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Optimization of temperatures for trapping of aqueous bioactive compounds from ginger in innovative drying system and development of ginger based functional drink

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Abstract
In this research study, the effect of different temperatures (60 ℃, 70 ℃, 80 ℃) on bioactive properties such as total phenolic content (TPC), total flavonoid content (TFC) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) antioxidant activity and physiochemical properties (pH, specific gravity and color) of ginger extract trapped from Innovative drying system were investigated. The ginger extract trapped at 80ºC showed increased TPC (i.e., 12.22±0.02^{a}mg GA/ml) and TFC (i.e., 6.26±0.01^{a}mg QE/ml). The increase in phenolics indicated the positive relationship with DPPH antioxidant activity of ginger extract obtained at 80 ℃. Specific gravity of obtained ginger extract was found to be 0.98. The pH value slightly decreased with rise in temperature. The L*, a* and b* value of the ginger extract obtained at 80ºC were 5.41±0.01^{c}, -0.19±0.01^{b}, and -0.52±0.02^{c}, respectively. The flavoured novel functional ginger drink was developed by blending of amla extract at various ratios between 2.5 to 7.5% on the obtained ginger extract. From this, the optimized drink was acquired based on the analysis of phenolics, flavonoids and sensory evaluation. The results obtained, indicate that the developed flavoured drink possess high antioxidant and anti-inflammatory rich bioactive compounds.

Keywords: Ginger extract, temperatures, total phenolics, total flavonoids, flavoured drink

Introduction
Since ancient times, numerous plants and their components have been utilized to cure various illnesses. Ginger (*Zingiber officinale Roscoe*), a member of the Zingiberaceae family, is perennial herbs cultivated in many tropical and subtropical areas and is extensively used as a spice and nutritional supplement around the world (J. Wang *et al*., 2019) [18]. Ginger is an traditional medicine and is utilized by Polynesians for more than two thousand years to cure high blood pressure, fitness issues, diabetes, cancer, and many other conditions (Ghasemzadeh *et al*., 2010) [9]. Ginger has been discovered to have biological properties including antioxidants, anti-inflammatory, antibacterial, and anticancer properties (An *et al*., 2016). The volatile components of ginger, particularly the aromatic molecules, are valued for their pungent, spicy, and delightful odour (Purnomo *et al*., 2010) [29]. Phenolic compounds are significant secondary metabolites found in ginger. Several researchers have looked at the possible health advantages of phenolics, such as their anti-tumor properties and antioxidant activities (Ghafoor *et al*., 2020; Kahkonen *et al*., 1999) [8, 13].
In order to prevent the growth of microbes and to slow down unfavourable browning processes, perishable agricultural raw materials are dried. This simple preservation technique extends the shelf life of fresh products (Muhlbauer & Muller, 2020) [25]. Numerous studies reported that the temperature of drying and conditions had an impact on the destruction of phytochemical compounds (Lachowicz *et al*., 2019; W. Wang *et al*., 2018) [19, 3]. Selecting an appropriate temperature for extraction and processing of crops is of significant importance in the case of food and pharmaceutical sectors since a detrimental method might have a negative impact on the quality of the final product and other nutritional qualities. The innovative drying system is used to trap the water molecules that evaporated from the drying system by creating a partial vacuum and drying the product comparable to a solar-powered drying system (Madhura *et al*., 2021) [21]. Several investigations have showed that the variations in essential oil concentrations and components in herbal plant extracts were considerably impacted by drying temperature from ginger and thyme (An *et al*., 2016; Mashkani *et al*., 2018) [23].

Flavoured beverages are available in different flavours, dietic, carbonated, and energy contents. The additional calories and other ingredients put into beverages to increase market acceptability might be harmful to the human body. A refreshing, thirst quenching, and cooling effect can be produced by mixing the natural flavoured extract with water (Larionov *et al*., 2020) [20]. Low-calorie, low fat, non-carbonated drinks are getting more and more popular as people become more health conscious. Food industries can make use of the advantages of bioactive ingredients while also adding value to regularly supplied mineral water (Kapp *et al*., 2020) [15].

Indian Gooseberry, also called as Emblica officinalis and is a member of the Euphorbiaceae family. It is generally referred to as "Amla" in India and has been utilized in Ayurvedic medicine as a "rejuvenating herb" for centuries. Several antioxidants found in amla extract, including emblicanin A and B, ellagic acid, and vitamin C, are being used to cure several medical ailments (Mahendran & Lanka, 2018) [22]. Additionally, it has several properties including those that are analgesic, antipyretic, immunostimulatory, antifungal, antibacterial, cardioprotective, and may raise haemoglobin levels (Middha *et al*., 2015) [24].

Therefore, the current study aims to explore how different temperatures can affect the biologically active compounds, antioxidant capacity, and the color of ginger extract obtained utilizing the Innovative drying system and using this extract for the development of bioactive compound rich flavour drink.

Materials and Methods

Materials

Ginger rhizomes were procured from a regional market in Thanjavur, India. These rhizomes were properly washed with running tap water and kept at 25 ± 2 ℃. Then, these ginger rhizomes were chopped into thin slices (2 mm) before being subjected to different temperatures in an Innovative drying system to trap the ginger extract.

Equipment

The Innovative drying system is completely made of stainless steel -304, which is specifically selected for its non-corrosive property. The vacuum pump was connected with the drying system to create vacuum. Other side, condenser is connected with drying unit to condense the water vapours which then collected in volumetric flask enclosed with airtight rubber cork (Figure 1). This drying system has developed to entrap the water vapours along with bioactive compounds that are evaporated in the food products by establishing a vacuum from the vacuum pump and drying the product (Harini *et al*., 2019; Madhura *et al*., 2021) [11, 21].

Preparation of novel Ginger extract- based drink

Fifty grams of amla was mixed with 175 ml of distilled water. Then this sample was kept in hydro distillation unit to acquire the amla extract. For preparing drink, this hydro-distillated amla extract was combined at various concentrations such as 2.5, 5, and 7.5% with the 50 ml ginger extract (Adesokan *et al*., 2013) [1]. The control was taken as unblended ginger extract and stored in refrigerated condition. The drink has been used for analysing various bioactive properties.

Chemical Reagents

The experiment employed the following chemical reagents: Folin Ciocalteu reagent, Sodium carbonate (NaCO3), Gallic acid, Quercetin, 1,1-diphenyl- 2-picrylhydrazyl (DPPH), Sodium nitrate, Aluminium chloride, Sodium hydroxide, Methanol. All the chemicals were purchased from SRL Chemicals Pvt. Ltd.

Total phenolics (TPC)

Folin-Ciocalteu reagent (FCR) was utilised to assess total phenolic content, as calculated by Ghafoor *et al*., (2020) [8] with slight modifications. One ml of sample solution was combined with 5 ml of FCR (diluted with water in the ratio of 1:10 v/v) and followed by addition of 4 ml of sodium carbonate (7.5%). Then, this mixture is allowed to stand for 30 minutes at 30 °C. A spectrophotometer (UV Spectrophotometer- Shimadzu Corporation, Japan- UV 1800) was used to examine the sample solution at 760 nm.

Total flavonoids (TFC)

With slight changes, total flavonoids were measured in the method given by Papoutsis *et al*., (2017). 2.7 ml of sample solution was added with 0.15 ml of NaNO2 (5%) and incubated at room temperature for 6 minutes. Afterward, 0.15 mL of AlCl3 (10%) was introduced and left to stand at room temperature for 6 minutes. Then, 2 ml of NaOH (4%) were added, and the mixture was allowed to kept for another 15 minutes. A spectrophotometer was used to examine the sample solution at 510 nm.

DPPH radical scavenging activity

The antioxidant activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test, as done by Zhang *et al*., (2017) with minor modification. At a concentration of 0.2mM, a DPPH solution was prepared. 3 ml of DPPH was added with 1 ml of methanol considered as control and 3 ml of DPPH was mixed with 1 ml of sample. Methanol was taken as blank. This mixture was kept dark for ½ hour. A spectrophotometer was used to examine the sample solution at 517 nm.

pH

A digital pH meter (Eutech Instruments Pvt. Ltd., Singapore) was used to determine the pH of the samples (Chen *et al*., 2018) [5].

Color

The color value of the sample solution was measured by using the Hunter Lab Colourimeter (Hunter Association Laboratory, Inc., USA). The color values were measured as L* (lightness/ brightness), a* (redness/greenness), and b* (yellowness/blueness) were seen on the screen (Madhura *et al*., 2021) [21].

Specific gravity

The specific gravity of the ginger extract that was collected using the system was measured using a pycnometer (Oyebadejo & Solomon, 2019) [26].

Sensory Evaluation

10 semi-trained panellists performed a qualitative interpretation of the drink formulations. Based on 9-point hedonic scale, the quality parameters were assessed for intensity of appearance, flavor, and general acceptability (Bembem & Agrahar-Murugkar, 2020) [4]. Every examination includes chilled samples, which are kept in the refrigerator. Panellists complete a scoring card, and all scores are

examined using a statistical approach.

GCMS

The ginger sample was extracted with methanol/DCM/ethyl acetate/hexane and 2 µl of sample was injected and analyzed through GCMS (8890GC/5977B GC/MSD-Agilent coupled with a Single Quadrupole Mass Spectrometer detector) and NIST library (Version-2020) were used for the identification of volatile compounds (Król & Dudziak, 2019) [18].

Statistical Analysis

The experimental results were presented as the average of three measurement readings and the standard deviation. To conduct the statistical analysis, Minitab 17 is utilized. For comparing means at $p<0.05$, one-way ANOVA (Turkey's test) is used.

Results & Discussion

Effect of different temperatures on total phenolics

The total phenolics of ginger extract at 60 ℃, 70 ℃, and 80 ℃ were reported to be 9.55,10.91 and 12.22 mg GAE/ml, respectively (Table 1). There is a significant difference ($p<0.05$) between all ginger extracts. The elevation in TPC found at extreme temperature might be owing to the lignin–phenolic acid linkages splitting or the degradation of lignin itself, resulting in much more phenolic acid being produced (Antony & Farid, 2022) [3]. To agree with our findings, Hossain *et al*., (2011) [12] observed that total phenolics enhanced as the temperature rose from 66 to 200 ℃ in marjoram, rosemary, and oregano. Similar results were discovered from grapes (Palma *et al*., 2001) [27].

Effect of different temperatures on total flavonoids

From table 1, the total flavonoids for different temperatures (i.e., 60 ℃, 70 ℃, and 80 ℃) were recorded, to be 4.88, 5.79, and 6.26, respectively. There is a significant difference ($p<0.05$) between all ginger extracts. An increase in flavonoids has been observed when the temperature rose from 60 ℃ to 80 ℃. Significantly raising temperature enhances the total flavonoids which is consistent with a previous study reported by Guthrie *et al*., (2020) [10] from kiwifruit peel and by Ko *et al*., (2014) from fruits and vegetable peel.

Effect of different temperatures on DPPH antioxidant activity: The DPPH radical scavenging potential of ginger extract at different temperatures was found to be varying from 42.16 to 48.01% respectively (Table 1). All the obtained ginger extracts had a statistically significant difference ($p<0.05$). This finding showed that the antioxidant capacity of the ginger extracts is proportional to the number of phenolics present (Kheirkhah *et al*., 2019) [16]. Similar findings have been for lemon myrtle by Kang *et al*., (2020) [14].

pH

As given in figure 2, the pH value of the ginger extract at 60 ℃, 70 ℃, and 80 ℃ was found to be 7.24, 7.20, and 7.19, respectively. There is a significant difference ($p<0.05$) observed between 60 ℃ and other samples. As the temperature goes on increasing in the drying system, accordingly the pH value is influenced. The reason may be due to temperature increase, vibrations of molecules increase, allowing water to ionize and generate additional hydrogen ions. Thus, the pH of the ginger extract will decrease. Similar results have been reported in black carrots (Türker & Erdoganˇdu, 2006).

Color

The table 2, provides the value of L*, a*, and b* of ginger extracts obtained at different temperatures. The L*value, shows a significant difference ($p<0.05$) among all ginger extracts. The negative a* value shows the ginger extract has a greenish hue whereas the negative b* shows a bluish hue. Unlike with L* and b* values, the a* value for ginger extracts at various temperatures did not differ much. There is no significant difference in a* value between ginger extract collected at 70 ℃, and 80 ℃. Similar findings has been reported in ash gourds (Madhura *et al*., 2021) [21].

Specific gravity

The ginger extract of different temperatures was analyzed for specific gravity to check the aqueous state. The results obtained are in the range of 0.98 (Table 1). There is no significant different ($p<0.05$) in specific gravity between the ginger extract. An earlier research in ash gourd showed the results which are similar to our findings (Madhura *et al*., 2021) [21].

Optimization of Ginger flavor drink

Considering the total phenolic and flavonoid content, the ginger flavour drink was optimized. The influence of various ratios of amla extract on total phenolics and flavonoids of ginger flavour drink is given in the Table 3. The total phenolics and flavonoids of all drink showed a significant difference. Based on the TPC and TFC, the ginger flavour drink at amla concentration 7.5% is considered to be the best because of its higher phenolic and flavonoid content when compared to all other drink.

Sensory evaluation

Ginger-flavoured drinks were exposed to a sensory analysis by using a hedonic scale to determine consumer acceptability and preference (Table 4). There is no much significant difference ($p<0.05$) in color between the samples. Therefore, the ginger flavour drink at amla concentration 7.5% is considered to be the best because of its higher acceptability rate when compared to all other drink.

GCMS

Table 5 shows the bioactive compounds discovered in optimized ginger flavour drink, along with their chemical formula, time of retention and area percentage. The results indicated existence of important bioactive compounds such as neral (8.32%), endo borneol (5.76%), 2,6-Octadienal,3,7-dimethyl- (12.64%), 2,4-Di-tert-butylphenol (9%), cyclotetradecane (7.24%) respectively. The findings showed that the concentration of bioactive compounds in drink were increased when it is added with amla extract which is in positive correlation with the increase in the total phenolics and flavonoid content. The current study on the bioactive components of the drink are in strong accordance with those of earlier literature (Choudhari, 2013; Exp *et al*., 2016) [6].

Table 1: Effect of different temperatures on ginger extract

	60 ^{0}C	70 ^{0}C	80 ^{0}C
TPC	9.55±0.02^c	10.91±0.04^b	12.22±0.02^a
TFC	4.88±0.04^c	5.79±0.02^b	6.26±0.01^a
DPPH	42.16±0.04^c	45.14±0.02^b	48.01±0.02^a
Specific Gravity	0.98±0.00^a	0.98±0.01^a	0.98±0.01^a

*There is significant difference ($p<0.05$) in values having superscript.

Table 2: Effect of different temperature on color

Temperature		60 ^{0}C	70 ^{0}C	80 ^{0}C
Color	L*	5.41±0.01^c	5.61±0.01^b	5.80±0.01^a
	a*	-0.19±0.01^b	-0.17±0.00^{ab}	-0.16±0.01^a
	b*	-0.52±0.02^c	-0.43±0.02^a	-0.47±0.00^b

*There is significant difference ($p<0.05$) in values having superscript.

Table 3: Influence of different concentration of amla on total phenolics and flavonoids of ginger flavour drink.

Various ratios of amla on ginger drink	TPC (mg GAE/ml)	TFC (mg QE/ml)
Control	12.19±0.01^d	6.25±0.02^d
2.5%	15.13±0.15^c	7.40±0.10^c
5%	18.03±0.15^b	7.99±0.05^b
7.5%	24.03±0.05^a	9.13±0.15^a

*There is significant difference ($p<0.05$) in values having superscript.

Table 4: Sensory evaluation of ginger flavour drink

Various ratios of amla on ginger drink	Color	Flavour	General acceptability
Control	8^b	6.0^d	7.9^c
2.5%	8.1^b	6.3^c	8.2^b
5%	8.3^a	7^b	8.2^b
7.5%	8.5^a	7.5^a	8.6^a

**Hedonic scale varies from point 1 (dislike extremely) to 9 (like extremely)

*There is significant difference ($p<0.05$) in values having superscript.

Table 5: GCMS analysis of optimized amla blended ginger flavour drink.

Compounds	Formula	Retention time	Area peak
Neral	C10H16O	6.2592	8.32
Endo- borneol	C10H18O	5.2006	5.76
2,4-Di-tert-butylphenol	C14H22O	10.184	9.00
Undecane	C11H24	4.0276	5.41
6-Octen-1-ol,3,7-dimethyl-, (R)- (Citronellol)	C10H20O	5.9846	0.95
Cetene	C16H32	11.1687	7.84
beta-Farnesene	C15H24	10.77	0.67
Hexadecane	C16H34	11.2546	1.47
2,6-Octadienal,3,7-dimethyl-, (E)- (Citral)	C10H16O	6.7055	12.64
Cyclotetradecane	C14H28	8.41	7.24

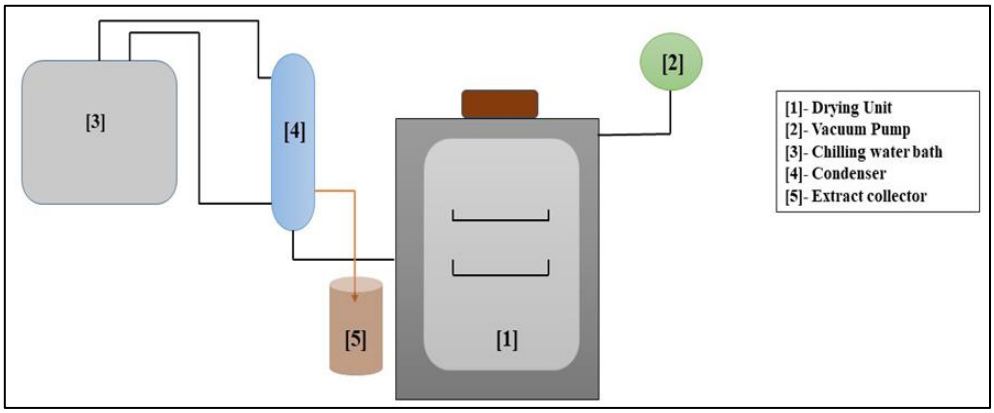

Fig 1: Innovative drying system

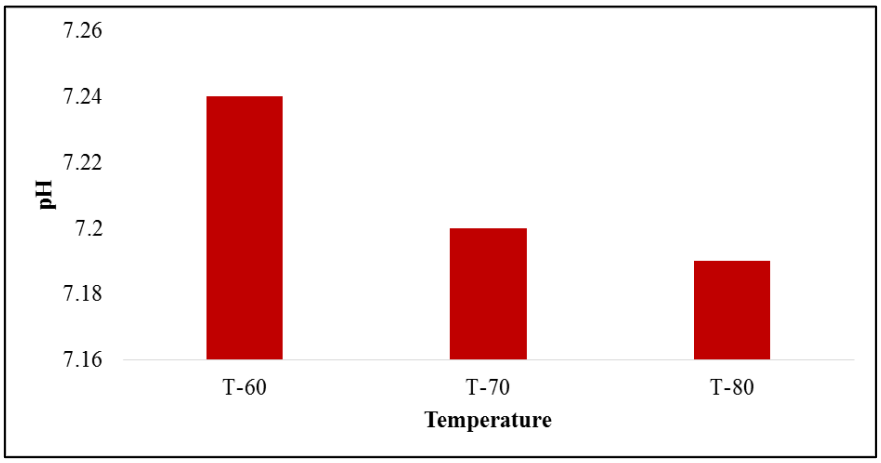

Fig 2: pH of Ginger Extract

Conclusion

The temperature plays an important role during any drying system so the drying temperature should be minimal to avoid any nutritional losses and to obtain better extraction efficiency. In our study 80 ℃ proved to be best temperature to obtain the maximum bioactive compounds in ginger using Innovative drying system. The aqueous ginger extract was trapped at this temperature was showed a better physicochemical and biological properties such as total phenolics, flavonoids, DPPH radical scavenging activity, color, pH, and specific gravity. By using this ginger extract, the ginger flavour drink was prepared with the addition of hydro-distillated amla extract. It showed to improve the additional flavor with overall increase in phenolic and flavonoid content of the drink that has proven to have therapeutic health benefits like antioxidant, antiviral and anti-inflammatory properties. Furthermore, this extract can also be used for new product development which can varied application in incorporation into beverages and snack foods.

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