International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Extraction of Antioxidants from Fruit Peels and Its Utilization in Ghee

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ABSTRACT

In this research, bio-active compounds found in pomegranate(*Punica granatum*) and orange(*Citrus X sinensis*) peels were extracted using ethanol (80 percentage).Natural antioxidants have gained considerable interest in recent years for their role in preventing the auto oxidation of fats, oils, and fat containing products. In the present study, peels of pomegranate and orange used as a source of natural antioxidants and ethanolic extracts of these peels were prepared. Ethanolic extract of pomegranate exhibited a high percentage of antioxidant activity and phenolic content compared to ethanolic extract of orange. Ethanolic extracts of orange and pomegranate were added to ghee at 200ppm concentration. BHA was also added to ghee at concentration of 200ppm for comparison. All samples were incubated at 60 - 80˚C for 20 days. The result revealed that, ethanolic extracts of pomegranate gave good antioxidant activity and less peroxide value during accelerated oxidative incubation of ghee. Ethanolic extract of pomegranate showed relatively comparable activity to BHA. Therefore, these extracts could be used as preservative ingredients in the food industries.

INTRODUCTION

Fruits and vegetable processing in India generates substantial quantities of waste. It had been previously reported that these waste and by products of fruits are an abundant source of antioxidant polyphenols. The peels are the source of sugars, minerals, and organic acids which have wide range of actions which includes antioxidants, antimutagenic, antibacterial and antiviral activities. A number of natural antioxidants have been added during food processing and have elongated the shelf life and oxidative stability of food products. A huge amount of plant biomass waste is produced yearly as by products from the agro food industries. These wastes are attractive source of polyphenol and antioxidants may have considerable economic benefit to food processors.

The high concentration of phenolic compounds present in peels, skin, seeds supports the utilization of these residues as a source of natural antioxidants. The research indicated that the natural phenolic compound can be extracted from raw material or waste products of food industry. Pomegranate has been extensively in the folk medicine of many cultures and its consumption has grown tremendously especially in the last decade. The peels of some fruits have higher antioxidant property than pulps.

Antioxidants are the chemical substance that reduces or prevent oxidation and have the ability to counteract the damaging effects of free radicals in tissue, and thus are believed to protect against cancer,

heart disease and several other diseases. They scavenge radicals by inhibiting and breaking of chain reaction, suppressing formation of free radicals by binding to the metal ions, reducing the hydrogen peroxide and quenching superoxide and singlet oxygen. Antioxidant (natural and synthetic) play significant role in retarding lipid oxidation reactions in food products. The detrimental effects of excessive lipid oxidation such as formation of off- flavors and undesirable oxidized chemical compounds (aldehydes, ketones and organic acids). Synthetic antioxidants (BHA and BHT) are widely used as food additives, but their application has been reassessed because of possible toxic or carcinogenic components formed during their degradation. This degradation leads to cause several diseases and also affect the shelf life of food products. A number of natural antioxidants have been added during food processing and have elongated the shelf life and oxidative stability of stored products. These types of natural antioxidants were extracted from different fruit peels like pomegranate and orange peels. The ethanolic extraction of pomegranate and orange were added to this type of food products which leads to their shelf life by preventing lipid oxidation. The present study was done to explore with the objectives to extract antioxidants in the form of phenols and flavanoids from fruit peels like pomegranate and orange to determine the antioxidant activity.

Antioxidants are used as food additives to guard against food deterioration. These are added to food products like oil, bread, cookies, biscuits and dairy products like sandesh, paneer, ghee etc. to enhance their shelf life by preventing lipid peroxidation and protecting from oxidative damage. Exposure to oxygen and sunlight are the two main factors in the oxidation of food, so food is preserved by keeping in the dark and sealing it in containers or even coating it in wax, as with cucumbers. These antioxidants are an especially important class of preservatives because like bacterial or fungal spoilage. Oxidation reactions also occur relatively rapidly in frozen or refrigerated food causing their spoilage.

The research mainly based on the food product natural ghee to improve their shelf life by incorporating antioxidants to it. Ghee is a form of highly clarified butter that is traditionally used in Asian cooking. Like butter, ghee is typically made from cow's milk. Ghee is made by melting regular butter. The butter separates into liquid fats and milk solids. Ghee is the most traditional dairy product which undergoes oxidative degradation during storage, resulting in alteration of major quality parameters affecting its suitability for consumption. Development of rancidity or hydrogen peroxide reduces the shelf life of the ghee, which ultimately affect theconsumer acceptability.

The antioxidant were extracted from pomegranate and orange peel using ethanol and which are added to the natural ghee to increase their shelf by preventing lipid oxidation or hydrogen peroxide formation. Among the ethanol extracts of fruit peels the pomegranate extracts exhibit higher antioxidants compared to orange peel. The incorporation of natural antioxidants to the ghee to improve their shelf life, which doesn't cause any side effects or disease to the consumers. The antioxidant extracts were further studied for their effects on ghee.

AIM AND OBJECTIVES

Fruits processing in India generate substantial quantities of waste, so these wastes and byproducts of fruits are an abundant source of antioxidant polyphenols. These peels and pomace are a source of sugars, minerals and organic acids, dietary fibers and phenolics which have a wide range of actions which includes antioxidants, antimutagenic, cardio preventive, antibacterial and antiviral

activities. Use of waste as a source of polyphenols and antioxidants may have considerable economic benefit to food processes and it is cheap and efficient by comparing with synthetic antioxidants generally used in food products like BHT and BHA are toxic and may cause health problems. By using natural antioxidants from these peels extracted and then utilization in ghee can increase the shelf life by preventing peroxide formation , and so the natural antioxidants can be added to any food product containing fat and oil to increase the shelf life by preventing rancidity. With this view, the work is carried out by using natural antioxidants with the following aims and objectives.

Experimental set up

A. Collection of fruit peels from pomegranate and orange.
B. The extracts can be prepared from dried powdered fruit peels.

This can be done by:

- Evaluating different extracts from pomegranate peels (PP), orange peels (OP) as a source of natural antioxidant.
- Characterizing the composition and content of phenolics in different extracts.
- Evaluating the efficiency of using agro food wastes ethanolic extracts in improving the quality, overall acceptance and oxidative stability of ghee during storage under thermaloxidative conditions.

Experimental procedure

A. Extraction of antioxidants from peels of pomegranate and orange.
B. Determination of extraction yield.
C. Determination of total phenolic content.
D. Determination of flavanoid content.
E. Determination of antioxidant activity by FRAP assay.
F. Determination of peroxide value of ghee.

REVIEW OF LITERATURE

Natural antioxidants

Antioxidants (natural and synthetic) play a significant role in retarding lipid oxidation reactions in food products. The detrimental effects of excessive lipid oxidation such as formation of off-flavors and undesirable oxidized chemical compounds (aldehydes, ketones and organic acids) are well known (Saad etal.,2007).Synthetic antioxidants(e.g., TBHQ, BHA and BHT) are widely used as food additives, but their application has been reassessed because of possible toxic or carcinogenic components formed during their degradation(Jo et al.,2006,Pitchaon et al.,2007).A number of natural antioxidants have been added during food processing and have elongated the shelf life and oxidative stability of stored products (Chennet al.,2008,Ebrahinabadi et al.,2010,Jang et al.,2012,Xiaowei et al.,2011).

Antioxidant property of plant products

A huge amount of plant biomass waste is produced yearly as by-products from the agro-food industries. This waste are attractive sources of natural antioxidants. The high concentration of phenolic compounds present in peels, skins and seeds supports the utilization of these residues as a source of natural antioxidants. Phenolic compounds exhibit a wide range of physiological properties such as anti-allergenic, anti-athrogenic, anti-inflammatory,anti- microbial,antioxidant,anti-thermobiotic,cardio protective and vasodialatory effects (Balasundran et al.,2006). Phenolics could be extracted by water or solvents and the extraction conditions need to be optimized with respect to solvent polarity and physical conditions(Nepote et al.,2005).In addition,research has indicated that natural phenolic compounds can be extractedfrom raw materials or waste products of food industry (Peschel et al.,2006).

Pomegranate

Pomegranate has been used extensively in the folk medicine of many cultures and its consumption has grown tremendously especially in the last decades (Li et al., 2006, Cam et al., 2009). The peels of some fruits have higher antioxidant activity than pulps (Guo et al., 2003, Fuhrman et al., 2005)

Tulsi leaves

Sharma (1997) isolated the antioxidant principle of tulsi (*Ocimum sanctum Limm*)leaves a pre-extraction. The anti-oxygenic compounds of tulsi leaves were extracted into methanol and then vacuum dried. The dried materials were further fractionated into water insoluble fraction which was then treated with mixture of silica gel and charcoal and designated at SCF. Addition of SCF pre-extract at the level of 0.6%(w/v)was found to be more effective than the addition of BHA at the level of 0.02%.The phenolic compounds appeared to be the main contributory factors in enhancing the oxidative stability of ghee.

Arjuna bark extract

Parmar et al., (2013) reported that extract of Arjuna bark increased the shelf life of ghee as compared to control sample during storage at 8˚C.Their findings also suggested that freshly prepared ghee from cow milk added with Arjuna bark has good potentiality to act as free radical scavenger.

Coriander

Patel et al., (2013) assessed the antioxidant activity of coriander extract in ghee and reported that coriander extract added ghee gave better oxidative stability of ghee during storage as compared to control sample but they also suggested that for ghee BHA is more effective antioxidant than coriander extract.

Sorghum grain powder

Kaur et al., (2001) studied the use of Sorghum (Sorghum bicolor L.) grain powder in enhancing the oxidative stability of ghee. Direct addition of Sorghum grain powder(SGP) at different levels in ghee was elevated the phospholipids as well as water extractable phenolic compounds of ghee. The results

also revealed that addition of SGP at a level of 1% (w/v) and above have higher effect than the addition of permitted level of BHA. The proactive action of SGP in ghee could be attributed to the transfer of phospholipids and phenolic compounds in SGP.

De-husked ragi powder (DRP)

Mehtha (2006) reported that addition of methanol pre-extract of de-husked ragi powder (DRP) at the rate of 0.1%, 0.25% and 0.5% resulted in a corresponding increase(over control) in phospholipids content and water extractable phenolics content of ghee. The anti- oxygenic in dics calculated from the induction periods of ghee samples stored at 80±2°C in comparison with sample of ghee DRP gave better result than control sample in order to prevent oxidative rancidity. This result suggested that the phospholipids and the phenolic compounds DRP transferred to ghee enhance its oxidative stability.

Tomato seed powder

Tomato seed powder added at 5.0% level in ghee inhibited oxidation and ensured its stability practically to the same extent as 0.01% of BHT or BHA (Guleria et al., 1983).

Onion skin extract

Jain (1996) elucidated the effect of addition of antioxidant principles of onion (*Allium cepa*) skin via pre-extract on the oxidative stability of ghee. The anti-oxygenic compounds of onion skin were extracted into methanol and dried. The dried material was mixed with ghee at a rate of 0.5% (w/v) addition of such extracts at different levels was found to be almost at par with addition of BHA at 0.02% in protecting ghee. Quercetin and anthocyanin, the phenolic compounds appeared to be the main contributory factors in enhancing theoxidative stability of ghee.

Vidarikand (Extracts)

Gandhi et al., (2013) evaluated antioxidativeproperties of Viadarikand ethanolic extract in ghee and reported that ethanolic extract of the vidarikand was more effective for preventing the development of the peroxide value and conjugated diene value in ghee during storage. Vidarikand ethanolic extract showed the higher induction period as compare to controlghee sample.

Betel, curry and drumstick leaves

Betel and curry leaves when added at 1.0 per cent level to ghee showed higher resistance to oxidative deterioration than BHA and BHT mixture. The antioxidative properties of betel and curry leaves were attributed to phenolic compounds, predominantly hydroxyl chavicol (Patel and Rajorhia, 1979). These leaves also contained some ascorbic acid which might work as synergist (Sethi and Aggarwal, 1956). When betel, curry and drumstick leaves were added at 1.0 and 3.0 per cent levels to ghee, which was subsequently stored for 12 m at ambient temperature, only curry leaves could protect ghee from hydrolytic rancidity and nonecould prevent oxidative deterioration (Thakar et al., 1984).

Mango seed kernel

A study was initiated by Parmar (1984) to elucidate the effect of addition of mango (*Mangifera indica*) seed kernels or its pre-extract on oxidative stability of ghee. Dried mango seed kernel powder (MSKP) added at 1.0, 1.5, 2.0 and 2.5 per cent (w/v) levels and butylated hydroxyl anisole added at 0.02 per cent level to buffaloes' milk ghee had antioxidant potentialities in the orders : 2.5 per cent MSKP > 2.0 per cent MSKP > 1.5 per cent MSKP >
0.02 per cent BHA > 1.0 per cent MSKP. The main antioxidant principles were indicated to be various types of phospholipids and the phenolic compounds of mango seed kernels. In addition to these compounds, the other possible agents were stated to be sterols, vitamin C, carotene and the interaction products of carbohydrates and protein generated during the heating process (Parmar and Sharma, 1990). Dinesh et al. (2000) isolated the antioxidant principles namely phenolics and phospholipids from MSKP using organic solvents.

These compounds were dissolved in ghee to prepare phenolic and phospholipids extracts separately and in combination. Addition of extract in combination was more effective than individual extract. Moreover the phenolics were more effective than phospholipids in prolonging the induction period of ghee. Addition of extracts either individually or in combination at a level of 5 per cent or above were more effective in increasing the stability of ghee than addition of BHA at a 0.02 % level. It was concluded that the phenolic compounds in MSKP seemed to be the main antioxidative compounds which along with phospholipids gavethe maximum stabilizing effect to ghee against oxidative deterioration.

Seed phospholipids

Bhatia et al., (1978) isolated phospholipids from sunflower seed, groundnut seed and cotton seed and added to ghee. The antioxidant potentiality of whole phospholipids from these sources was in order: sunflower > groundnut > soybean > cotton seed. This was in order of decreasing phosphatidyl ethanolamine content. Gupta et al. (1979) isolated lecithin and phenolic compounds from gram seeds (Cicerarietinum). They observed that phospholipids from this source could be good antioxidant for ghee. Kauret al. (1982) compared the seed phosphatides and synthetic compounds as antioxidants for cow and buffalo ghee. They found that antioxidant efficiency of sunflower seed oil phosphatides and synthetic compounds were in order: phosphatidylethanolamine> propyl gallate> palmitoylascorbate> BHA> phosphatidyl choline. The authors concluded that seed phospholipids were more effective than many synthetic antioxidants in controlling oxidative and lipolytic deterioration of ghee duringstorage.

Spices and condiments

Semwal et al., (1997) studied anti- or pro-oxygenic activity of turmeric (Curcuma longa) by adding its fractions (volatile oil and curcumin) in ghee at 37 ºC. The ground spice and water-soluble fraction of the spice showed anti-oxygenic activity. On the other hand curcumin, water-insoluble fraction, acetone soluble, ethanol soluble and insoluble fractions of turmeric showed moderate pro-oxidant activity.Volatile oil of turmeric also exhibited slight anti-oxygenic activity. Combination of alpha-tocopherol and curcumin showed moderate pro- oxygenic activity. Soni (2011) reported that addition of curcumin in ghee provided better storage life of ghee than control sample. He reported that addition of curcumin powder at 0.4% gave ghee higher flavor value and lower peroxide value as compare to control sample during accelerated storage and he also reported that addition of curcumin had not

create any colour defect in ghee.

The antioxidant property is present in the plant materials due to many active photochemical which include the vitamins, flavonoids, terpenoids, carotenoids, cumerins, lignin, saponin, plant steroids etc. The citrus fruits and their juices are an important source of the bioactive methanol, the compound are an important to human nutrition which including the antioxidants such as absorbic acid, phenolic compounds, flavanoids and pectin.

MATERIALS AND METHODS

EXTRACTION OF ANTIOXIDANTS FROM FRUIT PEELS

Sample collection

Pomegranate and orange were collected from local market. The peels were separated, washed and dried under sunlight. The dried peels were then ground into fine powder.

Separation of fruit peel extract

The dried powders of peels were extracted by cold percolation method using ethanol as a solvent. 10 g of the dried powder was taken in 100 ml of ethanol in conical flask, plugged with cotton wool and then keep in an orbital shaker at 120 rpm for 24 hours. After 24 hours the extract was filtered through Whatman filter paper No.41 for removal of peel particles and concentrated under vacuum at 40°C. The dried extract was stored at 4°C.

DETERMINATION OF EXTRACTION YIELD

The residues obtained after filtration were weighed to obtain the extraction yield.

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the residue} \times 100}{\text{Total weight of the peel powder}}$$

BIOCHEMICAL REACTIONS

Determination of total phenolic content

The concentration of total phenolic content was measured using UV spectrophotometer, based on colorimetric oxidation/reduction reaction using Folin-Ciocalteu reagent. Specifically, 2ml of diluted extract was mixed with 0.5ml of Folin-Ciocalteu reagent and 1.5ml of 20 % Na_2CO_3. The sample was incubated for 5 minutes at 50°C then cooled for 5 minutes. It was made up to 10ml using distilled water. The absorbance was measured at 760nm. Gallic acid was used as a positive control.

Determination of total flavonoid content

The flavonoid content was determined according to aluminium chloride colorimetric method.

The reaction mixture consisting in a final volume of 3 ml, 1.0 ml of sample (1 mg/ml) 1.0 ml ethanol and 0.5 ml of (1.2%) aluminium chloride and 0.5 ml (120 mM) potassium acetate was incubated at room temperature for 30 minutes. The absorbance of all the samples was measured at 415 nm. Quercetin was used as a positive control.

Determination of antioxidant property by FRAP assay

The ferric ion reducing power of petroleum ether and ethanol extracts of fruit peels was determined by modified ferric ion reducing antioxidant power [FRAP] assay. The principle of this method is based on the reduction of a ferric-tripyridyltriazine complex into its ferrous, colored form in the presence of antioxidants. Briefly, FRAP assay was done by adding 1.5ml of 1M HCl,1.5ml of 1%potassium ferricyanide,0.5ml of 1% SDS and 0.5ml of 0.2% $FeCl_2$ to the different aliquots of stock solution prepared and kept for water bath at 50°C for 20 minutes. After cooling, the absorbance of all the samples was measured at 700nm. The final result was expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1mmol/L of $FeSO_4$. The increase in absorbance is used to measure the reducing powerof peel extract solution.

Percentage of ferric reducing activity of the test solution was calculated using the following formula:

$$\text{Inhibition } \% = (1\text{-}A/A1) \times 100$$

Where,
A is the absorbance of control (blank, without extract) and A1 is the absorbance of the sample or extracts.

PREPARATION OF GHEE WITH ANTIOXIDANT EXTRACTS

Fresh standardized ghee containing free fatty acid (FFA) as oleic acid is 3%, moisture content not more than 0.3%, specific gravity of 0.9340 and viscosity being 30.893CP was collected from Milma diary, Kallepully branch, Palakkad, Kerala. One sample was prepared by adding 200ppm of pomegranate in ethanolic extract and other was prepared by adding 200ppm orange extract. A control was also prepared with no extracts added in ghee. 200ppm of synthetic additive BHA was added to one sample. It was stored in a hot air oven at 60-80°C for 20 days. Peroxide value was checked after every four days for these samples.

PEROXIDE VALUE DETERMINATION OF FRESH GHEE

Evaluation of lipid peroxidation

Ghee was collected. An accelerated oxidation test was performed to accelerate lipid oxidation in ghee sample. Before analyzing the peroxide value, ghee sample was kept in an oven maintained at 60-80°C for 20 days. Lipid oxidation was determined according to the changes in peroxide value.

Determination of peroxide value

A clean dry boiling tube was used to measure 1g of hot oxidized ghee sample.1g of powdered

potassium iodide was added and then 20 ml of solvent (2 vol. glacial acetic acid + 1 vol. chloroform) was added into the tube. The tube was placed in boiling water such that the mixture boils within 30 seconds and then allowed to boil vigorously for more than 30 seconds before it is poured quickly into a flask containing 25 ml of water and the mixture in the flask was titrated against with 0.01 N sodium thiosulphate solution using starch as an indicator. The blank was performed at the same time. The experiment was repeated and calculation can be done as;

$$\text{Peroxide value} = \frac{[S-B] \times N \times 1000 \times 8}{w \times 1000}$$

Where,
S= Volume (ml) of sodium thiosulphate required in titration of sampleB= Volume (ml) of sodium thiosulphate required in titration of blank N= Extract normality of sodium thiosulphate solution
w= Weight (gm) of sample

RESULT AND DISCUSSION

Extraction Yield (%) of fruit peels

The table shows the extraction yield of pomegranate and orange peel.

Fruit peels	Extraction yield (%)
Pomegranate peel	51.4
Orange peel	49.8

Among the two fruit peels, maximum yield of antioxidants using ethanol as a solvent was extracted with pomegranate peel which showed the maximum yield of 51.4 % and orange peel showed an extraction yield of 49.8 %. The extraction yield of antioxidants from fruit peels depends upon the solvent used for extraction. Ethanol and water are the most widely used solvents for hygienic and abundance reasons, respectively.

6.2 Total phenolic content and total flavanoid content

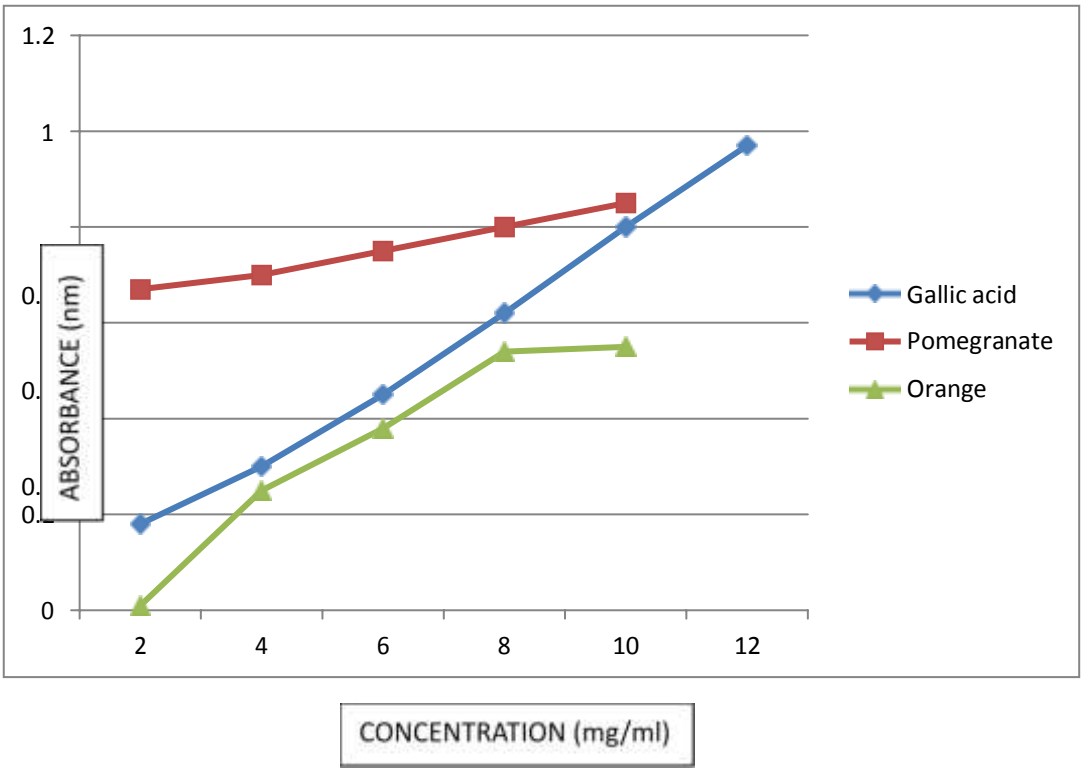
The total phenolic content was found to be maximum in pomegranate peel and minimum in orange peel. Ethanol was used as the solvent in the extraction of phenolic compounds due to its ability to inhibit the reaction of PPO that causes oxidation of phenolic compounds and its ease of evaporation as compared to water. Total flavanoid content was found to be maximum in pomegranate peels and minimum in orange peels.

The giventables provide the total phenolic content in,

Sample	Optical density

(pomegranate extract)	
S1	0.67
S2	0.70
S3	0.75
S4	0.80
S5	0.85
Sample (orange extract)	Optical density
S1	0.01
S2	0.25
S3	0.38
S4	0.54
S5	0.55

The graph provided below gives a comparison between the two samples with gallic acidstandard,



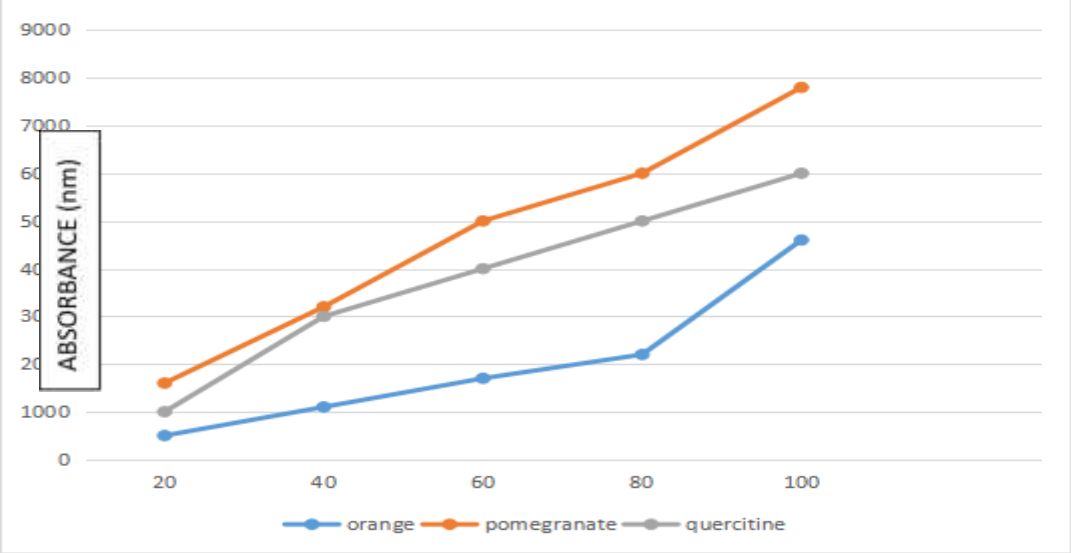
Flavonoids are a diverse group of phytonutrients found in almost all fruits and vegetables. Along with carotenoids, they are responsible for the vivid colors in fruits and vegetables. Like other phytonutrients, flavonoids are powerful antioxidants with anti- inflammatory and immune system benefits. The total flavanoid content assay performed with the two samples shows a maximum in pomegranate peels and minimum in orange peels, usingquercetin as standard.

The below tables provide the total flavanoid content in,

Sample	Optical density at 415 nm

(pomegranate extract)	
S1	0.10
S2	0.20
S3	0.30
S4	0.40
S5	0.48
Sample (orange extract)	Optical density at 415 nm
S1	0.08
S2	0.16
S3	0.25
S4	0.30
S5	0.39

The graph below provides a comparison between the two samples with quercetin standard,



CONCENTRATION

Total antioxidant activity

Ferric reducing antioxidant power (FRAP) assay is a widely used method that uses antioxidants as reductants in a redox-linked colorimetric reaction, wherein Fe3+ is reduced to Fe2+.The FRAP assay is high-throughput, adaptable and can detect antioxidant capacities as low as 0.2mM Fe^{2+} equivalents. The yellow color test solution changes to green or blue depending on the reduction capacity of extracts or compounds. The reduction was determined by decrease in absorbance at 550nm. The given table shows the % of antioxidant activity of the samples.

Sample	Optical density at 550nm	Antioxidant activity (%)

(pomegranate extract)		
S1	0.483	35.81
S2	0.750	58.67
S3	0.970	68.04
S4	1.184	73.81
S5	1.475	78.98
Sample (orange extract)	Optical density at 550nm	Antioxidant activity (%)
S1	0.432	28.24
S2	0.478	35.14
S3	0.520	59.61
S4	0.573	54.10
S5	0.601	51.58

Effect of antioxidant extracts on the peroxide value of ghee

Peroxide Value is commonly used to determine the rancidity of a sample containing fat or oil subject to oxidation.BIS method of expressing the peroxide value is done by calculating the amount of 0.002N sodium thiosulphate used in titration.

From the peroxide value, the quality of ghee can be assessed as follows:

Peroxide value in terms of volume in ml of 0.002N sodium thiosulphate used per gm offat	Quality of ghee
< 1.5	
1.6 – 2.0	
2.1 – 2.5	Very goodGood Fair Poor
2.6 – 3.5	Not acceptable
3.6 – 4.0	

200ppm concentration of ethanolic extracts of orange, pomegranate and BHA was added to the ghee. Peroxide values of ghee samples were calculated after a 20 day period of auto-oxidation. Ghee sample with BHA was also maintained as a control. BHA Butylated Hydroxyanisole is a synthetic antioxidant that is used to prevent fats in foods from going rancid. The peroxide values of ghee after addition of peel extracts on different days are as follows:

DATE : 27/01/2020 Sample used	Peroxide value
Blank	0
Ghee with ethanolic extract of pomegranate	0.0448
Ghee with ethanolic extract of orange	0.064
Ghee with BHA	1.60
Control	0.064

DATE : 31/01/2020	Peroxide value
Sample used	
Blank	0
Ghee with ethanolic extract of pomegranate	0.056
Ghee with ethanolic extract of orange	0.0688
Ghee with BHA	1.67
Control	0.0688

DATE : 03/01/2020	Peroxide value
Sample used	
Blank	0
Ghee with ethanolic extract of pomegranate	0.061
Ghee with ethanolic extract of orange	1.2
Ghee with BHA	1.701
Control	1.28

DATE : 07/02/2020	Peroxide value
Sample used	
Blank	0
Ghee with ethanolic extract of pomegranate	0.082
Ghee with ethanolic extract of orange	1.8
Ghee with BHA	1.76
Control	1.90

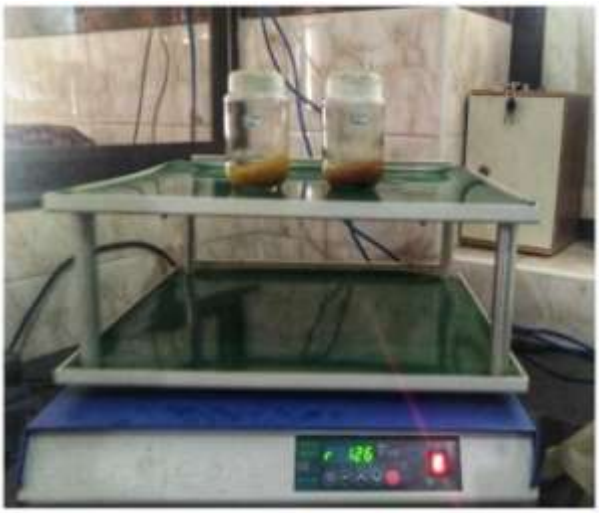
DATE : 11/02/2020	Peroxide value
Sample used	
Blank	0
Ghee with ethanolic extract of pomegranate	1.1
Ghee with ethanolic extract of orange	2.4
Ghee with BHA	1.78
Control	2.088

DATE : 18/02/2020	Peroxide value
Sample used	
Blank	0
Ghee with ethanolic extract of pomegranate	1.9
Ghee with ethanolic extract of orange	4.8057
Ghee with BHA	2.07
Control	4.7

DATE : 20/02/2020	Peroxide value
Sample used	
Blank	0
Ghee with ethanolic extract of pomegranate	2.004
Ghee with ethanolic extract of orange	4.988
Ghee with BHA	2.09
Control	5.9904

From the above data it can be concluded that the ethanolic extract of pomegranate shows less peroxide value due to its high antioxidant property, which is evident on comparison with BHA. Whereas, the ethanolic extract of orange shows more peroxide value than pomegranate and BHA thus showing less antioxidant property. While the control with no synthetic additive- BHA and ethanolic extracts of pomegrante and orange shows high peroxide value resulting inrancidity very easily.

1. EXTRACTION OF ANTIOXIDANTS FROM FRUIT PEELS

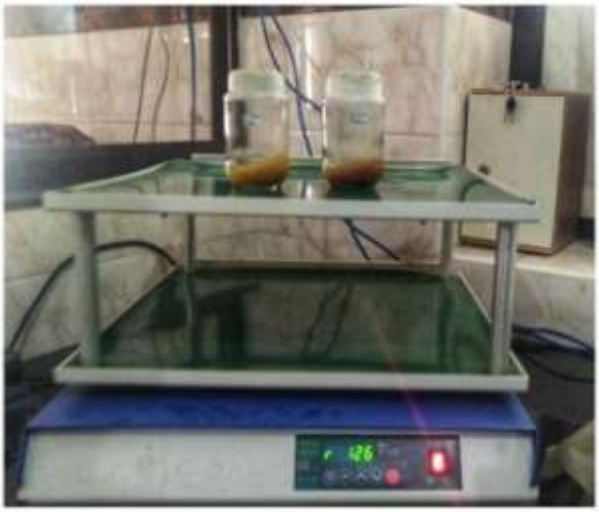



DETERMINATION OF EXTRACTION YIELD

2. BIOCHEMICAL REACTIONS

a. DETERMINATION OF TOTA PHENOLIC CONTENT


b. DETERMINATION OF TOTAL FLAVONOID CONTENT




PREPARATION OF GHEE WITH ANTIOXIDANT EXTRACTS

3. PEROXIDE VALUE DETERMINATION OF FRESH GHEE

SUMMARY AND CONCLUSION

Pomegranate and orange peels are good natural antioxidants due to presence of polyphenolic compound which are known to be potent antioxidant. Pomegranate peels and orange peels extracts were prepared using ethanol and *in vitro* antioxidant activity of each extract was investigated in ghee. Since ghee is fat rich products, antioxidant plays an important role in preventing rancidity. In general it was observed that extracts with higher antioxidant capacity were in parallel with their higher phenolic contents. Ethanol 80% showed better characteristics as a solvent for phenolic compound extraction. Thus, for use in the food industry, ethanol 80% would be a more appropriate solvent. The maximum antioxidant activity was found in pomegranate and minimum in orange peels. It can be concluded from the study that pomegranate peels due to high antioxidant activity and phenolic content may prove to be better substitute in place of synthetic antioxidants in extending shelf life of ghee by preventing peroxide formation. In addition natural antioxidants are safe and impact health benefits like protection against the cell damage that the free radicals cause known as oxidative stress. As long as they are consumed in moderate concentrations, they have been proven to have many positive health effects, such as preventing plaque formation in the arteries and preventing other chronic conditions such as cancer and heart disease. These beneficial properties have put natural antioxidants on the forefront of recent food advertising and public levels of awareness concerning natural antioxidants and their positive effects have increased significantly.

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