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# Optimizing carrot extract serum (*Daucus carota* L.) for anti-aging: efficacy in moisturizing and pore size reduction using Box-Behnken design method

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**ABSTRACT** : Carrot extract (*Daucus carota L.*) contains various bioactive compounds, including vitamins A, B, and C, alkaloids, flavonoids, tannins, anthraquinones, saponins, diterpenes, steroids, beta-carotene, phenols, terpenoids, and minerals, all of which possess antioxidant properties. These compounds are known to help slow down the aging process. The aim of this study was to determine the optimal concentrations of gelling and alkalizing agents, assess their interactions, and evaluate the anti-aging effects of the most effective serum formulation. The formulation was optimized using the Box–Behnken design with two key factors: Carbopol 940 concentration (0.5–1%) and triethanolamine (0.5–1%). The effects on pH, adhesion, and spreadability were evaluated. The best formulation was achieved with 0.837% Carbopol 940 and 0.855% triethanolamine, showing a pH error of 2.45%, adhesion error of 0.76%, and spreadability error of 1.01%, all within acceptable limits (errors < 10%). After four weeks of stability testing, the formulation remained stable, well-mixed, with a pH of 5.16, an adhesion time of 1.60 seconds, and spreadability of 6.73 cm, with no discomfort. The combination of Carbopol 940 and triethanolamine improved the physical properties, enhancing anti-aging effects compared to the base formula. The optimized serum increased skin moisture by 84.62%, reduced pore size by 64.71%, lightened spots by 62.79%, and reduced wrinkles by 67.50%. This indicates the optimized carrot extract serum is stable, safe, and effective as an anti-aging agent, making it a promising natural skincare product.

**KEYWORDS**: Anti-aging; Box-Behnken design; *Daucus carota* L.; optimization; serum.

## INTRODUCTION

Aging is a process in which the functions and capabilities of the skin gradually decline. Exposure to ultraviolet (UV) rays is the primary cause of oxidative stress on the skin and thus constitutes an important risk factor for the development of skin issues, such as the formation of wrinkles, lesions, and cancer [1]. One way to prevent and address skin aging caused by free radicals is to increase the use of additional antioxidants from outside the body. Antioxidants are compounds that can counteract or neutralize the effects of free radicals, thereby preventing degenerative diseases in the skin [2]. Anti-aging is part of cosmetic products that contain ingredients that can reduce wrinkles and increase moisture levels in the skin. The main function of anti-aging preparations is to reduce wrinkles and spots [3].

Carrots (*Daucus carota* L) are thought to be beneficial in combating free radicals and possess antioxidant properties. Research findings indicate that carrot extract functions pharmacologically as an antioxidant, comprising chemical compounds such as vitamins A, B, and C, alkaloids, flavonoids, tannins, anthraquinones, carbohydrates, saponins, diterpenes, steroids, beta-carotene, phenols, terpenoids, and minerals. Carrots (*Daucus carota* L.) are a source of β-carotene and phenolic compounds that have high antioxidant activity. These compounds play a role in neutralizing free radicals, thereby reducing oxidative stress, one of the main factors contributing to premature aging of the skin. Previous studies have utilized carrot extract in topical formulations, including lotions and spray serums, to assess their antioxidant activity [5].

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Serum is a low-viscosity preparation, typically considered neither too thick nor too thin due to its low viscosity. The composition of the gelling agent added to the serum preparation influences the viscosity of the preparation. Serum has the advantage of having a high concentration of active ingredients, providing a more comfortable effect and easier spread on the skin surface due to its moderate viscosity [6]. The use of serum is preferred over cream because the active ingredients in serum are easier for the skin to absorb [7].

The antioxidant activity of carrot extract (*Daucus carota* L.) exhibited $IC_{50}$ values below 50 ppm, specifically $IC_{50}$ values of F1 (1%) at 41.312 ppm, FII (3%) at 34.649 ppm, and FIII (5%) at 28.804 ppm [5]. Its antioxidant properties are frequently utilized in anti-aging treatments and to avert premature skin aging. The study indicates that there were no skin irritation reactions observed. This application is very efficacious as an anti-aging treatment at a 15% concentration, yielding progressively excellent results, consequently refining skin texture, diminishing pores, alleviating blemishes, and reducing wrinkles.

A good pharmaceutical preparation must be useful, effective, stable, and easy to use. The formulation process needs to be improved in order to fulfill these standards. Form keyed optimization is done to get the optimum formulation with goodFlying characteristics with fewer tests. pH, spreadability, adhesion, and organoleptic qualities are some of the physical properties under question. These physical parameters must be right for a successful serum formulation [9].

Carbopol 940 and triethanolamine (TEA) were the materials chosen for the optimization process. Carbopol is a gel base that, when mixed with other ingredients, makes a clear gel that spreads smoothly on the skin, cools it down, doesn't clog pores, and can be readily rinsed off with water. Carbopol, on the other hand, has an acidic pH (2.5–4.5), hence triethanolamine is needed to bring the pH of carbopol 940 back to neutral [10]. Triethanolamine works best as an alkalizing agent at a concentration of 2–4% [11].

Optimization was conducted to determine the ideal concentration of the gelling agent (carbopol 940) and the alkalizing agent (triethanolamine) in the serum formulation. The RSM experimental design comprises Box-Behnken and Composite Central Design (CCD). The Box-Behnken Design offers advantages over the Central Composite Design due to its greater efficiency, requiring fewer tests while maintaining the same number of variables, therefore lowering testing expenses [12]. This optimization research utilizes the Box Behnken Design (BDD) within the Design Expert 13 software to assess the impact of carbopol 940 and triethanolamine concentrations on the formulation's physical characteristics and stability [11], 12].

This study intends to optimize and formulate a serum preparation from carrot extract (*Daucus carota* L.) with different quantities of carbopol 940 and triethanolamine, employing the Box-Behnken Design (BBD). The best serum composition will thereafter be assessed for anti-aging capabilities via a skin analyzer, evaluating criteria like skin hydration, pore size, skin blemishes, and wrinkles.

## ▪ MATERIALS AND METHODS

### Materials

The materials used consisted of carrot extract (*Daucus carota* L.) obtained from PT. Lansida Group, Yogyakarta, Indonesia; carbopol 940 (Corel Pharma Chem, Ahmedabad, India); propylene glycol (SK picglobal, Ulsan, South Korea); triethanolamine (Petronas, Kuala Lumpur, Malaysia); DMDM hydantoin (Nguyen BA Co., Ltd, Ho Chi Minh City, Vietnam); sodium EDTA (Merck, Darmstadt, Germany); methanol, concentrated sulfuric acid, concentrated hydrochloric acid, chloroform, and ammonia (all from Emsure, Darmstadt, Germany); magnesium metal; anhydrous acetic acid; Mayer and Dragendorff reagents; DPPH (Sigma Aldrich, St. Louis, Missouri, USA); and ascorbic acid (Weisheng Pharmaceutical, Shijiazhuang, Hebei, China) and distilled water. For the closed patch test, sterile gauze (Medica, Jakarta, Indonesia) and waterproof plaster (Medica, Jakarta, Indonesia) were used.

### Phytochemical screening test of carrot extract

A 0.5 grams of concentrated extract is dissolved in 5 mL of ethanol. Then, 5 mL each of distilled water and chloroform (1:1) are added. The mixture is shaken vigorously and left to settle until two layers form. The aqueous layer (top layer) is used for testing flavonoids, phenolics, and saponins. The chloroform layer (bottom layer) is used for testing terpenoids and steroids. Meanwhile, testing for alkaloids has its own procedure [13].

***Flavonoid test***

A few drops of water layer are added using a few drops of concentrated HCl, then 0.2 mg of Mg metal is added. The result is considered positive if an orange, pink to red color appears within 3 minutes [13] ,[14].

***Phenolic test***

A few drops of water layer are placed in a test tube, then a few drops of 1% $FeCl_3$ are added. The result is considered positive if a purple to dark blue color appears [12], [13].

***Saponin test***

A few drops of the aqueous layer are placed in a test tube, then shaken vigorously until foam forms and persists for 5 minutes. The result is considered positive if foam persists for 5 minutes.

***Terpenoid and steroid test***

The chloroform layer is filtered through a pipette containing norit, which serves to attract color pigments, making it easier to identify the color of the test results. Then, drop it onto a drop plate. For the terpenoid test, the chloroform layer is added with 1-2 drops of Liebermann-Burchard reagent (2 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid), which yields a positive result if it produces a red color. For the steroid test, the chloroform layer is added with 1-2 drops of Liebermann-Burchard reagent, and the result is considered positive if it produces a green-blue color [14], [15].

***Alkaloid test***

For the alkaloid test, 0.5 grams of extract is added to 10 mL of chloroform, then another 10 mL of 0.05 M ammonium chloroform solution is added, stirred and filtered, then 1 mL of 2 N sulfuric acid is added to the reaction tube, the tube is shaken for 2 minutes, left to form two layers, and then separated. The acid layer (top) is taken and 1-2 drops of Dragendrof reagent are added; a positive result is indicated by the formation of a white precipitation [14], [15].

**Antioxidant activity test of carrot extract**

***Preparation of DPPH reagent***

10 mg of DPPH was dissolved in 10 mL of methanol solution, shaken until homogeneous, and stored in a dark bottle to obtain a solution with a concentration of 1000 µg/mL. It was diluted to 40 µg/mL by pipetting 400 µL and adding methanol to 10 mL in a measuring flask [12], [16].

***Sample Solution Preparation***

Carrot extract was weighed at 10 mg and dissolved in 10 mL of methanol to obtain a stock solution with a concentration of 1000 µg/mL. The test was conducted in 6 concentration series: 1000; 500; 250; 125; 62.5; 31.25 µg/mL. The test was repeated 3 times [14], [15].

***Preparation of Vitamin C Standard Solution***

Vitamin C was weighed at 10 mg and dissolved in 10 mL of methanol to obtain a stock solution with a concentration of 1000 µg/mL. A dilution with a concentration of 100 µg/mL was then prepared by pipetting 1 mL of the stock solution into a 10 mL volumetric flask, followed by the addition of methanol up to the mark. The testing was conducted with six concentration series: 100; 50; 25; 12.5; 6.25; and 3.125 µg/mL. Each concentration test was repeated three times. The dilutions were performed on a 96-well microplate [13,14].

***Antioxidant Activity Using the DPPH Method***

A total of 100 µL of sample solution with a concentration of 1000 µg/mL was added to the wells in row A. A total of 50 µL of methanol was pipetted into the wells in rows B-H. Then, 50 µL of the extract solution in row A was pipetted using a multichannel micropipette and added to row B, 50 µL of the solution in row B was pipetted and added to row C, and so on until row F. Next, 50 µL of the solution in row F was pipetted and discarded, resulting in test solutions with concentrations of 1000 µg/mL (row A), 500 µg/mL (row B), 250 µg/mL (row C), 125 µg/mL (row D), 62.5 µg/mL (row E), and 31.25 µg/mL (row F). Rows A-G were added with 40 µL of 40 µg/mL DPPH solution. Row H contained only methanol as a blank. The test solutions were incubated for 30 minutes at room temperature and in the dark by covering the 96-well microplate with aluminum foil. After 30 minutes, the absorbance of the test solutions was measured at a wavelength of 517 nm using a microplate reader [14].

### Optimization design of carrot extract serum formulation

A Box-Behnken statistical design with two factors and 17 experiments was used for the optimization study with the help of Design Expert software version 13. The variables used were modified from the research by Rudianti et al [15], namely the concentration of Carbopol 940 and Triethanolamine, which function as the main components in forming the gel in gel-based serum preparations. The dependent variables (responses) observed included pH, adhesion, and spreadability [11], [14].

**Table 1.** Run of formula and responses used using box behnken design.

| Run | Factor 1 A:Carbopol 940 | Factor 2 B:TEA | Response 1 pH | Response 2 Adhesion test (seconds) | Response 3 Spreadability test (cm) |
|---|---|---|---|---|---|
| 1 | 0.50 | 0.50 | - | - | - |
| 2 | 1 | 0.50 | - | - | - |
| 3 | 0.50 | 0.75 | - | - | - |
| 4 | 1 | 0.75 | - | - | - |
| 5 | 0.75 | 0.50 | - | - | - |
| 6 | 0.75 | 0.75 | - | - | - |
| 7 | 0.75 | 0.75 | - | - | - |
| 8 | 1 | 0.75 | - | - | - |
| 9 | 0.75 | 0.75 | - | - | - |
| 10 | 0.75 | 0.5 | - | - | - |
| 11 | 0.75 | 0.75 | - | - | - |
| 12 | 0.75 | 0.75 | - | - | - |
| 13 | 1 | 1 | - | - | - |
| 14 | 0.50 | 0.75 | - | - | - |
| 15 | 0.75 | 1 | - | - | - |
| 16 | 0.75 | 1 | - | - | - |
| 17 | 0.50 | 1 | - | - | - |

### Preparation of carrot extract serum formulation

For the formulation of carrot extract serum formulation follow this formulation design (Table 2):

**Table 2.** Serum preparation formulation design.

| Composition | Concentration (%) |
|---|---|
| Carrot Extract (*Daucus carota* L.) | 15 |
| Carbopol 940 | 0.5-1 |
| Propylene glycol (PG) | 15 |
| Triethanolamine (TEA) | 0.5-1 |
| DMDM Hydantoin | 0.5 |
| Na EDTA | 0.1 |

### Response testing

Several stages in testing serum evaluation used as a dependent variable or response are as follows.

#### *pH testing*

pH testing is carried out using a pH meter calibrated with standard solutions of pH 4, pH 7, and pH 10. The electrode is then washed with distilled water and dried with tissue paper. One gram of the sample is weighed and dissolved in 10 mL of distilled water. The electrode is then immersed in the solution and left until the digital reading stabilizes at a constant pH value, which is the pH value of the formulation. The test is repeated three times for each formulation. The physiological pH range for human skin is 4.5–6.5 [16].

***Spreadability test***

Prepare a flat glass and place 0.5 grams of facial serum on it, then place another glass on top and apply a 150-gram weight for 1 minute. Measure the diameter formed. The test is conducted with three replicates for each formula. The requirement for spreadability is a spread diameter of 5–7 cm. The higher the spreadability value, the more the active ingredient can come into contact with and spread across the skin [17].

***Adhesion test***

The adhesion test was conducted by weighing 0.5 grams of serum, then placing it between two glass objects with a weight of 1 kg for 5 minutes. The weight is lifted, and the time until the two glass objects separate is recorded. The standard adhesion strength for good serum is >1 second. The test is performed with three replicates for each formula [18].

**Validation of formula optimization model**

The parameters for producing carrot extract serum were optimized using Design Expert software version 13 by setting selection criteria in the form of optimal concentrations of gelling agent and alkalizing agent. The output of the response generated was pH, spreadability, and adhesiveness. Desirability is a function value that indicates the program's ability to meet desires based on the optimization criteria set for the final product. A desirability value close to 0.9 or 1.0 indicates a higher probability of producing the desired product, while a desirability value of 1.0 indicates that the objective has been achieved [19].

**Evaluation of optimal serum preparation**

The stages of serum preparation evaluation testing are as follows.

***Organoleptic test***

Organoleptic testing is conducted to examine the physical appearance of the serum. Organoleptic testing is performed by observing the texture, color, and smell of the serum preparation [20].

***Homogeneity test***

The preparation is tested for homogeneity by applying it to a glass slide. Check for the presence of coarse particles that are not yet homogeneous [21].

***pH testing***

The pH evaluation of the best formula resulting from optimization was carried out according to the procedure in the Response Testing section.

***Viscosity test***

The viscosity of the preparation was tested by placing the serum preparation into a viscometer using spindle number 4 and reading the viscosity using a rotor rotating at a speed of 30 rpm [22]. The optimal viscosity conditions for gel-based serum preparations are between 2,000 and 4,000 cPs [18]. The testing was conducted with three replicates for each formula.

***Spreadability test***

The spreadability evaluation of the best formula resulting from optimization was carried out according to the procedure in the Response Testing section.

***Adhesion test***

The adhesion evaluation of the best formula resulting from optimization was carried out according to the procedure in the Response Testing section.

***Skin ırritation test***

The ethical clereance was approved with number 507/KEP-UNIVRAB/III/2025 by Faculty of Medicine Abdurrab University. A closed patch test was conducted on three healthy volunteers (25-30 years old) after signing a written informed consent and approved by the Ethics Committee. The serum was applied to the inner upper arm, covered with gauze and a waterproof plaster for 24 hours. After removal, redness, spots, or edema were observed. Each symptom was scored 0–3, and the Primary Irritation Index (PII) was calculated as the average score of all volunteers. The PII scale is: 0–0.4 no irritation; 0.5–1.9 mild irritation; 2–5 moderate–severe irritation. This method ensures the safety of the formulation before widespread use [21].

***Stability Test Using the cycling test***

The cycling test method involves weighing 0.5 grams of serum preparation for each formula and placing it in a tightly sealed vial. The serum samples in the vials are subjected to the cycling test , where the vials are stored at 4°C for 24 hours, then removed and placed at 40°C for 24 hours; this process constitutes one cycle. Repeat this process for up to 6 cycles and observe any changes in organoleptic properties and pH of the preparation in each cycle. The preparation is considered stable if it has undergone 6 cycles without any changes in organoleptic properties and pH [23].

**Anti-aging effectiveness testing**

Anti-aging effectiveness testing was conducted using two volunteers. Prior to testing, the panelists filled out a consent form agreeing to participate as volunteers. The criteria for volunteers included not using other serums, having normal skin, being female, engaging in the same activities, and being between the ages of 25 and 30. The initial skin condition of each volunteer was measured on the back of the palm, including parameters such as moisture levels, pore size, number of spots, and wrinkles using a skin analyzer. After the measurements were taken, the serum was applied evenly to the skin area. The serum was applied to the back of both the right and left hands. The application was performed twice daily, in the morning and evening, for four weeks. Skin condition changes were measured at week 0 (initial condition), week 1, week 2, week 3, and week 4 using a skin analyzer.

**Data Analysis**

The test data was processed using Design Expert 13 Box Behnken Design software to obtain the optimum formula from optimization based on the test parameters of pH, spreadability, and adhesiveness. The data were presented in tables and figures to conclude the effect of concentration differences on the use of carbopol 940 and triethanolamine in carrot extract serum preparations (*Daucus carota* L.). The anti-aging efficacy test data were analyzed using ANOVA (Analysis of Variance) statistics.

## ▪ RESULTS

**Phytochemical screening test of carrot extract**

The phytochemical screening test of the carrot extract confirmed the presence of various bioactive compounds. The results of the qualitative analysis are summarized in Table 3.

**Table 3.** Results of phytochemical testing of carrot extract.

| Parameter | Result | Reaction |
|---|---|---|
| Flavonoid | + | Orange color formed |
| Fenolik | + | Blackish green color formed |
| Saponin | - | Stable foam not formed |
| Terpenoid | + | Brownish red color formed |
| Steroid | - | Blue/green color not formed |
| Alkaloid | + | Orange precipitate formed |

### Antioxidant activity test of carrot extract

The antioxidant activity of carrot extract and vitamin C as a positive control was tested using the DPPH method, and the results are presented in Tables 4 and 5.

**Table 4**. Antioxidant activity test of Vitamin C.

| Cons. (µg/ml) | Average ± SD | Abs. Sample | % Inhibisi | $IC_{50}$ value (µg/mL) |
|---|---|---|---|---|
| 100 | 0.079±0.002 | 0.013 | 96.02 | 5.29 (Very Strong Category) |
| 50 | 0.078±0.002 | 0.011 | 96.53 | |
| 25 | 0.077±0.001 | 0.011 | 96.74 | |
| 12.5 | 0.095±0.001 | 0.028 | 91.34 | |
| 6.25 | 0.219±0.009 | 0.152 | 53.41 | |
| 3.125 | 0.302±0.003 | 0.235 | 28.03 | |

**Table 5.** Antioxidant activity test of carrot extract.

| Cons. (µg/ml) | Average±SD | Abs. Sample | % Inhibisi | $IC_{50}$ value (µg/mL) |
|---|---|---|---|---|
| 1000 | 0.079±0.001 | 0.040 | 76.55 | 362.28 (Weak Category) |
| 500 | 0.111±0.002 | 0.072 | 58.33 | |
| 250 | 0.146±0.002 | 0.107 | 37.98 | |
| 125 | 0.173±0.001 | 0.134 | 22.29 | |
| 62.5 | 0.195±0.004 | 0.156 | 9.11 | |
| 31.25 | 0.199±0.001 | 0.160 | 6.98 | |

### Optimization design of carrot extract serum formulation

The formula predictions by Design Expert 13 software are shown in Table 6.

**Table 6.** Formula 17 run and response results using Box-Behnken Design.

| Run | Factor 1 A:Carbopol 940 | Factor 2 B:TEA | Response 1 pH | Response 2 Adhesion Test (seconds) | Response 3 Spreadability Test (cm) |
|---|---|---|---|---|---|
| 1 | 0.50 | 0.50 | 5.11 | 0.98 | 10.5 |
| 2 | 1 | 0.50 | 4.82 | 1.88 | 8.7 |
| 3 | 0.50 | 0.75 | 5.65 | 1.23 | 9 |
| 4 | 1 | 0.75 | 5.09 | 2.02 | 6.5 |
| 5 | 0.75 | 0.50 | 4.87 | 1.20 | 8.9 |
| 6 | 0.75 | 0.75 | 5.35 | 1.58 | 7.6 |
| 7 | 0.75 | 0.75 | 5.35 | 1.55 | 7.8 |
| 8 | 1 | 0.75 | 5.07 | 2.20 | 6.3 |
| 9 | 0.75 | 0.75 | 5.37 | 1.55 | 7.5 |
| 10 | 0.75 | 0.5 | 4.85 | 1.18 | 8.5 |
| 11 | 0.75 | 0.75 | 5.38 | 1.49 | 7.5 |
| 12 | 0.75 | 0.75 | 5.33 | 1.53 | 7.6 |
| 13 | 1 | 1 | 5.72 | 2.10 | 5.9 |
| 14 | 0.50 | 0.75 | 5.67 | 1.20 | 9.2 |
| 15 | 0.75 | 1 | 5.80 | 1.56 | 7 |
| 16 | 0.75 | 1 | 5.83 | 1.60 | 7.1 |
| 17 | 0.50 | 1 | 6.40 | 1.19 | 8.8 |

### Response testing

In this study, a desirability value of 1.000 was obtained for the recommended optimal formula, corresponding to a composition of carbopol 940 = 0.837% and triethanolamine = 0.855%, with predicted response values of pH = 5.451, adhesion = 1.746 seconds, and spreadability = 6.747 cm.

### Validation of formula optimization model

The verification results for the optimal formula of carrot extract serum yielded a pH error of 2.45%, adhesion strength of 0.76%, and spreadability of 1.01%.

**Table** 7. Optimum formula verification results.

| Preparation evaluation | Prediction design expert 13 | Average ± SD observation | % error |
|---|---|---|---|
| pH | 5.451 | 5.58±0.005 | 2.45% |
| Adhesion Test | 1.746 | 1.75±0.07 | 0.76% |
| Spreadibility test | 6.747 | 6.63±0.05 | 1.01% |

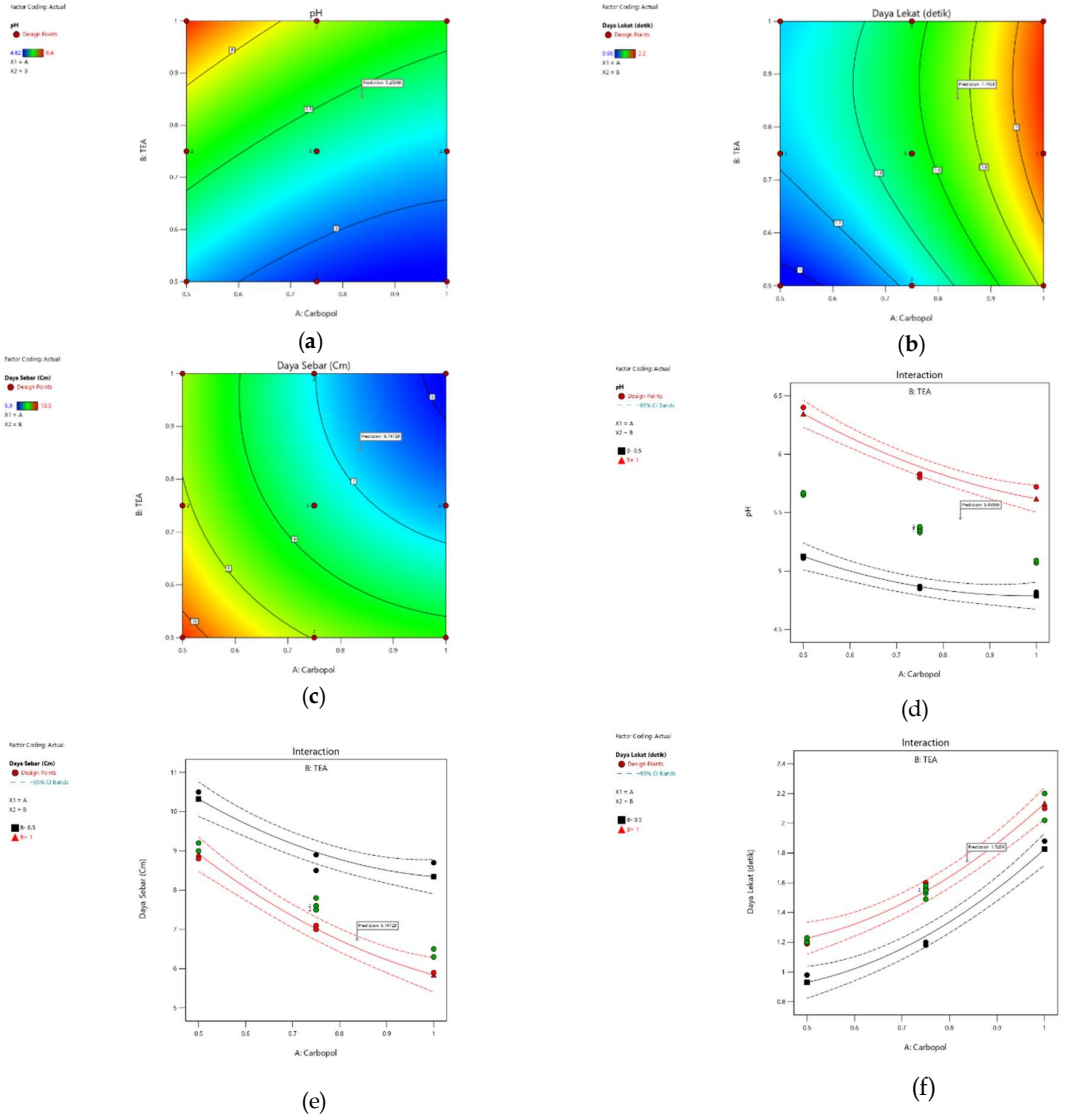


**Figure 1.** Contour plots of Carbopol 940 and TEA on pH (a), adhesive strength (b), and spreadability (c); and interaction plots of Carbopol 940 and TEA on pH (d), adhesive strength (e), and spreadability (f).

## Evaluation of optimal serum preparation

Table 8 provides a detailed summary of the evaluation results of the formulated optimum serum preparation, covering the main parameters assessed during the study.

**Table 8.** The evaluation result of optimum serum preparations.

| Parameter | Result (Average±SD) |
|---|---|
| Organoleptic | Brownish orange in color, with a distinctive carrot extract smell and Slightly thick |
| Homogeneity | Homogeneous |
| pH | 5.59±0.005 |
| Spreadability | 6.66±0.11 |
| Adhesion | 1.75±0.07 |
| Viscosity | 3.107±11.54 |

The long-term stability of the optimum serum formulation was assessed over a four-week period, and the results are presented in Table 9.

**Table 9.** The long term stability result of optimum serum preparations.

| Parameter | Result | | | | p-value |
|---|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 | |
| Organoleptic | Brownish orange in color, with a distinctive carrot extract smell and Slightly thick | Brownish orange in color, with a distinctive carrot extract smell and Slightly thick | Brownish orange in color, with a distinctive carrot extract smell and Slightly thick | Brownish orange in color, with a distinctive carrot extract smell and Slightly thick | - |
| Homogeneity | Homogeneous | Homogeneous | Homogeneous | Homogeneous | - |
| pH | 5.59±0.005 | 5.34±0.032 | 5.19±0.017 | 5.16±0.030 | <0.05 |
| Spreadability | 6.66±0.11 | 6.73±0.11 | 6.86±0.05 | 6.73±0.05 | >0.05 |
| Adhesion | 1.75±0.07 | 1.72±0.11 | 1.60±0.05 | 1.60±0.06 | >0.05 |
| Viscosity | 3.107±11.54 | 3.087±23.09 | 3.060±72.11 | 3.013±101.59 | >0.05 |

*Skin irritation test*

Closed patch testing on 3 healthy volunteers showed no redness, itching, or burning sensation, with a Primary Irritation Index (PII) = 0, thus categorizing the formulation as safe and non-irritating.

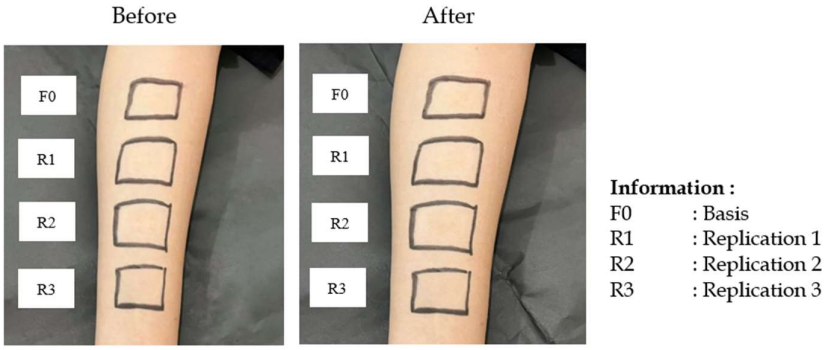


**Figure 2.** Irritation test results for carrot extract serum preparations.

*Stability test using the cycling test*

**Table 10.** The long term stability result of optimum serum preparations.

| Parameter | Cycle 1 (Average±SD) | Cycle 6 (Average±SD) | p-value |
|---|---|---|---|
| Organoleptic | Brownish orange in color, with a distinctive carrot extract smell and Slightly thick | Brownish orange in color, with a distinctive carrot extract smell and Slightly thick | - |
| pH | 5.43 ±0.01 | 5.08 ±0.005 | <0.05 |

**Anti-aging effectiveness testing**

The moisture content was measured on the dorsal side of the volunteers' palms using an Aramo skin analyzer. Before applying the serum formulation, the average moisture content on the backs of the hands across all groups was 6 and 12, indicating dehydrated skin (0-29). After 30 days of serum application, moisture content increased on the dorsal surfaces of the volunteers' hands for both the optimal and base formula users. The serum treatment led to an improvement in moisture levels, with averages of 39 and 32 for the participants' skin after serum application. Observations on day 7 showed that both formulas resulted in increased moisture content, with the optimal formula improving by 53.85% and the base formula by 25%. All formulations showed an increase in moisture from week 0 (before serum application) to week 4, with the optimal formula showing an 84.62% increase and the base formula a 62.50% increase (Figure 3).

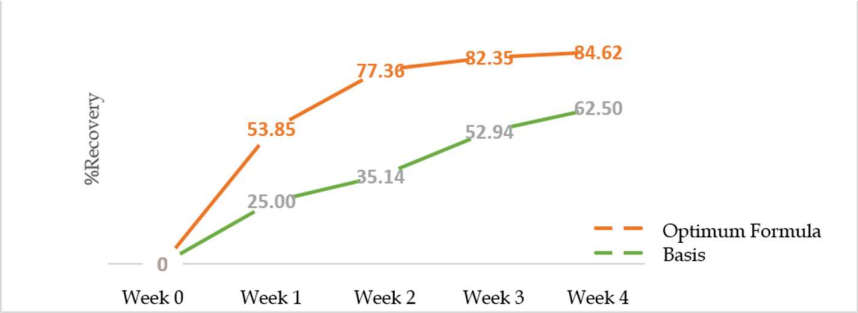


**Figure 3**. Graph of percent moisture recovery for one month.

The results of the percentage of pore size recovery for each formula each week can be seen in Figure 4.

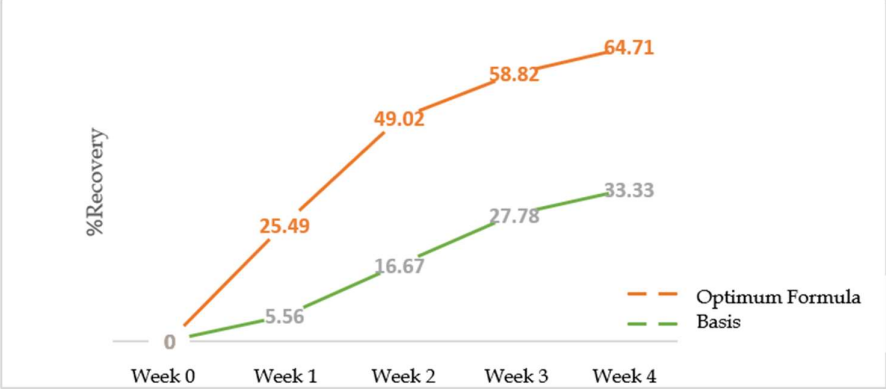


**Figure 4.** Graph of percent pore recovery for one month.

The data on the percentage of spot reduction recovery for each formula each week can be seen in Figure 5.

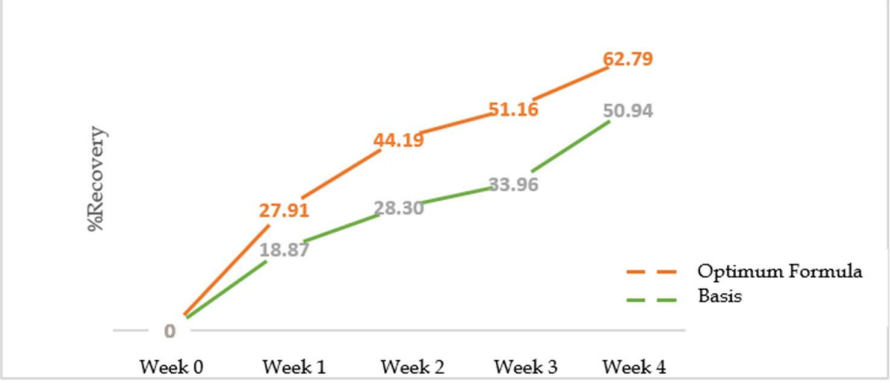


**Figure 5**. Graph of percent recovery of spots for one month.

The results of the percentage of wrinkle reduction for each formula each week can be seen in Figure 6.

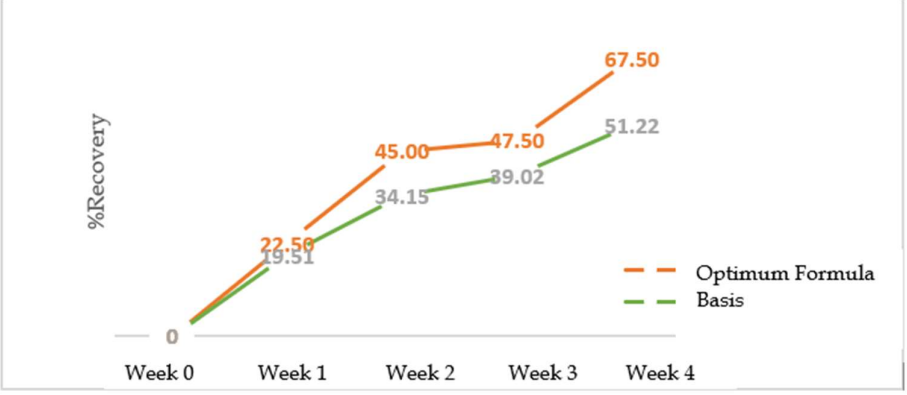


**Figure 6**. Graph of percent wrinkle recovery for one month.

## DISCUSSION

### Phytochemical screening test of carrot extract and antioxidant activity test of carrot extract

The results of this phytochemical screening can be seen in Table 3. The screening results of carrot extract show that it contains flavonoids, phenolics, terpenoids, and alkaloids. The table 4 shows that the $IC_{50}$ value for Vitamin C antioxidant activity testing is 5.29 µg/mL. This result falls into the category of very strong Vitamin C $IC_{50}$ values because it is <50 ppm. Table 5 shows the results of antioxidant activity testing of carrot extract, which showed weak antioxidant activity with an $IC_{50}$ of 362.28 µg/mL. The difference in antioxidant activity between vitamin C and carrot extract lies not only in the measurement results, but also in the chemical properties of the two substances. Vitamin C is a highly pure single molecule, so its antioxidant activity can work directly and consistently. In contrast, carrot extract is a complex mixture containing various compounds, with concentrations of active antioxidants such as β-carotene much lower than pure vitamin C. This explains why the antioxidant activity of carrot extract is relatively lower and indicates that the right dosage or extraction method is needed to maximize its potential [16], [17].

### Optimization design of carrot extract serum formulation

Formula optimization was performed by entering the variable data to be optimized into Design Expert software version 13. The variables in this study were the concentrations of Carbopol 940 (0.5-1%) and triethanolamine (0.5-1%). Data for each variable were entered into Design Expert software version 13, resulting in 17 formulas with various variable concentrations. The response of each formula was then evaluated based on pH, adhesion, and spreadability. The formula predictions by Design Expert 13 software are shown in Table 6 [14], [17].

### Response testing

The optimal formula recommended by the Design Expert software is determined based on the highest desirability contour plot. The higher the desirability value, the higher the suitability of the product formula to achieve the optimal formula with the specified response variables. In this study, a desirability value of 1.000 was obtained for the recommended optimal formula, corresponding to a composition of carbopol 940 = 0.837% and triethanolamine = 0.855%, with predicted response values of pH = 5.451, adhesion = 1.746 seconds, and spreadability = 6.747 cm. Furthermore, the carrot extract serum formula was reformulated for verification based on the response parameters used, namely pH testing, adhesion strength, and spreadability [17], [18].

### Validation of formula optimization model

The results of the One Sample T-Test show that there is a statistically significant difference between the predicted and actual values for the pH parameter ($p < 0.05$). Conversely, for the spreading power and adhesion power parameters, the p-value is > 0.05, so statistically there is no significant difference between the predicted and actual values for these two parameters. The One Sample T-Test was still used considering the relatively small sample size, so that parametric analysis could still be applied with careful interpretation [16,25].

Although there was a statistically significant difference in the pH parameter, the absolute value difference was relatively small, so a further analysis was performed in the form of a percentage error calculation to assess the practical significance of the difference. The results of the verification of the optimum formula for carrot extract serum (*Daucus carota* L.) showed a percentage error of pH = 2.45%, adhesion = 0.76%, and spreadability = 1.01%. These values indicate that there is no practical difference because all % errors are < 10% [25].

In the counter plot results, different contours and colors can be seen for each response. The counter plot showing red responses indicates maximum results, while blue indicates minimum results. In the pH counter plot diagram, there is a color gradient from blue to red, representing an increase in pH value as the concentration of carbopol 940 and triethanolamine changes. Blue indicates low pH areas, while red areas indicate higher pH. This contour pattern indicates that the synergistic increase in the concentration of both components enhances the pH value of the serum formulation. The optimal pH value is predicted to be 5.451, located in the graph area and marked with a prediction point. In the spreadability counter plot diagram, the color gradient from blue to red represents an increase in spreadability values as the concentration of Carbopol 940 decreases. The predicted spreadability value of 6.747 cm is obtained in the light blue area. On the counter plot diagram for adhesion strength, the color gradient from blue to red represents an increase in adhesion strength as the concentration of Carbopol 940 increases. The predicted adhesion strength value of 1.746 seconds was obtained in the green-colored area [26].

The interaction between factors, namely the concentration of carbopol 940 and triethanolamine on pH and spreadability, can be determined using Design Expert software version 13. The interaction between carbopol 940 and triethanolamine shows that an increase in the concentration of carbopol 940 tends to decrease the pH value, both at low (black line) and high (red line) concentrations of triethanolamine. From the discussion above, it is evident that carbopol 940 influences pH reduction, while triethanolamine plays a role in increasing the pH of the formulation. The interaction between these two ingredients determines the pH stability of the formulation, where an increase in carbopol without being balanced by triethanolamine causes the pH to decrease significantly. Conversely, at higher triethanolamine concentrations, the pH decrease caused by carbopol can be suppressed, resulting in a more stable pH formulation within the appropriate range for topical use. The interaction between Carbopol 940 and triethanolamine shows that increasing the concentration of Carbopol 940 tends to reduce the spreadability value, both at low triethanolamine concentrations (black line) and high concentrations (red line). This aligns with Carbopol's characteristics as a gel-forming agent that increases the viscosity of the formulation, thereby limiting the mobility and spread of the formulation on the skin surface. The interaction between carbopol 940 and triethanolamine shows that an increase in carbopol 940 concentration tends to increase the adhesive strength, both at low triethanolamine concentrations (black line) and high concentrations (red line) [26].

**Evaluation of optimal serum preparation**

*Organoleptic test*

The results of organoleptic testing on the shape, color, and odor of the optimal formulation are shown in Table 9. Based on observations conducted over 4 weeks, it was found that the serum formulation did not undergo changes and remained stable during 4 weeks of storage.

*Homogeneity test*

The homogeneity test of the serum preparation showed that no particles were found in the optimal serum formula of carrot extract (Table 9). A homogeneous preparation is characterized by the absence of clumped particles or coarse granules in the preparation [26], [27].

*pH test*

The desired pH level in the serum formulation is within the skin pH range of 4.5–6.5 [27]. The pH should not be too acidic, as this can cause skin irritation, nor should it be too alkaline, as this can cause dry, sensitive skin that is prone to infection [28]. The pH was measured weekly over a 4-week storage period using a pH meter. The pH test results showed that the pH of the optimal formula met the requirements; however, during the 4-week storage period, the pH decreased to 5.59–5.16 (Table 9). This change was statistically significant, with a p-value < 0.05, indicating that the decrease in pH between weeks did not occur by chance.

It can be seen that there was a change in pH, and the decrease in pH may be due to the use of carrot extract in the formulation, as the extract is acidic. It may also be caused by reactions occurring within the

formulation ingredients, influenced by environmental conditions such as temperature, light, and air humidity [29]. In formulations using a carbopol base, a decrease in pH may occur due to a reaction between the carboxyl groups in carbopol and water, resulting in the formation of $H_3O^+$ (acid), which increases in quantity, making the formulation more acidic [30]. Based on the results obtained, low carbopol concentrations produce higher pH values, while high carbopol concentrations produce lower pH values. This is because carbopol is acidic (2.5–3.0). The higher the carbopol concentration in the preparation, the more carboxylic acid groups are released, thereby lowering the pH of the preparation. Conversely, at low concentrations, there are fewer acid groups, resulting in a relatively higher pH.

*Spreadability test*

The spreadability test aims to determine the spreadability of carrot extract serum preparations when applied to the skin, as this can affect drug absorption and the rate of active ingredient release at the application site [31]. The larger the spreadability diameter value, the higher the serum spread rate with minimal application, thereby increasing drug contact with the skin surface [32]. In this test, a load of 150 grams was used to measure spreadability, yielding the following results: Week 1 = 6.66 cm, Week 2 = 6.73 cm, Week 3 = 6.86 cm, Week 4 = 6.73 cm (Table 9).

This indicates that the serum has a sufficiently wide spread, thereby demonstrating the active ingredient's ability to spread and make contact with the skin. Good spreadability results in effective drug release [31]. Spreadability is inversely proportional to viscosity; the higher the viscosity of the formulation, the lower the spreadability [32]. The decrease or change in spreadability between weeks was not statistically significant, with a p-value > 0.05, so it can be concluded that the spreadability of the serum remained stable during the 4-week storage period.

*Adhesion test*

Adhesion testing over a 4-week storage period at room temperature (15–30°C) yielded the following results: week 1 = 1.75 seconds, week 2 = 1.72 seconds, week 3 = 1.60 seconds, week 4 = 1.60 seconds (Table 9). Higher adhesion strength indicates better quality for serum preparations. Good adhesion strength for serum preparations is greater than 1 second [33]. The results show good adhesion strength. The decrease in adhesion strength from week to week is not statistically significant, with a p-value > 0.05, so it can be concluded that storage for 4 weeks does not significantly affect the adhesion strength of the serum. This stable adhesion strength indicates that the serum formulation remains of high quality during the storage period.

*Viscosity test*

The viscosity test aims to determine the thickness of the serum formulation. A good serum is one that is neither too thick nor too thin [34]. The viscosity test was conducted using a Brookfield spindle 4 viscometer at a speed of 30 rpm. Test results were obtained at week 1 = 3.107 cPs, week 2 = 3.087 cPs, week 3 = 3.060 cPs, week 4 = 3.013 cPs (Table 9).

These results indicate that the formula meets the viscosity requirements, with a suitable viscosity range for gel-based serum formulations being 2,000–4,000 cPs [18]. There was a decrease in serum viscosity from week to week, but statistical analysis showed a p-value > 0.05, meaning that the differences in viscosity between weeks were not significant. The viscosity test results showed a decrease in the viscosity of the carrot extract serum preparation. This was due to the relatively acidic pH of the carrot extract, so that the addition of carrot extract to the optimal serum formula base could cause the pH of the serum base to decrease further. the decrease in pH causes the gel to form less perfectly, resulting in a decrease in serum viscosity. The decrease in serum viscosity has an impact on the increased spreadability of the serum [35].

*Skin ırritation test*

Skin irritation testing of carrot extract serum was conducted on three panelists using the closed patch test method. Positive irritation reactions were indicated by redness, itching, or swelling on the inner arm skin. After 24 hours of observation, no such symptoms were found in the panelists, resulting in a Primary Irritation Index (PII) = 0 and the formulation being categorized as safe and non-irritating. This can be seen in Figure 2.

*Stability test using the cycling test*

cycling test stability testing aims to determine whether phase separation occurs in carrot extract serum preparations at extreme temperatures. After each cycle, the preparation is examined for phase separation, and

observations are made regarding organoleptic properties and pH. From the organoleptic examination, it was found that from cycle 1 to cycle 6, the formulation remained stable without any phase separation, with no changes in physical appearance, shape, odor, color, and a homogeneous composition (Table 10). This indicates that all the ingredients used in the serum formulation are well mixed, ensuring the formulation remains stable despite extreme temperature conditions.

Based on the results of testing over 6 cycles, the pH value of the serum preparation showed a decrease from 5.43 in cycle 1 to 5.08 in cycle 6, but was still within the appropriate pH range for facial serum (Table 10). There was a significant change in pH between cycles, with a p-value < 0.05, indicating that the difference was not accidental. This decrease in pH was likely caused by the use of acidic carrot extract in the formulation, chemical reactions between formulation components, or the influence of environmental conditions such as temperature, light, and humidity. These results emphasize the importance of controlling storage factors to maintain serum pH stability [29].

**Anti-aging effectiveness testing**

The moisture content was assessed on the dorsal surfaces of the volunteers' palms utilizing an Aramo skin analyzer. Prior to the application of the serum formulation, the average moisture content on the backs of the hands across all volunteer groups was 6 and 12, categorizing them within the dehydrated skin range (0-29) [36]. Following 30 days of application, an elevation in moisture content was seen on the dorsal surfaces of the volunteers' hands, applicable to both the optimal formula and the base formula users. The treatment utilizing the formula demonstrated an enhancement in moisture content, averaging 39 and 32 on the participants' skin post-application of the serum. Observations on day 7 indicated that each formula exhibited an increase in moisture content, with the ideal formula rising by 53.85% and the base formula by 25%. All formulations demonstrated an elevation in moisture content from week 0 (the original skin condition prior to serum treatment) to week 4, with the optimal formulation increasing by 84.62% and the base formulation by 62.50% (Figure 3).

Based on the results of statistical analysis using One-Way ANOVA, there was no significant difference in water content among the formulas. In the normality and homogeneity tests, the water content data showed a significance level >0.05, indicating that the data were normally distributed and the variance of the data from all treatment groups was the same or homogeneous. Based on the One-Way ANOVA test, the significance value was >0.05. If the significance value is >0.05, the interpretation is that there is no significant difference in the water content of the carrot extract serum formulation between the base and the optimal formulation.

UV rays are the biggest contributor to the formation of wrinkles. The appearance of wrinkles occurs due to a decrease in skin elasticity caused by a reduction in skin moisture content and thickening of the stratum corneum. For its physiological functions, the skin requires fat and water, both of which are closely related. The lipid layer on the skin's surface and the hygroscopic substances in the stratum corneum have the function of absorbing water and are in a functional relationship, known as the Natural Moisturizing Factor. The stratum corneum's ability to bind water is crucial for the skin's flexibility and elasticity. A thick skin layer allows the skin to retain moisture and elasticity, making it appear supple and preventing premature aging of the skin [37]. Serum formulations can enhance elasticity due to their antioxidant activity. Reactive free radicals can be inhibited by antioxidant compounds, thereby increasing collagen and elastin fiber production. Collagen and elastin fibers in the dermal layer of the skin help maintain elasticity. Skin elasticity is a key factor in preventing sagging skin and restoring skin density due to photoaging [38].

The serum base can increase moisture due to the presence of propylene glycol as a humectant, which attracts water to the skin and plays a major role in preventing dry skin, thereby making the activity of carrot extract in increasing water content in the skin more effective and helping to maintain skin moisture [39].

The size of pores on the skin is closely related to the smoothness of the skin and the quality of the skin. The smaller the size of the pores on the skin, the smoother the skin is, and vice versa, the larger the size of the pores, the rougher the skin is. One sign of premature aging is enlarged pores. If the skin is frequently exposed to sunlight, it can cause pores to enlarge due to the accumulation of dead skin cells [40]. In addition to age-related factors that cause pores to enlarge due to reduced skin elasticity, exposure to sunlight also plays a role.

The average pore size on the back of the volunteers' hands before serum application was 25,5 and 9, which falls into the moderate (20–39) and small (0–19) categories according to [36]. After 30 days of serum use, there was a reduction in pore size, indicating that the formulation can reduce skin pores. The average pore size

became 9 and 6 after use, indicating an improvement in skin condition. The optimal formula yielded better results than the base, with a pore recovery percentage on day 7 of 25.49% for the optimal formula and 5.56% for the base. By week 4, pore size recovery improved significantly to 64.71% for the optimal formula and 33.33% for the base (Figure 4), indicating the effectiveness of the optimal formula in improving skin pore condition.

Based on statistical analysis using One-Way ANOVA, there were differences in pore size recovery among the formulas. In the normality test of pore size recovery values, the significance was >0.05. Based on the homogeneity test and One-Way ANOVA, the significance value was <0.05. If the significance value is <0.05, the interpretation is that there is a significant difference in pore size between the base and the optimal formula of the carrot extract serum formulation.

The measurement data showed that the average number of spots on the back of the hands of all volunteer groups before using each serum preparation was 21.5 and 26.5. These figures fall within the range of moderate spots on the skin, which is 20-39 [36]. After 30 days of use, the number of spots on the backs of the hands of volunteers using the optimal formula and the base formula decreased to an average of 8 and 13, respectively, after using the serum. Based on observations on day 7, each serum formula showed recovery, with the optimal formula at 27.91% and the base formula at 18.87%. After 30 days of serum use, all formulas showed recovery, with the optimal formula at 62.79% and the base formula at 50.94% (Figure 5).

Based on statistical analysis using One-Way ANOVA, there were differences in spot reduction among the formulas. In the normality and homogeneity tests of water content data, the significance was >0.05, meaning the data were normally distributed and the variance of data from all treatment groups was the same or homogeneous. Based on the One-Way ANOVA test, the significance value was <0.05. If the significance value is <0.05, the interpretation is that there is a significant difference in the stain reduction values of the serum extract formulations.

Dark spots (hyperpigmentation) can appear on aging skin or even on younger skin due to various causes. The most common cause of dark spots on the skin is excessive exposure to sunlight. The more sunlight the skin is exposed to, the more active melanin production becomes [41]. UV exposure stimulates tyrosinase enzyme activity and increases the number of melanocytes that produce melanin. As a result, the transfer of melanosomes from melanocytes to keratinocytes increases, as does melanin production. Excessive melanin production and abnormal accumulation of melanin in certain areas of the skin due to excessive UV radiation exposure can cause hyperpigmentation spots or make some areas of the skin darker than others [42].

The results of wrinkle measurements on the backs of the volunteers' hands showed that the average wrinkle level on the backs of the hands of all volunteer groups before using the serum preparation was 20 and 20.5, which falls within the range of wrinkled skin (51–52) [36]. After 30 days of use, the wrinkle levels on the backs of the hands of volunteers, whether using the optimal formula or the base formula, showed an average reduction of 6.5 and 10, respectively. Based on observations on day 7, each serum formula showed recovery, with the optimal formula at 22.50% and the base formula at 19.51%. After 30 days of serum application, all formulas showed recovery, with the optimal formula at 67.50% and the base formula at 51.22% (Figure 6).

Based on the results of statistical analysis using One-Way ANOVA, there was no significant difference in wrinkle reduction among the formulas. In the normality and homogeneity tests, the wrinkle reduction values showed significance >0.05, indicating that the data were normally distributed and the variance of the data from all treatment groups was the same or homogeneous. Based on the One-Way ANOVA test, the significance value was >0.05. If the significance value is >0.05, the interpretation is that there is no significant difference in the wrinkle reduction values of the carrot extract serum formulation between the base and the optimal formulation.

The dermis layer is the layer of skin that plays a role in the elasticity and smoothness of the skin. When collagen production decreases in the dermis layer of the skin (due to aging and environmental factors), the skin will appear dry and lose its elasticity. As a result, the skin will appear wrinkled and saggy [43]. The aging process is a natural or internal process that leads to a decline or degeneration, causing the body to lose its ability and functionality, which includes the appearance of wrinkles and fine lines on the face or other parts of the body. Skin aging is a natural or internal aging process that is further stimulated by changes in skin elasticity [44]. Carrot extract is a major source of antioxidant compounds, particularly β-carotene, which acts as a photoprotective agent through specific mechanisms. The structure of β-carotene, consisting of a long chain

of conjugated double bonds, enables efficient absorption and quenching of ultraviolet radiation as well as neutralization of free radicals. This mechanism protects skin cells from photo-oxidative damage, a primary factor contributing to premature aging. Thus, the distinctive chemical structure of β-carotene directly underlies the anti-aging activity of carrot extract. Flavonoids, as antioxidants, function as a source of hydrogen that binds with free radicals. To address the dangers posed by free radicals, the body develops protective mechanisms to prevent the formation of free radicals and lipid peroxidation, as well as to repair damage, including that to the skin [45].

The overall results of the study indicate that the optimal formula exhibits anti-aging activity on the skin condition of the backs of volunteers' hands. The condition of the volunteers' hands showed an improvement in skin condition over the course of one month of using the carrot extract serum. This is evident from the average percentage increase in moisture content (84.62%), reduction in pore size (64.71%), reduction in spots (62.79%), and reduction in wrinkles (67.50%).

## ▪ CONCLUSION

Based on the research results, the ratio of carbopol 940 at a concentration of 0.837% and triethanolamine at a concentration of 0.855% was found to be the optimal serum formula obtained using the Box-Behnken design method. The combination of these two ingredients affects the pH value, adhesion, and spreadability of the carrot extract serum formulation produced. The optimal formula for the carrot extract serum formulation meets the requirements for formulation evaluation, including organoleptic properties, homogeneity, stability (cycling test), pH, viscosity, spreadability, adhesion, and skin irritation. The optimal formula of the carrot extract serum formulation exhibits better anti-aging activity compared to the base, with an average percentage increase in moisture content (84,62%), reduction in pore size (64.71%), reduction in spots (62.79%), and reduction in wrinkles (67.50%).

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