Research Article
Open Access

Journal of Life Science and Applied Research (JLSAR)
E-ISSN: 2959-8036

EFFECTS OF STORAGE TEMPERATURE AND PH ON THE PHENOLIC CONTENT, ANTIOXIDANT ACTIVITY, TURBIDITY AND COLOUR OF CHAMOMILE ENRICHED BEVERAGES

R. D. Mustafa [1]* and N. Harbourne [2]

[1] Department of Food Science and Quality Control, College of Agricultural Engineering Sciences, University of Sulemaniyah, Sulaimani, Iraq.
[2] School of Agriculture and Food Science, University College Dublin, Ireland.
*** Corresponding author:** Roza D. Mustafa, Department of Food Science and Quality Control, College of Agricultural Engineering Sciences, University of Sulemaniyah, Sulaimani, Iraq.
Email: roza.mustafa@univsul.edu.iq

Article Info:

Received: Feb. 19, 2024
Revised: Mar. 21, 2024
Accepted: Apr. 29, 2024
Published: Jun. 30, 2024

DOI:
https://doi.org/10.59807/jlsar.v5i1.94

How to Cite:
R. D. . Mustafa and N. . Harbourne, "EFFECTS OF STORAGE TEMPERATURE AND PH ON THE PHENOLIC CONTENT, ANTIOXIDANT ACTIVITY, TURBIDITY AND COLOUR OF CHAMOMILE ENRICHED BEVERAGES", JLSAR, vol. 5, no. 1, pp. 28–40, Jun. 2024.

Available From:
https://www.jlsar.com/index.php/journal/article/view/94

Abstract: In recent years, polyphenols have gained attention for their health-promoting properties in foods and beverages. Chamomile, a medicinal plant rich in polyphenols, is an ideal ingredient for functional foods. However, the stability of its bioactive ingredients during storage must be assessed. This study examines the effects of acidified aqueous chamomile extracts (pH 3 and pH 6) and storage temperatures (5°C and 20°C) on total phenolic content, antioxidant capacity, turbidity, and color over 6 weeks. Results indicate that phenolic content and antioxidant capacity in pH 3 extracts were stable at both temperatures, while pH 6 extracts showed significant changes at room temperature. Turbidity was higher in pH 3 extracts but remained acceptable. Storage temperature significantly affected the color, with extracts at room temperature changing from green-yellow to yellow, and slightly darker at pH 6. Therefore, acidifying extracts to pH 3 and storing them at 5°C is ideal for retaining phenolic content and color.

Keywords: Chamomile infusions, Phenols, Antioxidant, Stability, Beverages.

1. Introduction

Recently many studies have focused on the biologically active ingredients especially polyphenols in food and beverages because of their benefit for human health comprising antioxidant and anti- inflammatory activities. According to several researchers a high level of bioactive ingredients, including phenolic compounds, are commonly found in medicinal herbs [1], [2], [3]. Furthermore, as reported by World Health Organisation, more than 80% of the population of the world mostly depends on traditional medicinal plants for health [4]. Thus, these compounds would be ideal ingredients for functional beverages. Moreover, inclusion of bioactive ingredients in functional foods especially functional beverages is developing quickly in the market field [5], [6] and traditional products, including organic plant compounds are increasingly of interest to consumers [7].

Chamomile (Matricaria chamomilla L.) is an herbal flowering plant annually indigenous to Europe and is a daisy family (Asteraceae or Compositae) member. It has been in use traditionally as a medicinal plant mostly

due to its anti-inflammatory, antispasmodic, sedative and anti-microbial properties [8], [9], [10], [11]. [12] also suggested that chamomile has uses for many different healing purposes such as wounds treatment, ulcers, eczema, gout, skin irritations, bruises, burns, canker sores, neuralgia, sciatica, rheumatic pain, hemorrhoids, mastitis and other diseases. These properties are mainly because phenolic compounds are found in chamomile in accordance to [13], [14], [15] stated that there are significant variations in total phenolic content of herbal extracts. The range of results was between not detected to 46.46 mg/g of herbs and the highest contents of phenolic compounds (> 30 mg/g of herb) was among the processed tea, lemon balm, mate, green tea, black tea and peppermint which order from high to low. These results were in agreement with [2] who found the similar total phenol content in the same herbal extracts (green tea > black tea> peppermint > chamomile > fennel).

[15] mentioned that it is important to take into account that many factors may have an impact on the total content of phenolic components present in different herbal extracts, such as the preparation approach (concentration, processing of plant, temperature and time of extract), the characteristics of the cultivation (soil, climate, stresses) and the analysis method. Chamomile flowers are one of the oldest and most widely used in herbal tea and beverages. Therefore, it can be used in the beverages as a source of bioactive ingredients to improve nutritional value.

Meantime, before inclusion to the food products as functional juice, determination of the stability of the bioactive ingredients and other characteristics such as colour of medicinal plant extracts during all processing steps required to their incorporation into beverages and foods and possible storage conditions is extremely important. This is because they may have an effect on the potency and acceptance of the food product by the consumer. There are many factors affect the stability of bioactive ingredients and other attributes in the medicinal plant extracts such as the plant material preservation after harvesting it (drying, freezing, etc.), the method of preparing the extraction of plants, heat treatment of the food and beverages including these plants extracts and storage condition of the foods and beverages [16].

Previous researchers have studied the stability of bioactive ingredients and colour of the acidified feverfew infusions ($2.9 \leq pH \leq 6$) during storage at 5°C and 22°C and have found that parthenolide had more stability at infusions at pH6 stored at 22°C. However, there was a significant reduction in phenol content and progressive browning at this condition of storage. Acidified infusions ($pH \leq 4.6$) retained colour, total phenolic content and parthenolide during at 5°C and 22°C [17]. Therefore, storage condition can significantly affect phenolic content so it is important to study it.

Recent studies have shown that total phenolic content, formation of turbidity and colour of aqueous chamomile extracts affected significantly by extraction temperature and type of drying fresh chamomile flower. The amount of total phenol increased with increasing the temperature of extraction from 57°C to 100 °C. Colour and turbidity level also develop with increasing temperature from 57°C to 90°C but extracts became slightly darker and forming high level of turbidity at 100°C. Researchers have found that increasing in drying temperature of fresh chamomile flower from 40°C to 80°C led to a considerable decrease in amount of total phenolic compound and extracts prepared from chamomile flowers oven-dried at 80°C were the darkest colour [16]. However, there has been no data published on the stability of these bioactive ingredients in the aqueous chamomile extracts incorporated into acidic beverages during storage. Before incorporation into functional foods., it is important to have knowledge on factors affecting the phenolic content and antioxidant capacity of chamomile extracts during storage such as temperature and pH of beverages other characteristics have impact on the quality of extracts are colour and turbidity formation during storage.

The main objectives of this study were to assess the impact of pH on the colour change, degradation of total phenolic content and antioxidant capacity and formation of turbidity of acidified chamomile infusions during storage time at both 5 and 20 °C temperatures. The purpose of this study to determine ideal storage conditions

to maintain the total phenolic contents and colour of chamomile infusions and remain their effectiveness when the extracts incorporate into acidic beverages.

2. Materials and Methods

Materials: Dried chamomile flowers were purchased from the All in All Ingredients, Dublin, UK. Folin-Ciocalteu phenol reagent and Gallic acid were purchased from Sigma-Aldrich, UK. Sodium Carbonate anhydrous was purchased from Fisher Scientific, Leicestershire, UK. Citric acid, tri-Sodium citrate, Acetic acid ($C_2H_4O_2$), Sodium Acetate Trihydrate ($C_2H_4NaO_2.H_2O$), TPTZ (2,4,6-tripyridyl-s-triazine),hydrochloric acid (HCl) , Ferric Chloride Hexahydrate ($FeCl_3.6H_2O$), Ascorbic Acid, potassium sorbate, and Benzoic acid were purchased from BDH Laboratory Supplies, England.

Extraction: 2.5 g of chamomile flowers were immersed in 100 ml of distilled water in a Duran bottle and were put in water bath (Nickel Electro, Boro Labs Limited, Berks, UK) for 20min at 90 °C. Whatman no. 1 filter paper (Whatman Ltd, England) was used to filter the chamomile extract under vacuum after heating. Finally, the extract was put on ice to cool quickly as described by [16]. Extraction was done in triplicate.

Beverage preparation: Different concentrations of citric acid and tri-sodium citrate were used to adjust the pH of the extracts to obtain a pH of 3 and 6 and a final concentration of 0.06 M to be the similar to the citric content in fruit beverages. The pH of the beverages was determined with a pH meter (Orion star A111). 250 ppm of benzoic acid and 300 ppm of potassium sorbate was added as preservatives to reduce microbial deterioration during storage [17].

Experimental design: There were two treatments were applied to beverage pH (3 and 6) which were storage in the fridge (5°C) and at room temperature (20°C) for 6 weeks.

Total phenols: The total phenolic content of the extracts was determined by the Folin–Ciocalteu method according to [18]. The standard curve has the equation y = 0.0021x + 0.0639, R^2 = 0.9954, which y is absorbance at 760 nm and x is concentration of gallic acid (mg/L). All analysis was done in triplicate. 0.2 ml of extract, standard or blank (water) was added to 6.0 ml distilled water to a 10ml volumetric flask. 0.5 ml Folin-Ciocalteu reagent was added to the volumetric flask. After 3 minutes, 1.5 ml of the 20% Sodium Carbonate solution was added. Volumetric flask was filled to 10ml with water and vortexed (Whirilimixer) to mix. The mixture was left to stand for 2 hours at room temperature. The absorbance was measured at 760 nm on UV-Vis Spectrophotometer (Cecil 1021). The total phenol content was measured as gallic acid equivalents (mg GAE)/g dry weight of plant material [19]. All analysis was done in triplicate.

Colour: The colour of chamomile extract was determined using Colour Quest spectrophotometer (Hunter lab, Virginia, U.S.A). The Hunter Lab scale was expressed in L*(lightness), a*(redness-greenness) and b*(blueness-yellowness). Before doing measurements of colour, the Hunter Quest spectrophotometer was calibrated with a black calibration plate. The colour difference is expressed in Hue angle (h°) and is defined as red–purple: 0°, yellow: 90°, bluish-green: 180°, and blue: 270°. Chroma (C) is a measure of the intensity or colour saturation. Chroma and hue angle were calculated by these equations [20].

$$\text{Hue angle (h°)} = \arctan\left(\frac{b*}{a*}\right) \quad (1)$$

$$\text{Chroma (C°)} = [(a*)^2 + (b*)^2]^{1/2} \quad (2)$$

Turbidity: Turbidity was measured using a UV–visible spectrophotometer (Cecil 1021) at 800 nm. The percent transmittance (T %) was recorded and 100-T% was used as a measure of turbidity [21], [22].

Total antioxidant capacity (FRAP test): The total antioxidant power of chamomile extracts was measured by using the ferric reducing antioxidant power (FRAP) assay. The ferric reducing-antioxidant assay (FRAP) is based on the reduction at low pH of ferric 2, 4, 6-tris (pyridin-2-yl)-1, 3, 5-triazine [Fe^{III}-TPTZ] to the ferrous complex followed by spectrophotometric analysis [23], [24]. The stock solutions were prepared which included

300 mmol acetate buffer (3.1 g Sodium Acetate Trihydrate (CH_3COONa) dissolved in 500m l of water and then 16 mL Acetic Acid (CH_3COOH) added), pH 3.6, 10 mmol TPTZ (2,4,6-tripyridyl-s-triazine) dissolved in 40 mmol HCl and kept in fridge, and 20mmol $FeCl_3.H_2O$ solution. Working FRAP reagent was prepared freshly by adding 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution as described by [25]. 10µl of standard, blank or extract was added into a 96-well microplate and 300µl of FRAP reagent was added. The absorbance was measured immediately in the GENio Pro™ microplate reader (Columbus/Columbus pro washer) at 600nm at room temperature with Magellan software. The calibration curve was prepared from the equation $y = 0.0011x - 0.0185$, $R^2 = 0.9969$ where x was absorbance and y was ascorbic acid concentration (µmol). The standard curve was linear between 0 and 1000 µmol ascorbic acid. Results were expressed in µmol Fe (II)/g of extract and compared with that of ascorbic acid. Antioxidant activity was calculated and expressed as ascorbic acid equivalents (µmol/L) according to [26].

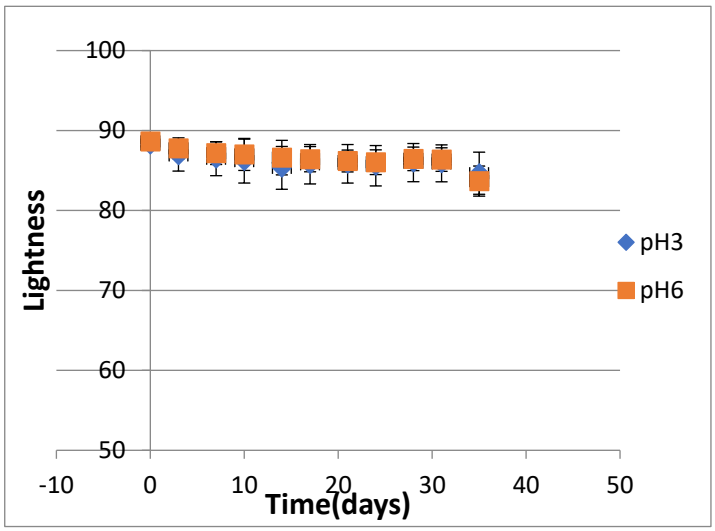
Statistical analysis: All of extractions and analysis of this experimental study was done in triplicate. Data from experiment results were expressed as mean ± standard deviation (SD). The means were compared by using the student's *t*-test (two-tailed) in Microsoft Excel in order to determine the significant differences between several storage parameters. Results with $p \leq 0.05$ were considered significantly different.

3. Results and Discussion:

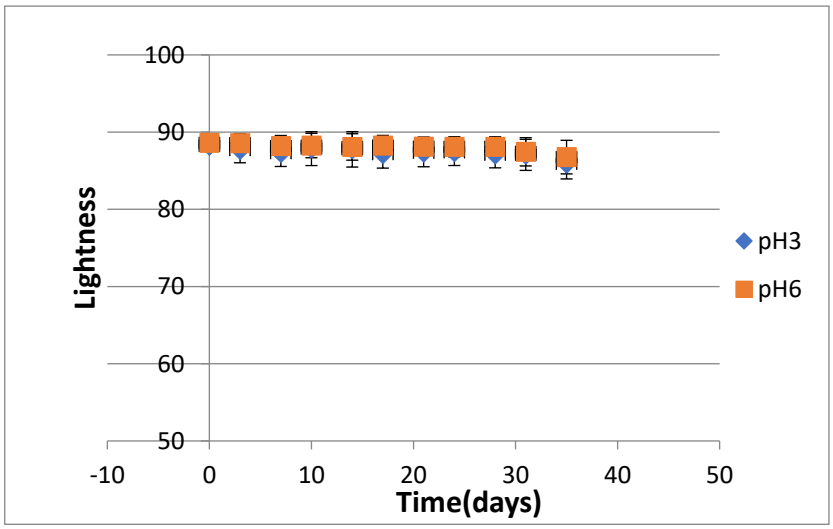
Colour of chamomile extracts and the effect of storage pH and temperature: Storage condition may affect the quality features of functional beverages. Unfavourable change in colour and loss of bioactive components may be a result of bad storage conditions of beverages [16]. Therefore, in this study, the effect of storage conditions such as pH and temperature on the colour of chamomile extracts was examined. At pH 3 and 6 the colour of chamomile extracts did not change significantly during 6 weeks storage in the fridge (5°C) but colour of extracts at room temperature (20°C) did change significantly. As shown in figure 1a, after 35 days of storage at room temperature (20°C) the lightness of chamomile extracts at pH 3 Did not decrease significantly ($p>0.05$) from 86.24 ± 0.03 to 84.66 ± 2.6 and at pH 6 decreased significantly ($p<0.05$) from 88.64 ± 0.005 to 83.68 ± 1.8 and became slightly darker. The reduction of lightness at 5°C at pH 3 and 6 were not significant ($p>0.05$) (figure 1b). The temperature of storage had a significant ($p<0.05$) impact on hue angle (h) of these chamomile extracts. The reduction of hue angle of chamomile extracts at pH 3 and 6 at room temperature storage (20°C) was higher ($p<0.05$) than fridge storage (5°C). There may be correlation between total phenol in the extracts and hue angle. As [27] pointed out that a raise in total phenol of the chamomile extract at high extraction temperature follow a hue angle reduction. Initially extracts were yellow-green colour with a hue angle of 96.68 ± 0.9 and 96.58 ± 0.1 changed to yellow colour with a hue angle of 92.79 ± 2 and 91.44 ± 1.5 at pH 3 and 6, respectively and after 35 days at room temperature storage (20°C) whereas the hue angle of the extracts at pH 3 and 6 stored at fridge (5°C) only changed from 96.68 ± 1.8 and 96.52 ± 0.2 to 95.39 ± 1.8 and 94.30 ± 2 is shown in figures 1c and 1d. This change in hue angel might be due to the degradation or/and oxidation of phenol content in the extracts. The chroma (saturation) of chamomile extracts at pH6 in both storage temperatures (5 and 20°C) was little higher ($p>0.05$) than those at pH3 during the storage time. The chroma of the extracts at pH 3 and 6 which stored at room temperature (20°C) increased slightly ($p>0.05$) from 34.52 ± 0.06 and 34.40 ± 0.1 to 36.30 ± 4.9 and 37.92 ± 5.1 respectively. However, the chroma of extracts at pH 3 and 6 remained stable during storage time (35 days) at fridge (5°C) as shown in figures 1e and 1f. [17] stated that browning of feverfew infusions at pH 6 stored at 22°C is due to phenol degradation and [28] also stated that lightness, hue angle and chroma of feverfew infusions were affected by extraction temperature. Infusions were brown colour at extraction temperature 20-70°C and it changed to yellow colour at 80-100°C extraction temperature as result of activity of polyphenol oxidase. This enzyme caused oxidation of some of non-tannin phenolic content at or below 70°C and formed brown colour with low amount of total phenol content. However, inactivation polyphenol oxidase occurred at or over 70°C

and caused light colour and high total phenol amount in the infusions. Other studies found that extraction condition and post-harvest processing affected the colour also [27]. In addition, [29] have shown that drying of chamomile flower at 80-95°C result in forming undesirable colour (caramel colour). [30] found that the colour of strawberry nectars is stable for long time at a storage temperature of 4°C.

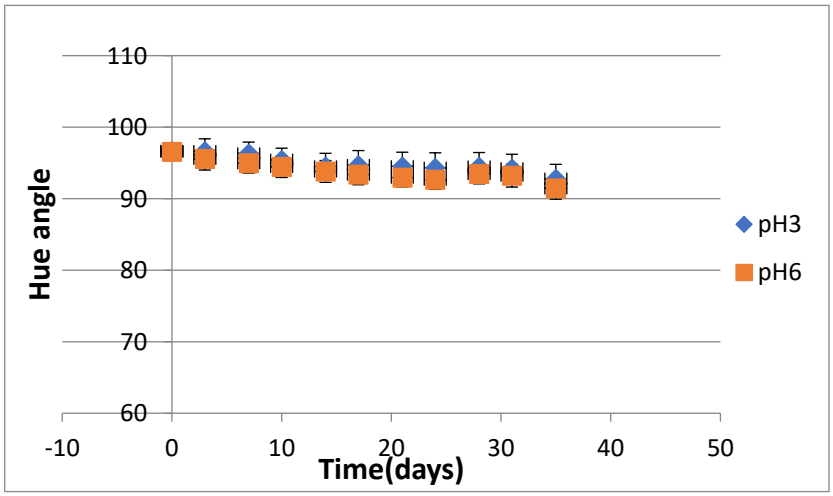
a.



b.



c.



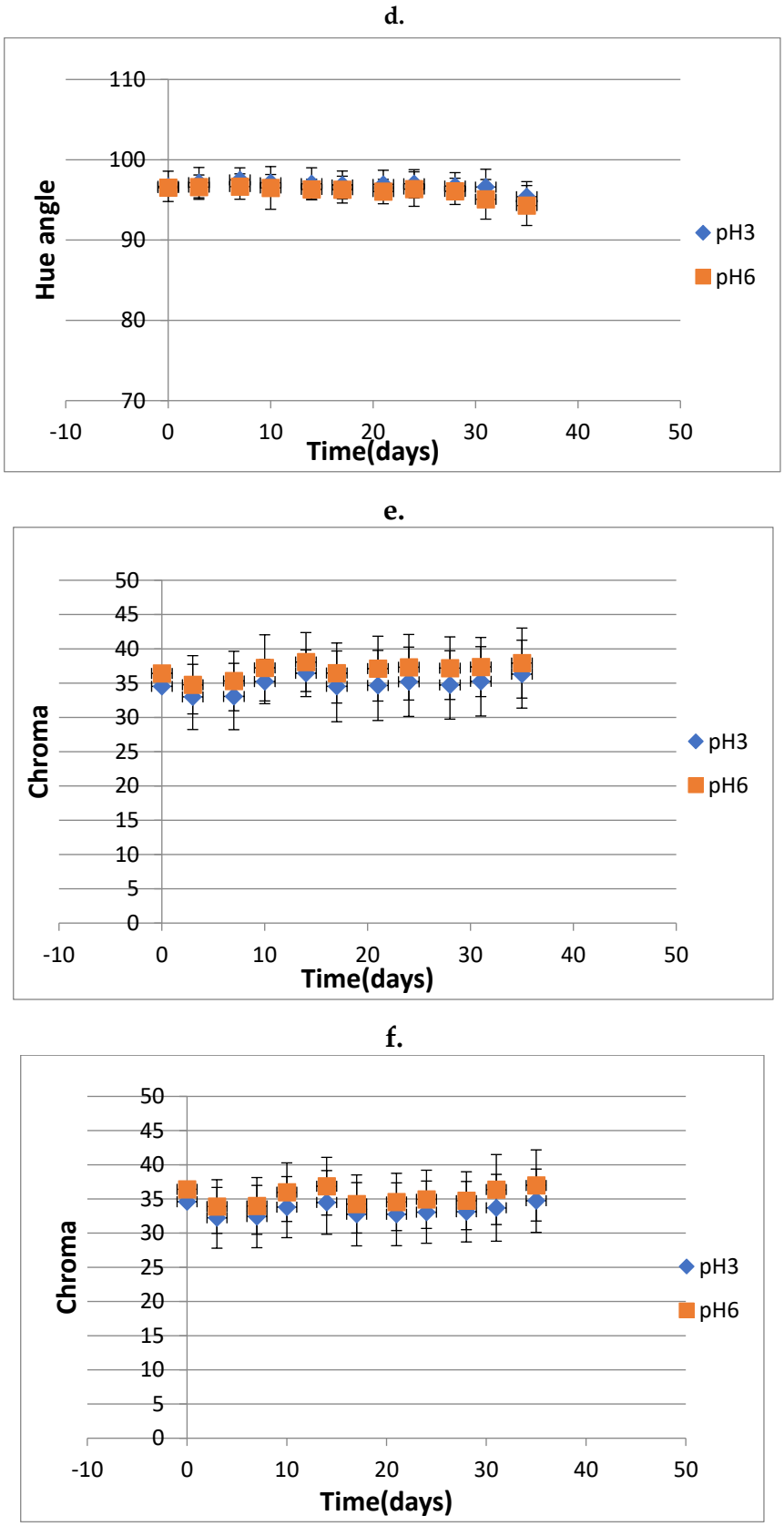


Figure 1: Colour of chamomile extracts at pH 3 and 6 during storage at fridge (5°C) and room temperature (20°C), a and b lightness, c and d hue angle, e and f chroma.

Effect of storage pH and temperature on the total phenolic content of chamomile extracts: The content of total phenols in the chamomile extract was 2.5g, whilst the amount of total phenol content of chamomile extract was 15.5 mg/ g of dry weight of chamomile extract. This result is in agreement with [16], [31], who stated that the content of total phenol in a range of chamomile herb is 12.7-24.5 mg/g of dry weight. On the other hand, [32]

stated that the content of total phenolic compounds in Chamomilla recutita was 14.2 mg/ g of dry chamomile herb. [31] also found total phenolic content in the Jordanian chamomile (Matricaria chamomilla) is more than 20 mg/g of dry weight of extracts.

In this study, the effect of pH and temperature of storage on chamomile extract were determined. The phenolic content of chamomile extracts at pH 3 and 6 stored at fridge (5°C) did not change significantly ($p>0.05$) after 35 days of storage (figure 2a). However, the phenolic content of the extract at pH 6 stored at room temperature (20°C) decreased significantly ($p<0.05$) from 357.5 ± 0.3 to 319.5 ± 2.5 mg/L GAE. There was not a significant change ($p>0.05$) in the phenolic content of the extract at pH 3 at room temperature storage (20°C) after 35 days of storage as shown in figures 2a and 2b. [17] found that reduction in total phenolic content in feverfew infusions at pH 6 was more than feverfew infusions at pH 2.9 and 4.6 stored at 22°C for 56 days and this reduction in total phenol compounds which coincided with browning of infusions was due to residual enzymatic activity. Furthermore, [28] showed that browning of feverfew infusions and lower polyphenols at extraction temperatures of 20–70°C caused by polyphenol oxidase enzyme. In plants, there are different optimum pH levels for polyphenol oxidase activity but nonetheless it is at or near neutral level [33], [34]. The chamomile was extracted at 90 C but it is possible that there was still some polyphenol oxidase activity. In addition, [35] found that lowering pH of the extraction of green tea causes an increase in flavanosls (type of polyphenol) concentration. Moreover, [36] stated that the Green tea catechins (type of natural phenol and antioxidant) is more stable at low pH solutions. Therefore, it is important for food industries filed to acidify the chamomile beverage to around pH3 and store in the fridge to retain colour and total phenolic content during storage time.

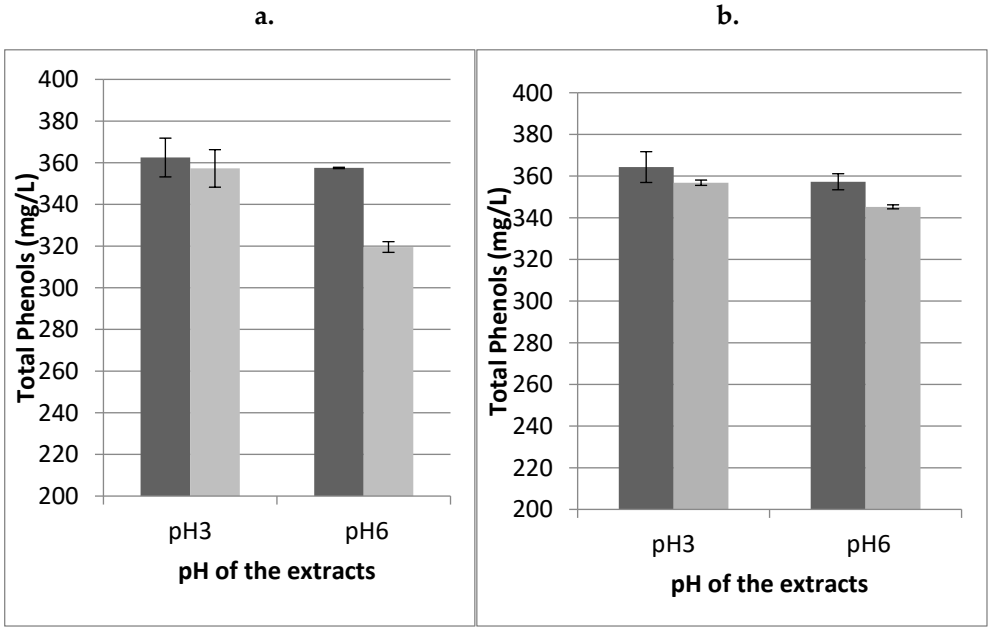


Figure 2: Total phenolic content of chamomile extracts and effect of pH levels and storage condition on it during storage time, (a) at room temperature (20°C) and (b) at fridge (5°C) at day 0 (dark bar) and day 35 (light bar).

Effect of storage pH and temperature on turbidity of chamomile extracts: Recent studies have shown that extraction time and temperature have a significant effect on formation of turbidity in chamomile extracts as the extracts have a low level of turbidity under 10% mark as a low level at 800 nm at the range of extraction temperature 57-90°C [27]. This is similar to the level found in the present study. Turbidity of chamomile extracts at pH3 were higher than chamomile extracts at pH6 ($p<0.05$) in both storage conditions room temperature (20°C) and fridge storage (5°C) all the time during storage time (35 days) as shown in figure 3. High turbidity in the

extracts at pH3 might be as a result of denaturation of protein. As supported by [37], Chamomile extracts contain protein. Other factor of forming turbidity in the extracts may be due to reaction between protein and other chemical compounds but it needs further experimental researches to be more accurate. [21] found that the level of turbidity in sugar beet protein and saponin solutions increase at pH2 as a result of the interaction between protein and saponin and forming acid beverage floc (ABF). Although there was no significant different in total phenol content between extracts at pH3 and 6 in this study, [38] reported that the presence of polyphenols and pectins mainly lead to the turbidity of a juice. In the present study, turbidity of chamomile extracts at pH 3 and 6 during storage time (35 days) at both fridge and room temperature storage was not significantly different ($p>0.05$). There may be correlation between turbidity and total antioxidant capacity of chamomile extracts. Consumers may decide on quality of beverages such as tea by the level of turbidity of the beverages. Therefore, beverages with high level of turbidity may be understood as a low quality and unfavourable beverage [39].

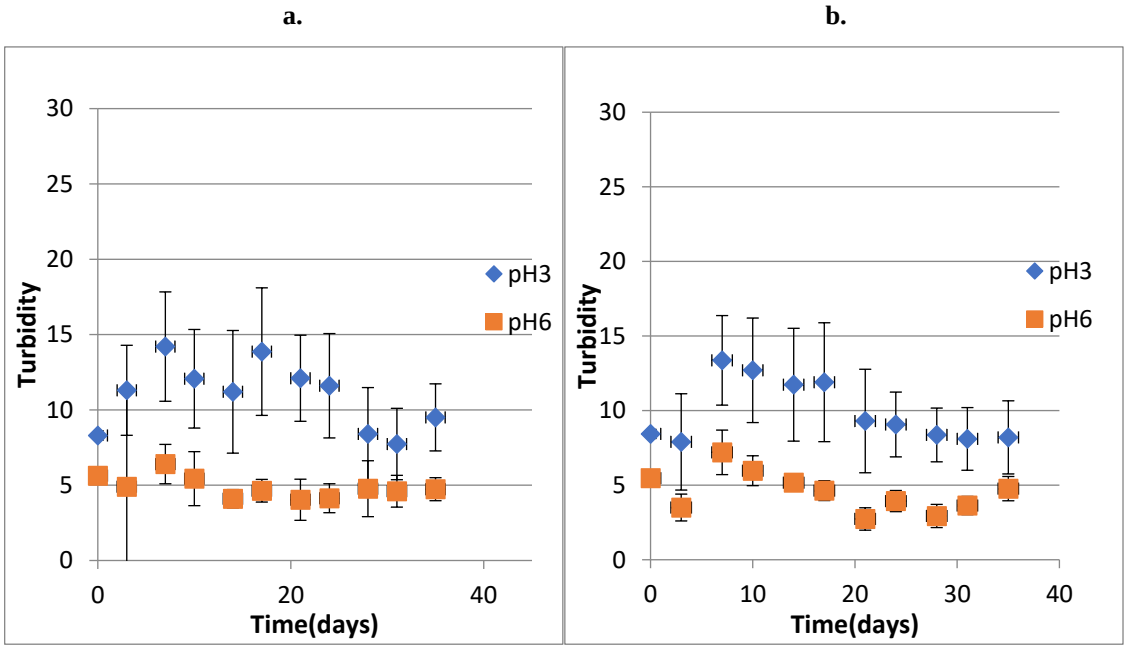


Figure 3: Turbidity of chamomile extracts and effect of pH and storage condition, (a) at room temperature (20°C) and (b) at fridge (5°C) during storage time.

Effect of pH and storage condition on total antioxidant capacity of chamomile extracts: In this study, Ferric-reducing antioxidant power (FRAP) was used to measure the antioxidant capacity of chamomile extracts at pH 3 and 6 stored at room temperature (20°C) and fridge (5°C) during 35 days. An antioxidant able to give a single electron to the colourless ferric complex (Fe^{3+}-tripyridyltriazine) would cause the reduction of this complex into the blue-coloured ferrous complex (Fe^{2+}-tripyridyltriazine) that absorbs strongly at 593 nm [25]. [40] showed that the lowest ferric reducing capacity (FRAP) among several plants extracts of industrial interest was chamomile extract (0.12 ± 0.01 mmol Fe^{+2}/g) and the highest antioxidant capacity (94.51% of DPPH inhibition) was pine (*Pinus maritima*) extract and the lowest antioxidant capacity (0.19%) was sage (*Salvia sclarea*) extract. In addition, [2] found that total antioxidant capacity determined by FRAP in chamomile was 2856 mmol/L. The FRAP values of chamomile extracts at pH6 always was lower ($p<0.05$) than chamomile at pH3 stored at room temperature (20°C) and fridge (5°C) storage during storage time (35 days) as shown in figure 4. This difference in FRAP values might be because of total phenolic content in the extracts as in this study was found that total phenol content in all samples decrease more at pH6 than 3 during storage. Therefore, total antioxidant capacity in chamomile extracts decreased more ($p<0.05$) at pH 6 than 3 after storage time at room temperature (20°C) and fridge (5°C) storage. [32] found that there is correlation between antioxidant capacity and total phenolic compounds in several herbal extracts. The relationship between total phenol compound and antioxidant

capacity is proportional. [40] have shown that there are a considerable relationship between total phenolic content and antioxidant capacity and pointing that the main contributor to the antioxidant properties of 30 plants of industrial interest including *Matricaria recutita* (chamomile) are phenolic compounds. [40] also found that the ferric reducing power, measured by the FRAP assay, correlated strongly with total phenolic content. This is in agreement with report of [41] that ferric reducing power can be related to total phenolic content. [42] concluded that the exhibition of strong antioxidant activity in aqueous leaves extract of L. leonurus may result of the high content of phenolics, flavonoid and proanthocyanidins antioxidant compounds.

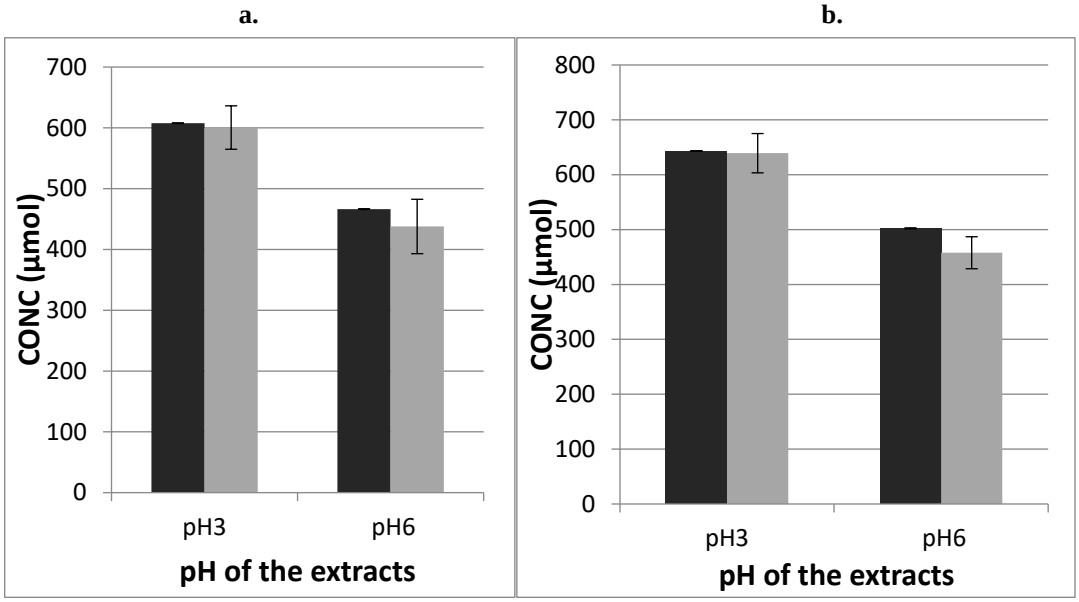


Figure 4: Total antioxidant capacity of chamomile extracts and effect of pH level and storage condition, (a) at room temperature (20°C) and (b) at fridge (5°C) during storage time at day 0 (dark bar) and day 35 (light bar).

4. Conclusion

The effect of two different pH levels (pH3 and pH6) and storage temperatures (room temperature (20°C) and fridge (5°C)) on the colour, total phenolic content, antioxidant capacity and turbidity of chamomile extracts was studied. Acidification of aqueous chamomile extracts to pH3 was resulted in total phenolic content and antioxidant capacity remained stable during fridge and room temperature but antioxidant capacity and total phenolic content at pH6 significantly decreased during storing at room temperature. The turbidity of chamomile extracts at pH3 was higher than turbidity at pH6 all the time during storage. However, the level of turbidity of chamomile extracts at pH3 was not as high as the level indicated low level (10%) of turbidity. Temperature of storage did not affect the level of turbidity. There was a significant different ($p<0.05$) between colour of chamomile extracts stored at room temperature and extracts stored at fridge as the colour of extracts at room storage changed from green-yellow to yellow but the darkness of extracts was higher in pH6 rather than pH3 at the end of storage. Therefore, according to these results of this study, acidifying aqueous chamomile extracts to pH3 and store at fridge is might be an optimal storage conditions for chamomile extracts to maintain the total phenolic content and capacity of antioxidant, acceptable turbidity level and desirable colour and chamomile extracts can be incorporate into model beverages as a functional food.

Declaration of Competing Interests:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

R. D. Mustafa ; methodology, writing—original draft preparation, N. Harbourne; writing—review and editing, N. Harbourne; paraphrasing. All authors have read and agreed to the published version of the manuscript.

Funding:

This research received no external funding.

Institutional Review Board Statement:

The study was conducted in accordance with the protocol authorized by the Department of Food Science and Quality Control, College of Agricultural Engineering Sciences, University of Sulemaniyah, Sulaimani, Iraq.

Informed Consent Statement:

No Informed Consent Statement.

Data Availability Statement:

No Data Availability Statement.

Conflicts of Interest:

The authors declare no conflict of interest.

Acknowledgments:

The authors are grateful to the staff of the Department of Food Science and Quality Control, College of Agricultural Engineering Sciences, University of Sulemaniyah, Sulaimani, Iraq.

Disclaimer/Journal's Note:

The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of JLSAR and/or the editor(s). JLSAR and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

5. References

[1] Y. Cai, Q. Luo, M. Sun, and H. Corke, "Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer," *Life Sci*, vol. 74, no. 17, 2004, doi: 10.1016/j.lfs.2003.09.047.

[2] V. Katalinic, M. Milos, T. Kulisic, and M. Jukic, "Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols," *Food Chem*, vol. 94, no. 4, 2006, doi: 10.1016/j.foodchem.2004.12.004.

[3] M. S. Butt, A. Nazir, M. T. Sultan, and K. Schroën, "Morus alba L. nature's functional tonic," 2008. doi: 10.1016/j.tifs.2008.06.002.

[4] World Health Organization, "WHO Traditional Medicine Strategy 2002–2005. World Health Organization, Geneva.," 2005.

[5] T. Bech-Larsen and J. Scholderer, "Functional foods in Europe: consumer research, market experiences and regulatory aspects," 2007. doi: 10.1016/j.tifs.2006.12.006.

[6] I. Dini, "An Overview of Functional Beverages," *Functional and Medicinal Beverages: Volume 11: The Science of Beverages*, pp. 1–40, Jan. 2019, doi: 10.1016/B978-0-12-816397-9.00001-7.

[7] J. Gruenwald, "Novel botanical ingredients for beverages," *Clin Dermatol*, vol. 27, no. 2, 2009, doi: 10.1016/j.clindermatol.2008.11.003.

[8] M. Rotblatt, "Herbal Medicine: Expanded Commission E Monographs," *Ann Intern Med*, vol. 133, no. 6, 2000, doi: 10.7326/0003-4819-133-6-200009190-00031.

[9] K. Bone and S. Mills, *Principles and practice of phytotherapy: Modern herbal medicine*. 2012. doi: 10.1016/C2009-0-48725-7.

[10] D. L. McKay and J. B. Blumberg, "A review of the bioactivity and potential health benefits of peppermint tea (Mentha piperita L.)," 2006. doi: 10.1002/ptr.1936.

[11] P. Gardiner, "Complementary, holistic, and integrative medicine: Chamomile," 2007. doi: 10.1542/pir.28-4-e16.

[12] J. A. Astin, K. R. Pelletier, A. Marie, and W. L. Haskell, "Complementary and alternative medicine use among elderly persons: One- year analysis of a blue shield Medicare supplement," *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, vol. 55, no. 1, 2000, doi: 10.1093/gerona/55.1.M4.

[13] M. F. Ramadan, L. W. Kroh, and J. T. Mörsel, "Radical Scavenging Activity of Black Cumin (Nigella sativa L.), Coriander (Coriandrum sativum L.), and Niger (Guizotia abyssinica Cass.) Crude Seed Oils and Oil Fractions," *J Agric Food Chem*, vol. 51, no. 24, 2003, doi: 10.1021/jf0346713.

[14] O. Maschi, E. Dal Cero, G. V. Galli, D. Caruso, E. Bosisio, and M. Dell'Agli, "Inhibition of human cAMP-phosphodiesterase as a mechanism of the spasmolytic effect of Matricaria recutita L.," *J Agric Food Chem*, vol. 56, no. 13, 2008, doi: 10.1021/jf800051n.

[15] R. A. Moraes-de-Souza, T. L. C. Oldoni, M. A. B. Regitano-D'Arce, and S. M. Alencar, "Antioxidant activity and phenolic composition of herbal infusions consumed in Brazil," *Ciencia y Tecnologia Alimentaria*, vol. 6, no. 1, 2008, doi: 10.1080/11358120809487626.

[16] N. Harbourne, E. Marete, J. C. Jacquier, and D. O'Riordan, "Stability of phytochemicals as sources of anti-inflammatory nutraceuticals in beverages - A review," Mar. 2013. doi: 10.1016/j.foodres.2011.03.009.

[17] E. N. Marete, J. C. Jacquier, and D. O'Riordan, "Feverfew as a source of bioactives for functional foods: Storage stability in model beverages," *J Funct Foods*, vol. 3, no. 1, 2011, doi: 10.1016/j.jff.2011.01.004.

[18] E. Joubert, M. Viljoen, D. De Beer, and M. Manley, "Effect of heat on aspalathin, iso-orientin, and orientin contents and color of fermented rooibos (Aspalathus linearis) iced tea," *J Agric Food Chem*, vol. 57, no. 10, 2009, doi: 10.1021/jf9005033.

[19] V. L. Singleton and J. A. Rossi, "Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents," *Am J Enol Vitic*, vol. 16, no. 3, 1965, doi: 10.5344/ajev.1965.16.3.144.

[20] R. G. McGuire, "Reporting of Objective Color Measurements," *HortScience*, vol. 27, no. 12, 2019, doi: 10.21273/hortsci.27.12.1254.

[21] P. A. J. Morton and B. S. Murray, "Acid beverage floc: Protein-saponin interactions and an unstable emulsion model," in *Colloids and Surfaces B: Biointerfaces*, 2001. doi: 10.1016/S0927-7765(01)00188-6.

[22] C. S. Heong, Kaur, Bhupinder, N. Huda, A. A. Karim, and A. Fazilah, "Effect of fermentation on the composition of Centella asiatica teas," *Am J Food Technol*, vol. 6, no. 7, 2011, doi: 10.3923/ajft.2011.581.593.

[23] P. M. Kuś, I. Jerković, C. I. G. Tuberoso, Z. Marijanović, and F. Congiu, "Cornflower (centaurea cyanus l.) honey quality parameters: Chromatographic fingerprints, chemical biomarkers, antioxidant capacity and others," *Food Chem*, vol. 142, 2014, doi: 10.1016/j.foodchem.2013.07.050.

[24] C. I. G. Tuberoso, E. Bifulco, I. Jerkovic, P. Caboni, P. Cabras, and I. Floris, "Methyl syringate: A chemical marker of asphodel (asphodelus microcarpus salzm. et viv.) monofloral honey," *J Agric Food Chem*, vol. 57, no. 9, 2009, doi: 10.1021/jf803991j.

[25] A. B. Tayade, P. Dhar, M. Sharma, R. S. Chauhan, O. P. Chaurasia, and R. B. Srivastava, "Antioxidant capacities, phenolic Contents, and GC/MS analysis of rhodiola imbricata Edgew. Root extracts from Trans-Himalaya," *J Food Sci*, vol. 78, no. 3, 2013, doi: 10.1111/1750-3841.12054.

[26] V. T. Aparadh Shri Pancham Khemaraj Mahavidyalaya and B. A. Kore, "EFFECT OF POWDERY MILDEW INFECTION ON DPPH RADICAL SCAVENGING ACTIVITY AND FERRIC-REDUCING ANTIOXIDANT POWER OF PLANTS," 2013. [Online]. Available: https://www.researchgate.net/publication/236888046

[27] N. Harbourne, J. C. Jacquier, and D. O'Riordan, "Optimisation of the extraction and processing conditions of chamomile (Matricaria chamomilla L.) for incorporation into a beverage," *Food Chem*, vol. 115, no. 1, 2009, doi: 10.1016/j.foodchem.2008.11.044.

[28] E. N. Marete, J. C. Jacquier, and D. O'Riordan, "Effects of extraction temperature on the phenolic and parthenolide contents, and colour of aqueous feverfew (Tanacetum parthenium) extracts," *Food Chem*, vol. 117, no. 2, 2009, doi: 10.1016/j.foodchem.2009.03.103.

[29] A. V. Borsato, L. Doni-Filho, and D. C. Ahrens, "Secagem da camomila [Chamomilla recutita (L.) Raeuchert] com cinco vazões específicas do ar," *Revista Brasileira de Plantas Medicinais*, vol. 7, no. 3, 2005.

[30] M. Gössinger *et al.*, "Effects of processing parameters on colour stability of strawberry nectar from puree," *J Food Eng*, vol. 90, no. 2, 2009, doi: 10.1016/j.jfoodeng.2008.06.018.

[31] M. P. Kähkönen *et al.*, "Antioxidant activity of plant extracts containing phenolic compounds," *J Agric Food Chem*, vol. 47, no. 10, 1999, doi: 10.1021/jf990146l.

[32] P. Trouillas *et al.*, "Antioxidant, anti-inflammatory and antiproliferative properties of sixteen water plant extracts used in the Limousin countryside as herbal teas," *Food Chem*, vol. 80, no. 3, pp. 399–407, Mar. 2003, doi: 10.1016/S0308-8146(02)00282-0.

[33] G. Gundo[notdef]ggmaz, S. Do[notdef]ggan, and O. Arslan, "Some Kinetic Properties of Polyphenol Oxidase Obtained from Various Salvia Species (Salvia Viridis L., Salvia Virgata Jacq. and Salvia Tomentosa Miller)," *Food Science and Technology International*, vol. 9, no. 4, pp. 309–315, Aug. 2003, doi: 10.1177/108201303036476.

[34] R. Yoruk and M. R. Marshall, "Physicochemical properties and function of plant polyphenol oxidase: A review," 2003. doi: 10.1111/j.1745-4514.2003.tb00289.x.

[35] B. F. Zimmermann and M. Gleichenhagen, "The effect of ascorbic acid, citric acid and low pH on the extraction of green tea: How to get most out of it," *Food Chem*, vol. 124, no. 4, 2011, doi: 10.1016/j.foodchem.2010.08.009.

[36] Q. Y. Zhu, A. Zhang, D. Tsang, Y. Huang, and Z. Y. Chen, "Stability of Green Tea Catechins," *J Agric Food Chem*, vol. 45, no. 12, 1997, doi: 10.1021/jf9706080.

[37] O. Singh, Z. Khanam, N. Misra, and M. K. Srivastava, "Chamomile (Matricaria chamomilla L.): An overview," 2011. doi: 10.4103/0973-7847.79103.

[38] M. Rinaldi, A. Caligiani, R. Borgese, G. Palla, D. Barbanti, and R. Massini, "The effect of fruit processing and enzymatic treatments on pomegranate juice composition, antioxidant activity and polyphenols content," *LWT*, vol. 53, no. 1, 2013, doi: 10.1016/j.lwt.2013.02.015.

[39] J. B. Hutchings, *Food Colour and Appearance*. 1999. doi: 10.1007/978-1-4615-2373-4.

[40] S. Dudonné, X. Vitrac, P. Coutiére, M. Woillez, and J. M. Mérillon, "Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays," *J Agric Food Chem*, vol. 57, no. 5, 2009, doi: 10.1021/jf803011r.

[41] C. C. Wong, H. Bin Li, K. W. Cheng, and F. Chen, "A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay," *Food Chem*, vol. 97, no. 4, 2006, doi: 10.1016/j.foodchem.2005.05.049.

[42] S. O. Oyedemi and A. J. Afolayan, "In vitro and in vivo antioxidant activity of aqueous leaves extract of leonotis leonurus (L.) R. Br," *International Journal of Pharmacology*, vol. 7, no. 2, 2011, doi: 10.3923/ijp.2011.248.256.