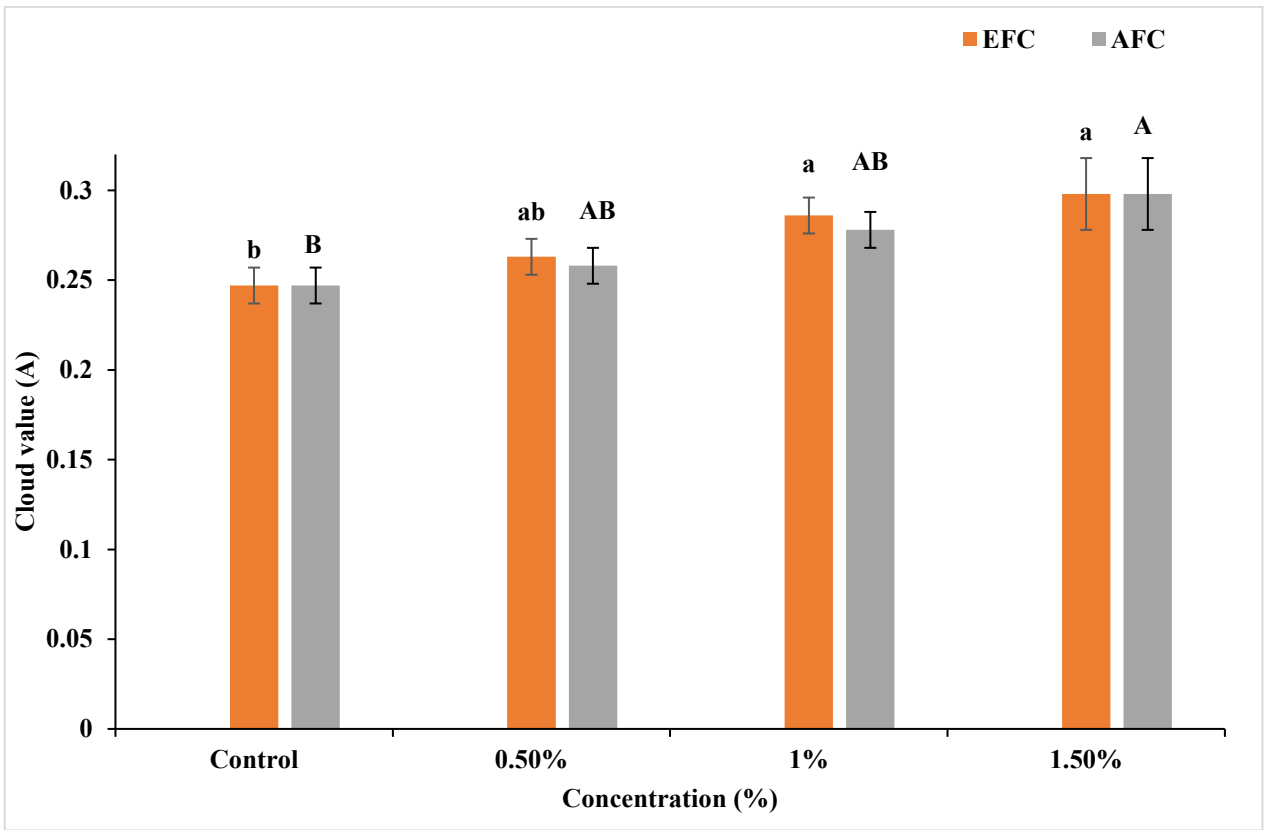


Fig. (5): Orange juice cloud value at different concentrations of both EFC and AF.

The different in capital letters refer to the significant differences among concentrations of AFC, and the different small letters refer to the significant differences among concentrations in EFC at significant level of $p < 0.05$.

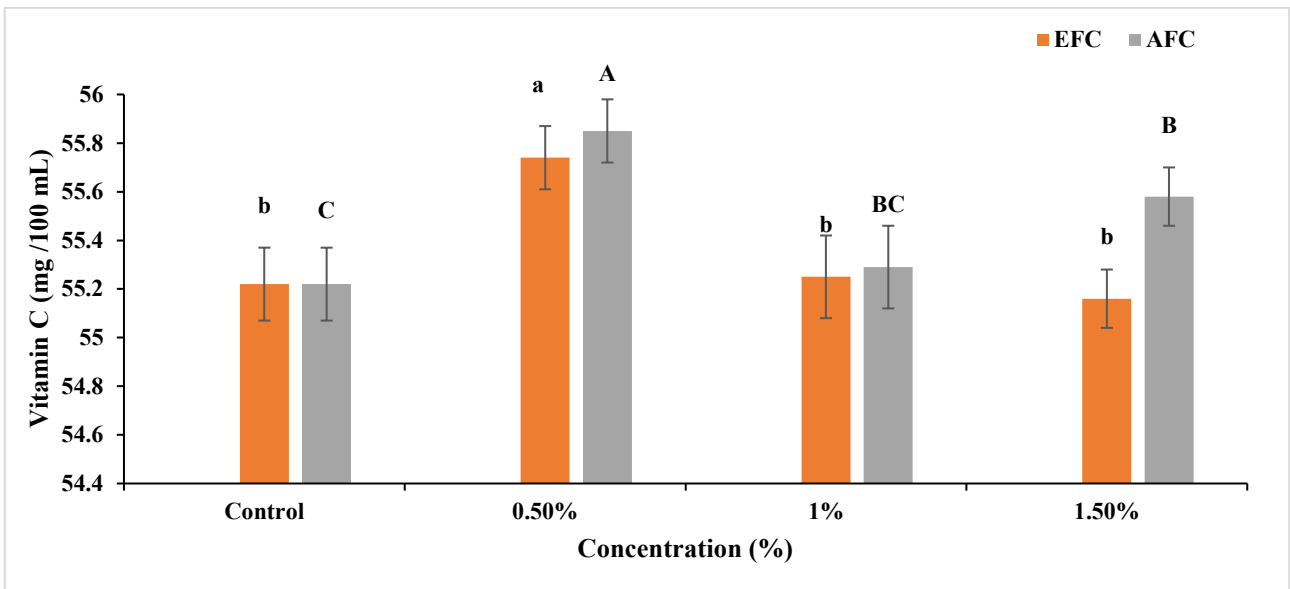


Fig. (6): Ascorbic acid of orange juice at different concentrations of both EFC and AFC.

The different in capital letters refer to the significant differences among concentrations of AFC, and The different small letters refer to the significant differences among concentrations in EFC at significant level of $p < 0.05$.

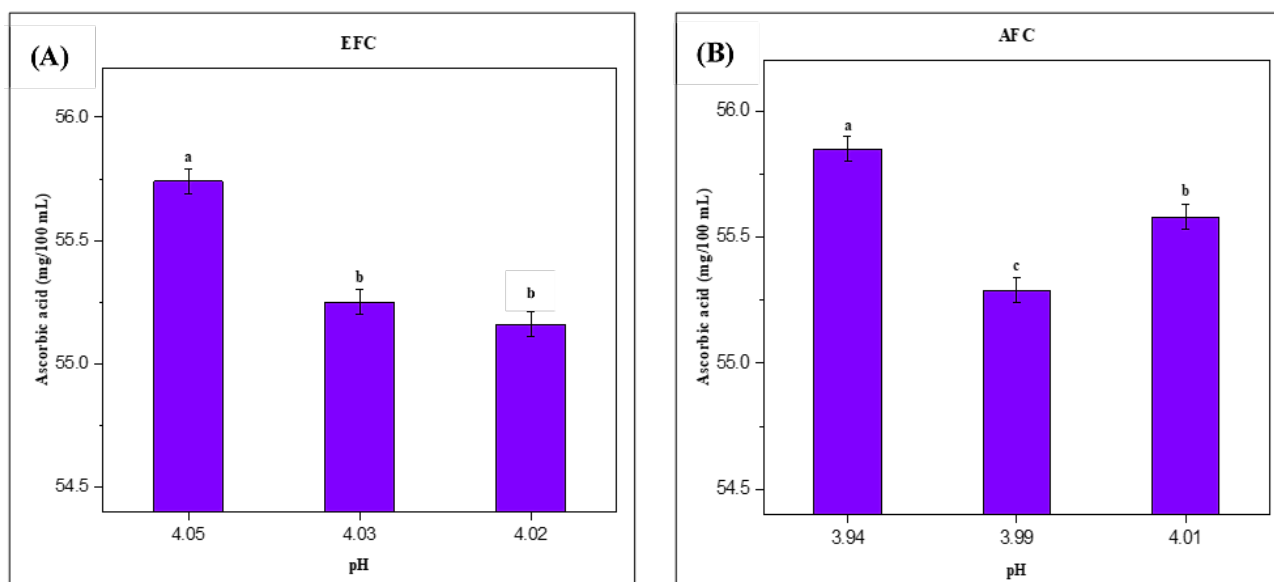


Fig. (7): Ascorbic acid and pH correlation after adding EFC (A) and AFC (B).

The different letters refer to significant differences among pHs at significant level of $p < 0.05$.

Pectin methyl esterase (PME)

One of the enzymes that break down pectin in fruits and vegetables is pectin methyl esterase which can affect viscosity, cloudiness, and texture of the juice (Abdelmaksoud *et al.*, 2022). Looking at fig. (8), the PME activity in the control sample measured at $46.89 \text{ U.mL}^{-1}.\text{min}^{-1}$. Upon adding EFC, the PME activity decreased to $43.92 \text{ U.mL}^{-1}.\text{min}^{-1}$ at 0.5%, $42.56 \text{ U.mL}^{-1}.\text{min}^{-1}$ at 1%, and $41.74 \text{ U.mL}^{-1}.\text{min}^{-1}$ at 1.5%. Conversely, when AFC was introduced, the PME activity increased to $44.24 \text{ U.mL}^{-1}.\text{min}^{-1}$ at 0.5%, then decreased to $42.12 \text{ U.mL}^{-1}.\text{min}^{-1}$ at 1%, and further dropped to $40.35 \text{ U.mL}^{-1}.\text{min}^{-1}$ at 1.5%.

These results suggest that EFC inhibits PME activity, given the decreasing trend with higher EFC concentrations. On the other hand, it was indicated by the increase occurred with increase in concentration that AFC stimulated PME activity. These results are similar to previous research on the effect of coacervates on PME activity in citrus fruits (Sahoo & Chakraborty, 2023). It was also noticed that PME activity decreased from $47.57 \text{ U.mL}^{-1}.$

min^{-1} to $5.38 \text{ U.mL}^{-1}.\text{min}^{-1}$ after pasteurization for 60s at 95°C based on Abdelmaksoud *et al.* (2019). Altogether, the results suggest that there were significant effects on PME activity after addition of coacervates to the orange juice. These effects can contribute to optimizing of orange juice processing and storage conditions.

Electrical conductivity (EC)

Electrical conductivity (EC) evaluates a solution's capacity to conduct electrical current, which is connected to its ion concentration, such as organic acids, sugars, and minerals in orange juice. Fig. (9) shows how EFC and AFC affect orange juice EC.

The control sample started with 0.278 s.m^{-1} EC. After adding 0.5%, 1%, and 1.5% EFC, the EC rose to 0.334, 0.347, and 0.375 s.m^{-1} , respectively. However, adding 0.5%, 1%, and 1.5% AFC yielded EC values of 0.325, 0.335, and 0.373 s.m^{-1} , respectively. These data show that EFC increased EC higher than AFC. High EFC and AFC concentration may raise EC because charged groups in EFC interact with orange juice ions, raising ion concentration

and EC. These EC results imply that greater EFC concentrations may raise orange juice EC, which might affect flavor and shelf life (Ahmed *et al.*, 2022). In this respect EC of

fresh orange juice was 0.272 s.m^{-1} and increased after pasteurization to 0.282 s.m^{-1} at 95°C for 60s (Abdelmaksoud *et al.*, 2019).

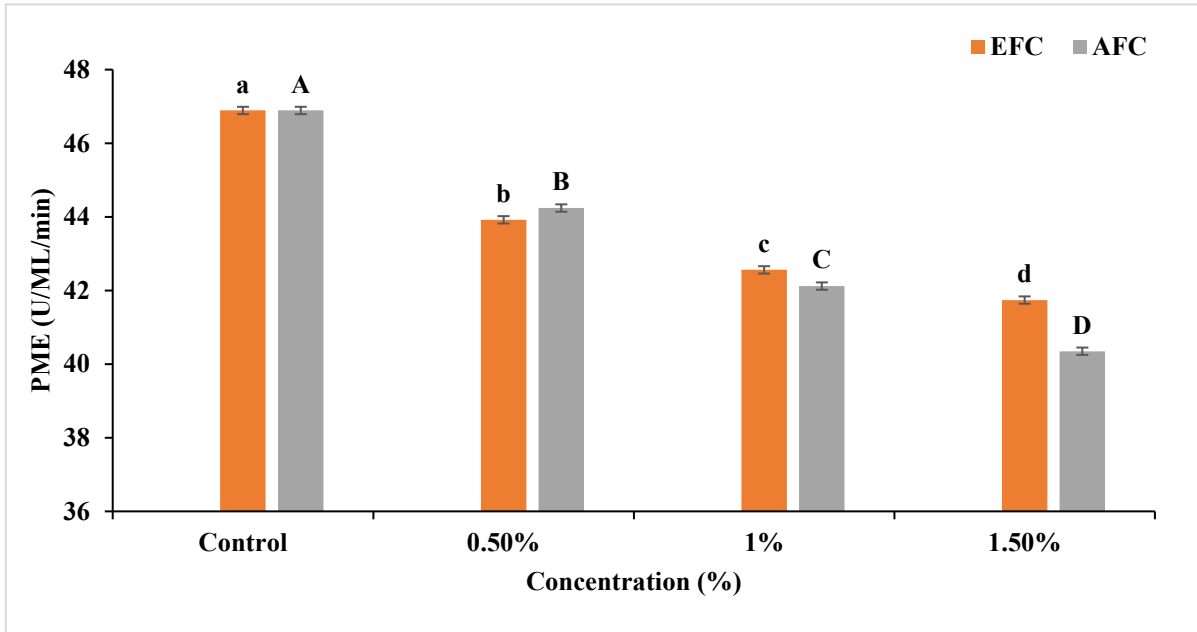


Fig. (8): Units of PME in orange juice at different concentrations of both EFC and AFC.

The different capital letters refer to significant differences among concentrations in AFC, and the different small letters refer to significant differences among concentrations in EFC at a significant level of $p < 0.05$

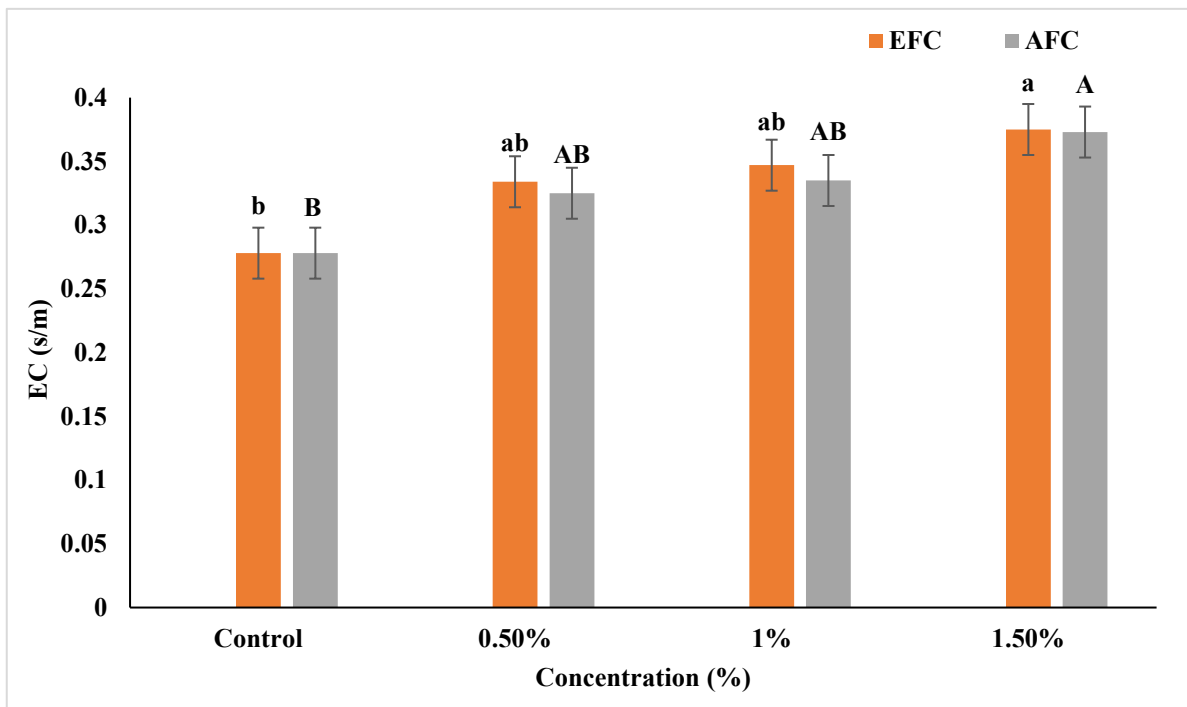


Fig. (9): Electrical conductivity of orange juice at different concentrations of both EFC and AFC.

The different capital letters refer to significant differences among concentrations in AFC, and the different small letters refer to significant differences among concentrations in EFC at a significant level of $p < 0.05$.

DPPH scavenging assay

The antioxidant capacity of orange juice was evaluated using the DPPH radical scavenging test, as illustrated in fig. (10). The control sample initially displayed an antioxidant activity of 60.68%. Upon adding EFC, the antioxidant activity increased as the concentration rose. Specifically, at 0.5%, the antioxidant activity reached 70.36%, at 1% it reached 74.36%, and at 1.5%, it reached 79.58%. Similarly, when AFC was introduced,

the antioxidant activity also increased with higher concentrations. At 0.5%, the antioxidant activity was 71.65%, at 1% it was 75.84%, and at 1.5%, it was 81.21%.

The higher bioactive content in EFC and AFC boosted free radical scavenging, making AFC at 1.5% the best orange juice antioxidant (Waley *et al.*, 2020; Wojtunik-Kulesza *et al.*, 2020). Therefore, the results of this study led to increased antioxidant activity and nutrition as a result of adding EFC and AFC to orange juice.

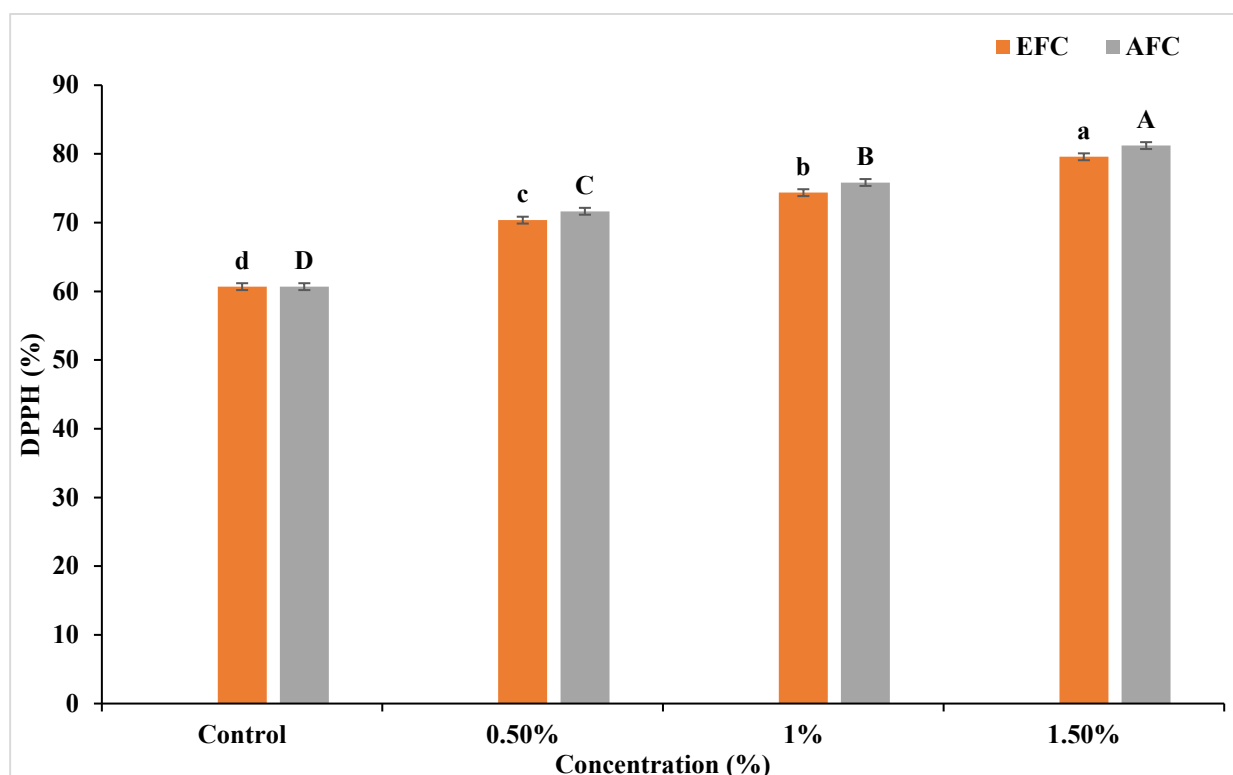


Fig. (10): Antioxidant activity of orange juice at different concentrations of both EFC and AFC.

The different capital letters refer to significant differences among concentrations in AFC, and the different small letters refer to significant differences among concentrations in EFC at a significant level of $p < 0.05$.

Microbial evaluation

Table (3) displays the total plate count (TPC) in orange juice samples after adding EFC and

AFC compared to the control sample ($2.32 \log \text{cfu.mL}^{-1}$). The counts also decreased after adding the coacervates.

Table (3) :Microbiological analysis after addition of 60% ethanol freeze-dried coacervates (EFC) and 100% ethanol freeze-dried coacervates (AFC) at different concentrations.

	Total plate count (log cfu.mL ⁻¹)			Mold and yeast (log cfu.mL ⁻¹)		
	Control	EFC	AFC	Control	EFC	AFC
Control	2.32	-	-	1.87	-	-
0.5%	-	2.12	2.18	-	1.5	1.53
1%	-	2.02	2.04	-	1.35	1.33
1.5%	-	1.78	1.84	-	1.1	1.12

TPC of 0.5, 1, and 1.5% concentration samples were 2.12, 2.02, and 1.78 log cfu.mL⁻¹, while they were 2.18, 2.04, and 1.84 log cfu.mL⁻¹ after adding EFC and AFC, respectively. Organic acid content in AFC facilitates microbial load and has resulted in an intensified impact on TPC (Ma *et al.*, 2020).

Table (3) shows that the addition of EFC and AFC to orange juice led to reduced microbial load including mold and yeast counts (Bagci & Temiz, 2011). Mold and yeast counts of control sample was 1.87 log cfu.mL⁻¹, while the counts were reduced to 1.5 log cfu.mL⁻¹ at 0.5%, 1.35 at 1%, and 1.1 at 1.5%. AFC extract led to a decrease in mold and yeast counts to 1.53 log cfu.mL⁻¹ at 0.5%, 1.33 at 1%, and 1.12 at 1.5%. As reported by Sari *et al.* (2020), the antibacterial effect of EFC and AFC led to inactivating mold and yeast growth, especially with an increase in the addition dose (Alam *et al.*, 2019). Therefore, the addition of EFC and AFC may be effective orange juice preservatives, controlling mold and yeast development, thus extending shelf life (Efenberger-Szmechtyk *et al.*, 2021).

Conclusion

The concluding remarks of this study can be pointed out as follows: the addition of 60% ethanol freeze-dried coacervates (EFC) and absolute ethanol freeze-dried coacervates (AFC) to fresh orange juice had significant effects on its quality parameters. Both EFC and AFC improved the antioxidant activity,

total phenolic content, and total flavonoids in the orange juice as the concentration of coacervates increased. On the other hand, the increase in concentration of EFC and AFC resulted in an increased cloud value and electric conductivity of the orange juice which is considered an undesired reflectance. The concentration of coacervates played a significant role in the changes observed in the quality parameters of the orange juice. The results suggest that EFC and AFC could be used as potential natural additives in the food industry to improve quality and functional properties of orange juice. Future studies can focus on optimizing the concentration of EFC and AFC and investigating their impact on the sensory properties of orange juice.

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M.I.Y.: Methodology, Software, data curation, writing—original draft, conceptualization, review and editing.

R.X.: Supervision, writing—review and editing.

Z.T.A.: Validation, writing—review and editing, methodology.

A.B.A. and F.C.: Supervision, writing—review and editing.

K.F.M.: Validation, writing—review and

editing

S.H.S.: Software, validation, formal analysis.

T.G.A.: Methodology, data curation, Software, writing—original draft, Conceptualization, review and editing, review and editing.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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تحسين خصائص جودة عصير البرتقال الطازج من خلال إضافة كواسرفات مستخلصات نبات الكاسيا جافانিকা المجفدة

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المستخلص: هدفت الدراسة الحالية إلى تحديد تأثير إضافة كواسرفات مستخلصات نبات الكاسيا جافانিকা المجفدة إلى عصير البرتقال الطازج بتركيز متفاوتة (0.5، 1، و1.5%) على الخصائص الفيزيوكيميائية والميكروبيولوجية للعصير. كانت كواسرفات إما كواسرفات مستخلصات نبات الكاسيا جافانিকা باستخدام إيثانول 60% (EFC) أو بالإيثانول النقي (AFC). أظهرت النتائج أن قيمة الاس الهيدروجيني واللزوجة وموصلية الكهرباء لعصير البرتقال لم تتأثر بشكل كبير ($p < 0.05$) بإضافة EFC أو AFC حيث لم تتأثر قيمة الاس الهيدروجيني عند التركيز 0.5% وارتفعت قليلاً من 3.99 إلى 4.01 عند التراكيز 1% و 1.5%. أما بالنسبة للزوجة، بعد إضافة AFC و EFC، كانت النتائج 52، 53، و 53 مل باسكال. ثانياً عند التراكيز 0.5، 1، و 1.5% على التوالي. هناك زيادة في الموصلية الكهربائية حيث كانت العينة الكنترول 0.278 سيمنز/متر وبعد إضافة EFC، بلغت النتائج 0.334، 0.347، و 0.375 سيمنز/متر عند التراكيز 0.5، 1، و 1.5% على التوالي. بينما بعد إضافة AFC، بلغت النتائج 0.325، 0.335، و 0.373 سيمنز/متر عند التراكيز 0.5، 1، و 1.5% على التوالي. ومع ذلك، بعد إضافة EFC، زاد محتوى الفينولات الكلية ليصبح 77.25، 115.96، و 154.95 ملليغرام/100 مل، وزاد محتوى الفلافونويد الكلي ليصبح 34.76، 52.18، و 69.73 ملليغرام/100 مل، وزادت النشاط المضاد للأكسدة ليصبح 70.36، 74.36، و 79.58% عند التراكيز 0.5، 1، و 1.5% على التوالي. أيضاً، بعد إضافة AFC، زاد محتوى الفينولات الكلية ليصبح 79.26، 117.78، و 156.25 ملليغرام/100 مل. كما زاد محتوى الفلافونويد الكلي ليصبح 35.67، 53.00، و 70.31 ملليغرام/100 مل. وايضا زاد النشاط المضاد للأكسدة ليصبح 71.65، 75.84، و 81.21% عند التراكيز 0.5، 1، و 1.5% على التوالي. بالإضافة إلى ذلك، بعد إضافتها إلى العينة الكنترول عند التراكيز 0.5، 1، و 1.5%، خفضت EFC العدد الكلي للبكتريا إلى 2.12، 2.02، و 1.78 لوغاريتم جرام/مل على التوالي وخفضت عدد الفطريات والخمائر إلى 1.5، 1.35، و 1.1 لوغاريتم جرام/مل على التوالي. أيضاً، سببت إضافة AFC انخفاضاً في العدد الكلي للبكتريا الإجمالي إلى 2.18، 2.04، و 1.84 لوغاريتم جرام/مل على التوالي وانخفض عدد الفطريات والخمائر إلى 1.53، 1.33، و 1.12 لوغاريتم جرام/مل على التوالي. بشكل عام، تشير النتائج إلى أن إضافة EFC أو AFC إلى عصير البرتقال الطازج يمكن أن يعزز من جودته التغذوية والميكروبيولوجية دون التأثير على جودته الحسية.

الكلمات المفتاحية: كاسيا جافانিকা، كواسرفات، عصير البرتقال.