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Studies on impact of different processing methods on phyto-chemical and antioxidant activity of dried ginger (*Zingiber officinale* L.) rhizome

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Abstract

Present work have been undertaken to study the effects of different processing methods on essential oil and oleoresin contents, phyto-chemical constituents and antioxidant activity of dried ginger rhizome. Ginger (*Zingiber officinale roscoe*) is branched and horizontal rhizome and its size is near about 5 to 15 cm in length, 3 to 6 cm width and 0.5 to 1.5 cm thickness. Ginger is grown in most Indian states namely Meghalaya, Kerala, Arunachal Pradesh, Sikkim, Nagaland Mizoram, and Orissa. Ginger is known for its analgesic, antimicrobial, antibacterial, antidiabetic, antiemetic, antifungal, anthelmintic, anti-inflammatory, antithrombic, antitumor, antitussive, antiulcer, antiviral properties. The medicinal properties of ginger are contributed by presence of bioactive components, polyphenolic compound, flavonoids etc. The dried ginger rhizome (*Sunth*) can be prepared by using commercially adopted technologies like Surat method, Malabar method and MPKV method. From the investigation it was observed that ginger processed by MPKV method got superior result over Surat and Malabar method with respect to essential oil, oleoresin and gingerol contents i.e. 1.61 percent, 4.28 percent and 79.04 µg/ml respectively. The dried ginger (MPKV method) possess highest content of poly phenolic compounds, total flavonoids and total alkaloids i.e. 80.43 µg/ml, 426.40 µg/ml and 2.52 percent respectively. It was observed that highest antioxidant activity was found in dried ginger processed by MPKV method i.e. 5.31 mg/ml. From the present investigation it was concluded that the ginger sample processed by MPKV, rahuri method found significantly superior over other processing method with respect to phyto-chemical constituents and antioxidant activity.

Keywords: Antioxidant activity, dried ginger rhizome, essential oil, oleoresin, total alkaloids, total flavonoids and total polyphenolic compounds

1. Introduction

Ginger originated in India, where it was introduced in Africa and the Caribbean, but there are no definite details available on the main ginger domestication center (Prabhakaran, 2013) [24]. It is now grown throughout the moist tropics (Meadows, 1998) [15], and it is the most commonly used spice in the world. It originated in India, and is now cultivated throughout the world in tropical climates; the major producers are Taiwan, China, Jamaica, Nigeria, Australia, Japan, Fiji, Saudi Arabia, Mauritius, and Australia. Ginger is grown in most Indian states. Together, however, states namely Meghalaya, Kerala, Arunachal Pradesh, Sikkim, Nagaland Mizoram, and Orissa contribute 70 per cent to the total output of the country. Jamaican and Indian ginger are found to be superior in terms of consistency followed by West African variety.

Ginger is commonly utilized in a different value added foods products because of its good nutritional profile and flavoring compounds. Ginger used in fresh form in kitchens for increasing taste and flavor of different types of food dishes. The ginger can be utilized for production of ginger powder, paste, dehydrated ginger, bleached ginger, glazed ginger etc. The different value added food products like ginger candy, alcoholic beverage i.e. ginger ale etc. ginger is known for food value as well as medicinal value. Ginger utilized in ayurvedic medicine from ancient time period for treatments of different kinds of diseases.

Ginger is a complex compound contains more than 60 derivatives and compounds (Srivastava *et al*, 2000) [29]. The rhizome of ginger has essential oil and resin collectively known as oleoresin. The composition of the essential oil varies depending on the geographical origin, but the main constituents are hydrocarbons of sesquiterpene which are responsible for the characteristic aroma. Gingerol is the principal phenolic compound which gives shogaols, zingerone, and paradol on hydrolysis.

The ginger possesses good antioxidant activity which scavenger the free radicals formed during oxidative reaction. More than three quarters of the world's population currently rely on health-care plants and plant extracts. Ginger is widely used in herbal medicine and folk

medicine (Srivastava *et al.*, 2000) [29]. Approximately 8000 herbal remedies have been codified in ayurvedic medicines and continue to be used in India. Herbal preparations often contain dry pepper and ginger. Ginger contains 1 to 2 percent oil that gives the spice a special flavor and has been studied by many researchers (Govindarajan, 1982) [10]. Antidiabetic, antibacterial, antifungal, anthelmintic, anti-inflammatory, anti-tumor, antithrombic, antiemetic, antitussive, antibacterial, antiviral, Analgesic, headaches, immune help, migraine headache, gas or flatulence, morning vomiting, diarrhea, thermoregulatory, sinus infection etc. have been reported by Sharma (2017) [28].

Ginger is perishable in nature and get spoiled if not stored or processed properly, hence it is recommended to process the ginger rhizome for increasing its shelf life. The drying and dehydration of ginger by using sun drying and or use of different mechanical drier results in increasing the shelf life of ginger by removing excess amount of water. Drying of ginger not only increases its shelf life but also increases its storage stability, reduces transportation cost etc.

2. Material and methods

2.1 Procurement of ginger (*Gingiber Officinale*)

The famous variety of ginger (*Gingiber Officinale*) "Kochin" was selected with concern of horticulturist. The best quality raw material (Ginger) which were need for present research collected from aurangabad.

2.2 Chemicals and Glassware

Analytical grade chemicals and glassware required for research work were available in the laboratory of Department of Food Engineering, College of Food Technology. Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani.

2.3 Methods

2.3.1 Determination of essential oil and oleoresin from dried ginger rhizome

0.2 Kg of the powdered dried ginger was kept in a round bottom volumetric flask and added with 1000 ml distilled water and allowed to hydro distillation in modified clewenger apparatus for about 8 hrs. The recovered oil was dried over anhydrous salts of sodium sulphate and keeps the oil in the refrigerator at 4°C prior to use (Bankole, 1997) [3].

2.3.1.1 Determination of essential oil content of dried ginger

The dried ginger after complete drying was grinded and converted into the powder. Then, 60 g of sample was mixed with 500 ml water and immediately transfer to hydro distilled for 7 hours. The extraction of oil was carried out from the distillate with methanol. After the filtration, the solvent was vaporized and removed by distillation under reduced pressure in a rotary evaporator at temperature of about 45°C. The essential oil content of dried ginger was given as (Costa *et al.*, 2011) [4].

$$\text{Essential Oil Content (\% w/w)} = \frac{\text{Weight of essential oil (g)}}{\text{Weight of initial product (g)}} \times 100$$

2.3.1.2 Determination of oleoresin content

1 g of dried ginger rhizome powder was kept in the thimble. Required quantity of food grade solvent was added in round bottom collecting flask in apparatus. The temperature of the hot plate was set somewhat more than boiling point of the food grade solvent. At the end of the process or completion of

siphoning the thimble was removed and heating was continued so that the condensed solvent drips through the sample in the thimble for a given period. This step includes distilling the extract solvent, and using it to rinse the material. The rinsing stop cock is closed at the end of the period, so that the condensed solvent is collected in the upper chamber. The rinsing stop cock was opened to flush the thimble and its contents enabling the elimination of the last traces of oleoresin adhering to the sample when most of the solvent is drained off. The oleoresin content of the ginger sample was calculated by using the formula given by (Joy *et al.*, 2016) [14].

$$\% \text{ oleoresin} = \frac{\text{Final weight of flask} - \text{Empty weight of flask}}{\text{Weight of sample}} \times 100$$

2.3.2 Determination of gingerol content of ginger

Extraction

5 g of the coarsely powdered of dried ginger was placed in 100 ml methanol on water bath for 15 min. Cool the mixture and filter through filter paper. Reflux the residue further with methanol until colorless extract get obtained, cool the solution and filter by passing through filter. Mix all the filtrate and concentrate until the volume reaches to 50 ml.

Analysis

The analysis of gingerol contents of dried ginger rhizome was carried out by use of high performance liquid chromatography (HPLC) method. The mobile phase used was Acetonitrile and water in 55:45 ratio. The column used for estimation was C18 - ODS (Octadecylsilane).

Standard preparation

Weigh accurately 100 mg of working standard which contains 40 percent of Gingerol and add to a 25 ml volumetric flask. Dissolve and make up to 25 ml with high performance liquid chromatographic grade methanol.

Sample preparation

Weigh exactly a dried ginger sample quantity equal to 40 mg of gingerol to a 25 ml volumetric flask. Dissolve and make up to 25 ml with high performance liquid chromatographic grade methanol.

Procedure

By injecting 10 µl of normal solution with the correct syringe. Monitor the chromatograms repeat the injections four more times, and measure the area's RSD. This is not to reach two percent. Inject 10 µl of prepared samples and record the chromatogram. Calculate the amount of gingerol that comes from peak areas. The method followed for estimation of gingerol content was given by Mishra *et al.* (2013) [18].

2.3.4 Phyto-chemical screening of ginger extract

Procedure for dried ginger rhizome extract preparation

Take 50 gram of dried ginger rhizome powder and extracted with aqueous, ethanolic, and perolium ether by use of soxhlet unit at 55 to 85 °C for around 8 to 10 hours in order to separate the polar and non-polar compounds (Elgorashi and Staden, 2004) [8]. For each extraction process the powdered get dried and then used for extraction process. Aqueous, ethanolic, and perolium ether based ginger extracts were used for qualitatively analysis of phyto-chemical present in ginger sample following the standard procedures (Harbone, 1973) [11].

i) Alkaloid test

Wagner's test: 10 mg of ginger extract was used and add some drops of Wagner's reagent in the extract. It was observed that development of reddish-brown color indicates that alkaloids are present in test sample. The wagner's reagent can be prepared by dissolving 2g of iodine and 6g of potassium iodate in 100 cm³ of water.

ii) Flavonoid test

Lead acetate test: 10 mg of ginger extract was added with some drops of 10 percent lead acetate solution. The development of yellow color of extract indicates that flavonoids are present in sample.

iii) Phenol test

Sodium hydroxide test: 5 mg of ginger extract was dissolved in 0.5 ml of 20 percent H₂SO₄ solution. Followed by addition of some drops of aqueous Na(OH)₂ solution. If the color changes to blue it indicates the presence of phenolic compound in the ginger extract.

iv) Carbohydrates test

Fehling's test: 5 ml of Fehling's solution was added to 0.5 mg of ginger extract and heated in a water bath. The formation of yellow or red color precipitate showed the presence of reducing power.

2.3.5 Determination of total poly phenolic content**2.3.5a Protocol for preparation of extract**

Approximately 50g of freshly prepared dried ginger powder which was in triplicates forms were kept in brown envelopes and complete dried at a 65°C temperature to constant weight. Using ambient temperature (30°C) of percolation, a 0.7g of ginger powder from each sample was taken separately to 25 ml of distilled water and maintained at this temperature with continuous shaking for around 4 hrs. Add Another 25 ml of distilled water was added to the mark and repeat the extraction process. The filtrates were collected to provide a 50 ml extract for each sample.

2.3.5.1 Determination of total phenolic content of ginger extract

According to the Pinelo *et al.* (2005) [23] total phenolic contents from the dried ginger extracts were determined using Folin-Ciocalteu's method. 5 ml of Folin Ciocalteu reagent was added in 1 ml ginger extract sample in tube. Then, 4 ml of 7.5 percent sodium carbonate was added. After 1 hr of incubation at ambient temperature the absorbance was read at 765 nm against blank. The produced results were taken as mg gallic acid equivalent per gram of fresh sample (mg GAE/g). The formula for calculating the total phenolic contents present in ginger extract samples as follows.

$$C = c V/m$$

Where, C = total poly phenolic content present (mg GAE/g)
 c = gallic acid concentration obtained from calibration curve (mg/ml)
 V = Extract volume (ml)
 m = Extract mass (g)

2.3.5.2 Determination of total flavonoid content of ginger extract

The total flavonoid content of dried ginger extracts was quantified by using a procedure reported by Meda *et al.* (2005) [16]. 0.5 ml of completely diluted ginger sample was mixed with 0.5 ml methanol, 50 µl of 10 percent of AlCl₃, 50

µl of 1 mol/l potassium acetate and 1.4 ml water and incubates at ambient temperature for half hour. The absorbance of the sample extract was subsequently measured at 415 nm. The total flavonoid was quantified by use of formula,

$$\text{Total flavonoids contents} = \frac{A \times DF}{A^{1\%}_{1\text{cm}} \times (w - ld)}$$

Where, A = Absorbance in spectrophotometer

DF = Factor of dilution

A^{1%}_{1cm} = Absorption by AlCl₃

w = plant material weight

ld = Drying loss

2.3.6 Determination of alkaloids content of ginger extract

5 g of the dried ginger sample was taken into a 250 ml beaker and 200 ml of 10 percent acetic acid in ethanol was added and kept aside for 4 min, and then it was filtered through filter paper and extract was concentrated on a water bath to 1/4th of its original volume. Add concentrated ammonium hydroxide drop by drop to the extract till it precipitate completely. The entire solution was allowed to settle down and obtained precipitate was collected and by using ammonium hydroxide it was washed and then filtered through filter paper. The residue then dried and weighed (Harbone, 1973) [11]. The obtained dried residue was alkaloids contents present in the ginger sample.

$$\text{Alkaloid (\%)} = \frac{W_3 - W_2}{W_1} \times 100$$

Where, W₁ = Initial weight of ginger sample,

W₂ = Extract weight

W₃ = Residue weight

2.3.7 Determination of antioxidant activity of ginger**2.3.7a Procedure for preparation of ginger extract**

The extract was prepared by keeping the ginger powder in 100 ml boiling distilled water for about 3 minutes. The ginger extracts then cooled, filtered through filter paper and the volume was make up to 100 ml using distilled water (Toda, 2011) [30].

2.3.7.1 Antioxidant activity assay

According to Zaeoung *et al.* (2005) [31] the antioxidant activity of the ginger extracts was analyzed by using the DPPH (Diphenyl picryl hydrazyl) assay. Add 0.02 g of 100 µm diphenyl picryl hydrazyl solution in ethanol to 2 ml of ginger extract and mixed well. The samples were then allowed to kept aside for 20 minutes for reaction with diphenyl picryl hydrazyl and the absorbance was measured at 520 nm after reaction was complete through spectrophotometer. 1 percent Vitamin C was taken as positive control whereas distilled water was taken as blank for all the ginger samples. Antioxidant activity was expressed as percent inhibition of the diphenyl picryl hydrazyl radical which may be expressed as mg/ml and observed by de pigmentation of the diphenyl picryl hydrazyl reagent to colorless from dark violet color. In the diphenyl picryl hydrazyl free-radical scavenging assay, antioxidants react with diphenyl picryl hydrazyl and change it to the yellow colored a, a-diphenyl-β-picryl hydrazine. The degree of discoloration showed the free-radical-scavenging properties of the ginger extract.

$$\text{Antioxidant activity (\%)} = \frac{\text{Absorbance of blank sample} - \text{Absorbance of test sample}}{\text{Absorbance of blank sample}} \times 100$$

3. Result and discussion

3.1 Essential, oleoresin and gingerol content of processed ginger rhizome

The data related to impact of different processing methods on essential oil, oleoresin and gingerol contents of dried ginger rhizome is summarized in table 1.

Table 1: Effects of processing on essential oil and oleoresin contents of dried ginger rhizome

Processing method	Essential oil (%)	Oleoresin (%)	Gingerol (µg/ml)
Fresh ginger	0.48	1.24	20.84
Surat method	1.52	3.97	38.21
Malabar method	1.56	4.15	52.61
MPKV method	1.61	4.28	79.04
SE±	0.016	0.046	4.694
CD at 5%	0.047	0.158	15.873

*Each value is average of three determinations

The data summarized in table 1 depict the essential, oleoresin and gingerol contents of dried ginger powder. Ginger oil is prepared generally by steam distillation of freshly ground or dried ginger rhizome. The percent yield of ginger oil varies from 1.5 to 3.0 percent. The obtained ginger oil is a greenish yellow to light yellow colored liquid which on aging becomes viscous (Purseglowe *et al.*, 1981) [25]. The essential oil contents of fresh ginger rhizome was 0.48 percent. From table 17 it was depicted that ginger sample processed by MPKV method is significantly superior to Surat method and Malabar method. The ginger processed by MPKV method obtained highest value for essential oil i.e. 1.61% comparatively Surat method (1.52%) and Malabar method (1.56%). The dried ginger rhizome powder is used for extraction of essential oil. According to Deans and Svoboda (1992) [5] the drying of ginger results in significant impact on essential oil content. The MPKV method will preferred for higher yielding of essential oil.

Ginger oleoresin is produced by solvent extraction of powdered dried ginger with suitable organic non polar solvents like hexane, alcohol, acetone and ethylene dichloride, etc. (Adamade *et al.*, 2017) [1]. The oleoresin contents present in fresh ginger rhizome was 1.24 percent. The oleoresin contents ginger sample processed by Surat method, Malabar method and MPKV method are 3.97, 4.15 and 4.28% respectively. The ginger sample processed by MPKV method

method obtained high yield for oleoresin as compare to other sample.

The similar results were obtained by Jayashree and Visvanathan (2011) [24], who studied physical and chemical quality attributes of fresh and dried ginger (*Gingiber officinale*) rhizome and observed that dried ginger contained 1.6 percent essential oil and 3.5 percent oleoresin. El-Ghorab *et al.* (2010) [7] found that fresh and dried ginger rhizome possessed 0.31 and 1.1 percent essential oil respectively.

The gingerol contributes the pungent taste to ginger and It was observed that the gingerol contents of fresh ginger rhizome was 20.84 µg/ml. The processing of the ginger rhizome results in increase in contents of gingerol of dried ginger rhizome. The highest value for gingerol was observed in the dried ginger processed by MPKV method i.e. 79.04 µg/ml followed by Malabar method i.e. 52.61 µg/ml and Surat method i.e. 38.21 µg/ml. Jayashree *et al.* (2014) [13] reported that the sun drying of ginger had significant influence of gingerol contents of dried ginger powder. The similar results were given by Mishra *et al.* (2013) [18]

3.2 Phytochemical screening of ginger rhizome

Qualitative phyto-chemical analysis was carried out to know the secondary metabolites present in the different extracts of ginger rhizome. The data related to phyto-chemical screening of ginger rhizome is summarized in table 2.

Table 2: Phytochemical screening of lemongrass (*Cymbopogon citratus*) leaves

Phytochemical Constituents	Extracts			Name of the Test
	Aqueous	Ethanol	Petroleum ether	
Alkaloid	+	+	-	Wagner's Test
Flavonoid	+	-	+	Lead acetate test
Phenol	+	-	+	Sodium hydroxide test
Carbohydrate	+	+	+	Fehling's test

The ginger extracts prepared by aqueous, ethanol petroleum ether were used to qualitatively identify the presence of secondary metabolites in ginger rhizome. The data present in table 2 revealed that aqueous extract of ginger contained alkaloid flavonoids, phenols and carbohydrate. However ethanolic extract of ginger contained alkaloids and carbohydrate. Aqueous extract of ginger rhizome showed the presence of flavonoids, phenol and carbohydrate. Each phyto-chemical component is known for conferring various medicinal properties and health benefits to mankind. Phenol is important in modifying the erythrocyte membrane. Alkaloids protect the body from chronic and life threatening diseases.

Flavonoids come under class of good antioxidants and can potentially scavenge the free radical produced by oxidative reaction in the body. Similar results were obtained by Osabor *et al.* (2015) [21] and Arawande *et al.* (2018) [2].

3.3 Phyto-chemical constituents of dried ginger rhizome

The major emphasis in the investigation of poly phenolic and total flavonoid content was because of the bioavailability of these phyto-chemical components related to antioxidant activity (Rufino *et al.*, 2010) [26]. According to Scalbert and Williamson (2000) [27] the RDI for polyphenol has been

showed one gram per day. The data related to phyto-chemical

contents of dried ginger rhizome is presented in table 3.

Table 3: Effects of different processing on phyto-chemical constituents of dried ginger rhizome

Processing methods	Total poly phenol ^a (µg/ml)	Total flavonoids ^b (µg/ml)	Total alkaloids (%)
Fresh	89.71	433.01	4.36
Surat method	26.95	403.16	3.58
Malabar method	15.12	368.02	4.28
MPKV method	80.43	426.40	2.52
SE±	3.221	6.983	0.271
CD at 5%	10.344	22.079	0.862

*Each value is average of three determinations

^a – µg of Gallic Acid Equivalent/ml

^b - µg/ml of Quercetin Equivalent

The data presented in table 3 depict the effects of different processing on phyto-chemical constituents of dried ginger. It was observed that the total poly phenolic compound of fresh ginger was 89.71 µg of Gallic acid/ml. The processing of ginger results in decreasing the polyphenolic contents of dried ginger. The maximum contents of polyphenol was observed in ginger processed by MPKV method i.e. 80.43 µg of Gallic acid/ml. The total polyphenol contents of ginger processed by Surat and Malabar method were 26.95 and 15.12 µg of Gallic acid/ml respectively. The dried ginger processed by MPKV method was at par with Surat and Malabar method with respect to total poly phenolic compound. Faten *et al.* (2018)^[9] observed that with increasing in the temperature of drying results in decreasing the polyphenolic contents.

The total flavonoids content of ginger rhizome expressed in µg/ml of quercetin equivalent. The total flavonoids content of fresh ginger rhizome was 433.01 µg of quercetin/ml. The maximum flavonoids contents was observed in dried ginger processed by MPKV method i.e. 426.40 µg of quercetin/ml. The minimum contents of flavonoids was observed in ginger processed by Malabar method i.e. 368.02 µg of quercetin/ml. Middleton and Kandaswami (1994)^[17] reported that plant possessing poly phenolic compounds including flavonoids which are excellent antioxidants and also offers anti-mutagenic and anti-carcinogenic properties.

Alkaloids are formed as by-products during metabolic reaction in plant growth and development. The alkaloids are had bitter taste and they possessed antibacterial properties. For antispasmodic analgesic, and antimicrobial properties an isolated plant alkaloids and their derivatives employed as medicinal and health promoting agents (Okwu, 2004). The alkaloids present in fresh ginger rhizome were 4.36 percent. The total alkaloids contents of ginger processed by Surat, Malabar and MPKV method were 3.58, 4.28 and 2.52 percent respectively. The similar results were obtained by Ozola *et al.* (2019)^[22].

3.4 Antioxidant activity of dried ginger rhizome

According to Nobuji, (2000)^[19] spices had different phyto-chemical components which are commonly known as antioxidants or free radical scavenger such as poly phenolic compound, tannins and flavonoids. The antioxidants are capable of neutralize or scavenge the free radicals formed during the oxidative metabolic activity in the human body by giving required electron to come in state of stabilization. So that, the presence of bioactive compound in ginger rhizome reflects the antioxidant activity of food products. The antioxidant activity of dried ginger was studied and presented in table 4.

Table 4: Effects of different processing on antioxidant activity of dried ginger rhizome

Processing methods	Antioxidant activity (mg/ml)
Fresh ginger	6.24
Surat method	4.55
Malabar method	2.09
MPKV method	5.31
SE±	0.266
CD at 5%	0.829

*Each value is average of three determinations

Ginger is a extremely good source of natural antioxidants in plant origin. These antioxidants are produced during metabolic activity during growth and development of plants and are also called as secondary products. The antioxidant activity of ginger was analyzed by using DPPH method. The data presented in table 4 showed the effects of different processing on antioxidant activity of dried ginger rhizome. The antioxidant activity of fresh ginger was 6.24 mg/ml. The antioxidant activity of ginger rhizome gets reduced on drying (Heat treatment), because of reduction of poly-phenolic compound during drying process. It was observed that highest antioxidant activity was observed in ginger processed by MPKV method i.e. 5.31 mg/ml.

The antioxidant activity of ginger processed by Surat and Mulbar method were 4.55 and 2.09 mg/ml respectively. The dried ginger processed by MPKV method found significantly

superior over Malabar method with respect to antioxidant activity. Faten *et al.* (2018)^[9] reported that the antioxidant activity of thyme leaves extract was reduced with an increase in temperature of drying which thereby reduces the contents of total phenols. The similar results were obtained by Denre (2014)^[6] and Ozola *et al.* (2019)^[22].

Conclusion

In the light of present investigation it was concluded that the ginger sample processed by MPKV rahuri method is significantly superior over ginger sample processed by Surat and Malabar method with respect to essential oil, oleoresin contents, gingerol contents, total poly phenolic compounds, total flavonoids, total alkaloids and antioxidant activity.

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