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Research Article

In vitro antioxidant potential and sensory characteristics of a common Cameroonian beverage made from a mixture of *Citrus medica*, ginger (*Zingiber officinale*) and honey

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Abstract

Ginger (0-100% w/v), honey (0-100% w/v), and citrus medica (0-100% w/v) were examined for their effects on the beverage's sensory qualities and in vitro antioxidant activities. From the data, models were created to forecast the response variables from the components assessed. Sensory analysis, total phenolic content, and antioxidant activity were all determined. The overall amount of phenolics in the beverage was raised by the three-ingredient mixture. The antioxidant activity of the drinks was boosted by combining the three components. Citrus, ginger, and honey have significant contributions to the response, according to the polyphenol coefficient. Ginger and citrus, however, work synergistically to affect the response. The formulation M6 (17% citrus, 67% *Zingiber officinale*, and 17% honey) was identified by the results. M3, which contains 50% honey and 50% *Zingiber officinale*, was the beverage that people liked the colour, taste, and smell of best.

1. Introduction

Herbal treatments are now considered a technique to treat and maintain health. Due to their affordable prices and accessibility, these drugs have become more and more popular, especially in developing countries [1]. 90% of COVID-19 patients treated in China with a mixture of ginger belamcanda, dioscoreae, and *Glycyrrhiza glabra* showed positive results. *Zingiber officinale* and Echinacea have been recommended together in order to lessen and manage the clinical symptoms in COVID-19 outpatients [1]. According to a review of research, honey may have therapeutic benefits in the COVID-19 pandemic scenario. However, in vitro and in vivo research are required to further explore the effects of honey on

SARS-CoV-2 replication and/or the human immune system [2]. The phytochemical components in *Citrus medica* and *Zingiber officinale* may lessen viral load and SARS-CoV-2 shedding in the nasal passages [3]. Although they play a crucial part in many physiological processes, free radicals have also been associated to the development of chronic diseases, such as COVID-19. Polyphenols are now understood to be essential bioactive molecules for human health because of their capacity to lessen the negative effects of free radicals on healthy physiology [4]. Shogasulphonic acid A, gingerenone, and caffeic acid are only a few of the polyphenols that are abundant in *Citrus medica*, *Zingiber officinale*, and honey. The primary protease enzyme of the virus is inhibited by these polyphenols [5], and they may help control a COVID-19 patient's free radicals. People in Cameroonian traditional villages used a concoction of *Citrus medica*, *Zingiber officinale*, and honey to treat some clinical symptoms such as fever, dry cough, and breathlessness. The research mentioned above has demonstrated the potential of each natural remedy in the treatment of COVID-19. Whether the combination of these three components can aid in the management of free radicals is the primary question addressed by this study. Finding out how combinations of citrus, ginger, and honey affect antioxidant activities, total phenolic compounds, and sensory characteristics is the goal.

2. Materials and methods

2.1. Materials

Honey, very ripe limes (*Citrus medica*), and ginger rhizomes (*Zingiber officinale*) were purchased at a local market. Limes (10 kg) were squeezed and filtered using a Whatman filter paper N°11 to obtain lime juice. Ginger rhizomes were washed and peeled. The peeled ginger (2 kg) was sliced and blended with 2 L of distilled water using a domestic blender and the mixture was filtered using a Whatman filter paper N°11 to produce ginger juice.

2.2. Production of different beverages.

Minitab 18.1 software was used to generate a mixture design of 3 liquids (honey, lime juice and ginger juice). Each liquid was introduced into the beverage formulations at proportions varying from 0 to 100%. A total of 10 beverages were formulated and presented in Table 1.

Table 1. Formulations of beverages

Beverage sample	Citrus (%)	Ginger (%)	Honey (%)
M1	67	17	17
M2	50	50	0
M3	0	50	50
M4	17	17	67
M5	33,3	33,3	33,3
M6	17	67	17
M7	50	0	50
M8	100	0	0
M9	0	100	0
M10	0	0	100

2.3. Evaluation of the total phenolic content of the beverages

The Total Phenolic Content of the 10 formulated beverages was assessed using Folin-Ciocalteu reagent as described by Singleton and Rossi's (1965) [6]. A calibration curve was plotted using gallic acid at various concentrations (0, 0.5, 0.6, 0.8, and 1 mg/ml) as standard. The results were expressed as mg GAE/ 100ml of beverage.

2.4. Evaluation of the Antioxidant properties of the beverages

2.4.1. Evaluation of the radical scavenging activity of the beverages

The radical scavenging activity of beverages was assessed using the discoloration of the free radical DPPH as described by Brand-Williams et al. (1995) [7]. Briefly, 125 mL of an ethanolic DPPH solution (24mg/100 mL of methanol) was added to each beverage (500 µL), and then the mixture was kept at room temperature in the dark for 60 min. After reading the absorbance at 517 nm against a blank, the following formula was used to determine the ability to scavenge the DPPH• radical:

Radical Scavenging Activity (%) = (AS/AC)*100

Where AS is the absorbance of the beverage mixed with the DPPH solution and AC is the absorbance of the control (DPPH• solution).

2.4.2. Evaluation of the total activity capacity of the beverages

The total antioxidant capacity of the beverages was determined using the phosphomolybdenum test as described by Herken and Guzel (2010) [8]. A volume of 100 µL of beverage was added to 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium

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Table 2. Estimated regression coefficients for Polyphenols, DPPH, FRAP, TAC.

Term	Polyphenols	DPPH	FRAP	TAC
Citrus	12.97	68.22	0.06	2.72
Gingier	16.37	21.59	0.27	2.84
Honey	15.28	56.00	0.29	8.32
Citrus*Gingier	1.497	127.5	-0.19	3.50
Citrus*Honey	-20.05	-59.21	0.44	11.08
Gingier *Honey	-10.51	140.0	0.0096	13.72
Citrus*Gingier*Honey	12.95	-95.19	1.04	16.06
Citrus*Gingier*(-)	-356.7	1468	-19.86	262
Citrus*Honey*(-)	393.4	-1622	19.78	-263
Gingier*Honey*(-)	-367.8	1822	-20.27	278
R Square	100	100	100	100

phosphate and 4 mM ammonium molybdate) and incubated for 90 min at 90°C. After cooling at room temperature, the absorbance at 695 nm was determined. A standard curve was created using ascorbic acid concentrations (0–3 mg/mL).

2.4.3. Evaluation of the ferric reducing antioxidant power of the beverages

The evaluation of the ferric reducing antioxidant power (FRAP) of the beverages was evaluated based on the reduction of ferricyanide as described by Henderson et al. (2015) [9] with minor modifications. Various beverage concentrations were combined with 625 µL of sodium phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide, the mixtures were incubated at 50°C for 60 minutes. The reaction mixture was allowed to cool to room temperature. Then, it was acidified with 10% trichloroacetic acid (125 µL) and centrifuged at 3000 rpm for 10 minutes. A volume of 250 µL of $FeCl_3$ (0.1%) and 1 mL of distilled water were added to 1 mL of the supernatant. A standard curve was created using ascorbic acid concentrations (0-3 mg/mL).

2.5. Sensory evaluation

Fifty (50) panelists were selected to evaluate the sensory qualities of the beverages. The sensory characteristics assessed were the colour, the odor and the taste. All sensory attributes were examined by the panelists and were graded on a 9-point hedonic scale. Water was provided to rinse the palate between the samples.

2.6. Statistical analysis

The mixture design was generated and the results were subjected to Analysis of Variance (ANOVA) using Minitab 18 software.

3. Results and discussion

3.1. Polyphenols content in juice.

Fig. 1 shows the amount of total phenolic components measured by the Folin-Ciocalteu method, which relies on the transfer of electrons from phenolic compounds to the Folin-Ciocalteu reagent in an alkaline medium.

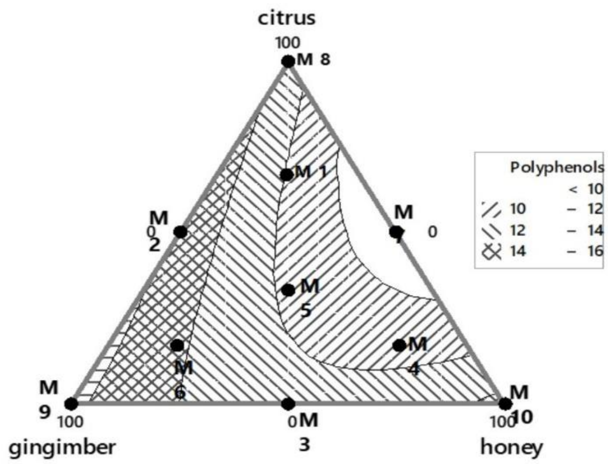

Figure 1. Total polyphenol content in the different mixture of beverage.

The highest level of phenol was found in beverage containing 100% ginger (M9), and it was greater than beverage containing 50% citrus and 50% honey (M7). Citrus, ginger, and honey alone have a significant contribution, according to the coefficient for the response polyphenols (Table 2). Ginger and citrus, however, worked synergistically to affect the response. However, the coefficient of the interaction between ginger and citrus is minor in comparison to the coefficient of each component. This indicated that,

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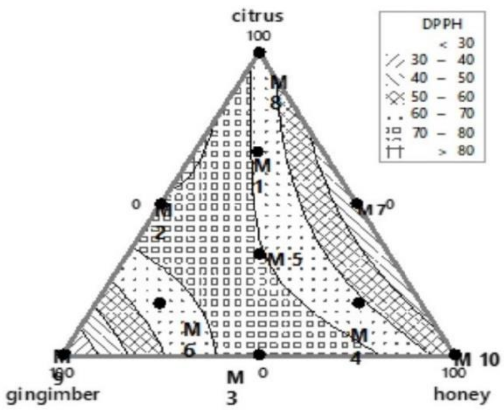
in contrast to the effects of ginger and honey, the addition of citrus to the recipe—even at 100%—did not significantly affect the phenol content. According to Mohammadzadeh Milani [4], the type and concentration of additives used determine the amount of polyphenols in black tea. They reported that the quantities of phenolic compounds were highest and lowest when honey and cloves were added to tea samples.

The mixture of Citrus-honey and the mixture of ginger-honey had the opposite effect on the response. The three components work together to enhance the reaction. Ginger significantly ($p<0.05$) affected the amount of polyphenols in juice samples. These studies showed that the juice, which included 100% ginger, enhanced the phenolic content. Ginger (M2 and M6) concentrations that are lower in juice indicate a small decline in phenol content. When honey was the only ingredient in the combination (M10), a considerable rise in phenolic compounds was seen. Lower levels of phenol chemicals (M4 and M7) were observed in the juice when honey was combined with other substances.

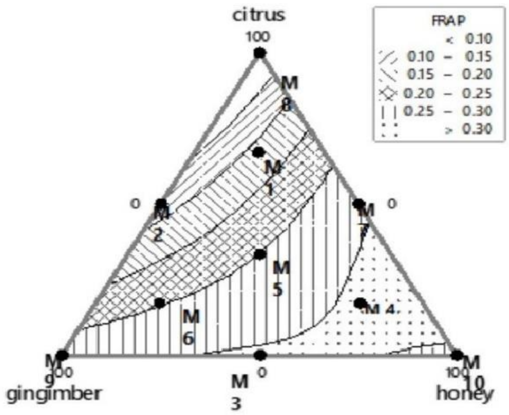
Many plants produce polyphenols as a secondary metabolite. Because of their antioxidative properties, they are receiving more and more increasing attention [10]. Due to their hydroxyl groups, they have the capacity to scavenge free radicals and quench active oxygen and may have advantageous impacts on human health [10].

3.2. Effect of citrus, ginger and honey on antioxidant activity.

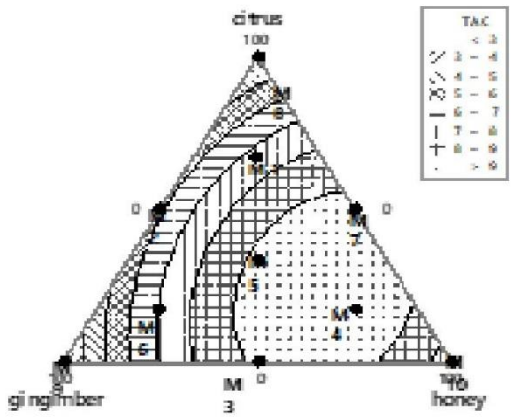
Data on antioxidant activity is presented in Fig. 2. The results showed that the mixture containing only 100% of citrus (M7), M2, M3, and M5 has the highest DPPH activity. The lowest DPPH activity was observed with the M9 mixture. Different results could be due to different amounts of additives added at different stages of the ingredients' preparation during juice preparation. As honey contains sugar, this may affect the antioxidant activity of the juice. Some studies have shown that the addition of sugar has reduced the antioxidant activity of black tea [11]; however, in another study, adding sugar has also increased the antioxidant activity of black tea [12]. The antioxidant activity of black tea was also dependent on the amount of ginger added [4].



A: Effect of Citrus, ginger and honey on DPPH activity.



B: Effect of Citrus, ginger and honey on FRAP activity



C: Effect of Citrus, ginger and honey on TAC activity.

Figure 2. Effect of Citrus, ginger and honey on the antioxidant activity of beverage.

The antioxidant activity of beverage was positively influenced by the addition of ginger, citrus, and honey. The highest DPPH activity was observed when the citrus was added. Some researchers reported that citrus, ginger, and honey exhibited antioxidant activity because of their natural antioxidant composition [13]. They contain some phenols such as phenolic acids, flavonol glucosides, phenolic volatile

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oils, and ascorbic acid. Another study reported that honey had antioxidant activity, which is related to enzymatic and non-enzymatic components and a lot of polyphenolic substances [14].

3.3. Effect of citrus, ginger and honey on sensory properties. Data on the sensory properties of beverage is presented in Fig. 3.

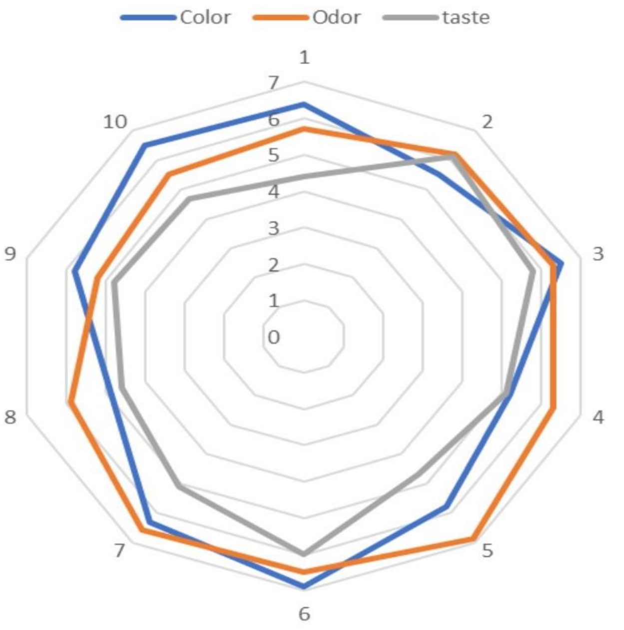

Figure 3. Effect of Citrus, ginger and honey on sensory properties.

The results showed that the mixture M6, M3 were the most accepted of the beverage in terms of color, odor, and taste. In terms of taste, the panelists rejected beverage 1. However, they appreciated the color and odor of the beverage. Wadikar et Premavalli [15] has reported that ginger-honey beverages can be beneficial for preventing colds and bronchitis. Panelists rated the beverage containing ginger and honey higher because it has a sweet taste. This sweet taste is due to the carbohydrate content in the ginger and honey. The sour taste of citrus may be the reason for the rejection of juice 1 by the panelists. It may also enhance their appetites. The preference of beverage 6 and 3 may justify their use in traditional medicine in Cameroon for preventing colds and bronchitis. It has been shown that ginger is used as a dietary supplement to boost immunity by enhancing the levels of interferon gamma (INF-α) and interleukin 2 (IL- 2) [16].

3.4. Model equations describing polyphenols, DPPH, FRAP and TAC as a function of citrus, ginger and honey. Table 2 displays the computed regression coefficients for polyphenols, DPPH, FRAP, and TAC. Citrus, ginger, and honey have significant ($p<0.05$) contributions to the response, according to the polyphenol coefficient. Ginger and citrus, however, work synergistically to affect the response. In contrast to the coefficients of each component, the interaction coefficient between citrus and ginger is minor. The mixture of citrus-honey and the mixture of ginger-honey had the opposite effect on the response.

4. Conclusions

The current study's findings showed that additive concentration significantly and favourably affected both phenol content, antioxidant activity and sensory properties of the beverage. The most antioxidant activity was found in citrus and ginger, whereas the lowest level was seen in beverages made just with honey. The beverage may be used to treat some clinical symptoms, including fever, dry cough, and breathlessness.

Authors' contributions

Conceptualization, methodology, visualization, writing (re-view and editing), project administration, P.A.E.; Writing (original draft), methodology, visualization, F.A.; Investigation, resources, formal analysis, methodology, visualization, writing (original draft), project administration, R.F.D.; Investigation, writing (review and editing) project administration, M.Y.D.; Investigation, writing (review and editing) project administration, C.B.B.; Investigation, writing (review and editing) project administration, A.E.M.; Supervision, methodology, writing (review and editing), project administration, C.M.M.

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Availability of data and materials

All data will be made available on request according to the journal policy

Conflicts of interest

The authors declare no conflict of interest.

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