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RESEARCH ARTICLE

UTILIZATION OF NATURAL ANTIOXIDANT FOOD PROCESSING WASTE MATERIAL FOR OXIDATIVE STABILIZATION OF GHEE

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ABSTRACT

The growing interest in the replacement of synthetic food antioxidants has led to multiple investigations in the field of naturally-sourced antioxidants. The search for cost-efficient natural antioxidants has led to the exploration with raw materials of residual origin. The present introduction of lipid oxidation and the antioxidant mechanisms, as well as the most recent research on the recovery and utilization of food processing wastes. Most studies found high levels of compounds with antioxidant activities in waste materials, encompassing a wide category of fruits and vegetables, roots and tubers, grains and seeds. Bioactive compounds found in granules, pallets and powder (peels of banana, apple, pomegranate, orange and tulsi leaves).

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INTRODUCTION

Ghee is an anhydrous milk fat, occupies a prominent place in the Indian diet. Chemically ghee is a complex lipid of mixed glycerides together with a small amount of free fatty acids, phospholipids, sterols and their esters, fat soluble vitamins (A, D, E and K), carotenoids, carbonyl compounds, hydrocarbons, charred casein, moisture and traces of trace elements like copper and iron. Oxidative rancidity is the major pathway by which ghee undergoes deterioration. This is referred to as autoxidation because the rate of oxidation increases as the reaction proceeds under usual processing and storage conditions. Several workers have done exhaustive work to improve the stability of ghee against autoxidation through feeding specific feed to milch animals (Tandon, 1977; Hagrass et al., 1983), altering processing parameters (Singh et al., 1979), using proper packaging materials and storage conditions (Chauhan and Wadhwa, 1987; Amr, 1990a), adding synthetic antioxidants, incorporating natural antioxidants from edible plant materials, spices and condiments, aromatic herbs etc. 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 et al., 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). Consequently, the search for endogenous protective ingredients in foods has been intensified where in their utilization requires only manipulation of food formulations. A number of natural antioxidants have been added during food processing and have elongated the shelf life and oxidative stability of stored products (Chenn et al., 2008; Ebrahimabadi et al., 2010; Jang et al., 2012; Xiaowei et al., 2011). A huge amount of plant biomass wastes are produced yearly as by-products from the agro-food industries. These wastes 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, antiatherogenic, anti-inflammatory, anti-microbial, antioxidant,anti-thermobiotic, cardio protective and vasodilatory 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 extracted from raw materials or waste products of food industry (Peschel et al., 2006). Studies were conducted to investigate antioxidant properties of Pomegranate, Tulsi leaves, Banana, Apple and Oranges has been used extensively in the folk medicine of many cultures and its consumption has grown tremendously especially in the

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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). The study, set of sample of granuine butterfat from three different localities were chosen and stored under the first-mentioned, more or less 'normal' condition.

Fruit Peels and leaves used: The peel and seed of three varieties of avocado (Shepard, Hass and Fuerte) showed activity against yeast, gram negative and gram-positive bacteria (Chia, Wah, & Dykes, 2010). The peel and seed extracts of avocado Hass with a minimum inhibitory concentration value of 104.2 µg/mL is the most effective against Salmonella enteriditis and Zygosaccharomyces bailii, respectively (Chia et al., 2010). Despite the high content of bioactive compounds in the skins and seeds of exotic fruits, attention must be paid to antinutritional and toxic factors, like high tannin content in these tissues (Abdalla et al., 2007). Tannins are considered nutritionally undesirable because they precipitate proteins, inhibit digestive enzymes and affect the utilization of vitamins and minerals. However, many tannin molecules have been reported to reduce the mutagenicity of a number of compounds and it all depends on the concentration at which it is used or consumed. To avoid these problems it is recommended that during the preparation of extracts from these byproducts, acidic and/or alkaline hydrolysis are recommended in order to inactivate these compounds.

Pomegranate: Pomegranate (*Punica granatum* L.) is native to the Mediterranean region and has been used extensively in the folk medicine of Indian subcontinent and many other countries. The world pomegranate production amounts to approximately 1,500,000 tons (FAO 2012). The pomegranate peel is considered as an agro-waste but it can be a potential source of antioxidants, phenols, flavanoids and also possesses antibacterial and antifungal activity. The peels (pericarp, rind or hull) amounts to approximately 60 % of the weight of the pomegranate fruit (Lansky and Newman 2007). The peel of pomegranate possesses higher total phenolic content and antioxidant activity than the pulp (Li et al. 2006). Elfalleh et al. (2012) reported that pomegranate peel contains higher antioxidant activity when compared with flower, leaf and seed. The antimicrobial and antioxidant potentials of pomegranate peel and seed extract were investigated in chicken products (Kanatt et al., 2010). Pomegranate peel extract (PE) showed excellent antioxidant activity while the seed extract did not have any significant activity, probably to the difference in the type and amount of bioactive compounds present in both tissues. Pomegranate peel extract showed good antimicrobial activity against Staphylococcus aureus and Bacillus cereus. In general, addition of pomegranate peel extract to popular chicken and meat products enhanced its shelf life by 2–3 weeks, during chilling temperature storage. PE was also effective in controlling oxidative rancidity in these ghee products (Kanatt et al., 2010).

Orange: Orange fruit, contributes up to 70 % of citrus fruits production, is widely cultivated citrus fruit in tropical and subtropical climates spreading over 130 countries. It is available both in winter and summer season. Sweet orange (*Citrus sinensis*) is the major citrus fruit produced worldwide (Rouseff, 2007) and processed commercially for orange juice in the industries generating huge quantity of peel (peel is the primary by-product range from 20 to 50 % of total fruit weight) which can efficiently be utilized for food supplements, food preservatives and food preparations (Kumar et al. 2011; Anagnostopoulou et al. 2006). Several researchers reported that orange peel is a good natural source of phytoconstituents which exhibit antioxidant activities by reducing the concentration of local free radicals, neutralizing free radicals and by chelating metals (Bombardelli and Morazzoni 1993). Orange peels contain high amount of total phenolics (a phytoconstituents) than edible portions of the fruits (Yassari and Yasari 2013). Antioxidant activities of citrus were due to the presence of total phenolic compounds (Mour et al. 2001). Antioxidant compounds of citrus peel not only play an important and physiological role but are also of commercial interest because of their multitude of applications in the food and pharmaceutical industries activities of the world. Therefore, nowadays, considerable interest is focused on the development and evaluation of natural antioxidants and radical scavengers from plant materials which are rich in polyphenolic compounds (Dubey et al. 2011; Mathur et al. 2011). Orange peel extract can be used as natural antioxidant (Bombardelli and Morazzoni 1993) in corn oil (Rehman 2006); in soybean oil (Gharahkhani et al. 2010; Abdelaal and Halaweish 2010); in canola oil (Yassari and Yasari 2013) for exhibiting antioxidant activities. The rapid increase in use of natural antioxidants in food industry (Beddows et al. 2001) has attracted the researchers to extract natural antioxidants from citrus peels and the use of those natural and safe substances in foods for the purpose of preventing rancidity and inhibiting lipid oxidation (Anagnostopoulou et al. 2006; Peschel et al. 2006; Rehman 2006; Yassari and Yasari 2013).

Tulsi: Sharma (1997) isolated the antioxidant principles of Tulsi (Ocimum sanctum Linn.) leaves via 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 per cent (w/v) was found to be more effective than the addition of BHA at the level of 0.02 per cent. The phenolic compounds appeared to be the main scontributory factors in enhancing the oxidative stability of ghee.

MATERIALS AND METHODS

Material used: The required sample for the product development are. Ghee (homemade), Fruits peel (banana, apple, orange, pomegranate), Tulsi leaves, Chemical- Acasia Which were taken in 1:1 ratio which is recommended amount of consumption of ghee and fruits peel in day to day life.

Collection of fruits peel: Orange , Pomegranate, Banana and Apple where purchased from local market of Lucknow area, then properly wash the fruits and peeling the fruits peel, and collect its properly.

Preparation of granules, pallets and powder form by fruits peel: This phase involve the whole idea of development of powder, granules and pellets preparation by using equal ratio of fruits peel and tulsi leaves 1:1:1:1:1 (i.e- 10gm apple peel, 10gm pomegranate peel, 10gm banana peel, 10gm orange peel and last 10gm tulsi leaves).

Antioxidants present in fruits peel: There are different attributes to classify the antioxidants. The first attribute is based on the function (primary and secondary antioxidants).

Table 1. Determination of Vitamin C in fruit peel

S.No	Parameter	Results	Test method
1	Vitamin C-mg/100g	40.45	IS:5886:1970(RA2010)

Table 2. Effect of fruits peel granules at different concentration on the PV (meq.O2 /kg fat) of ghee during storage

Treatments	Storage period (months)		
	0	3	6
C	0.87a± 0.02	1.95a± 0.2	2.22a± 0.04
T1	0.85a± 0.01	0.92d± 0.03	1.11ef± 0.02
T2	0.85a± 0.02	0.91d± 0.02	1.08f± 0.02
T3	0.86a± 0.02	0.91d± 0.01	1.08f± 0.02
Total	0.017	0.020	0.021

C: control without antioxidants.
T1: ghee treated with 200, 400 and 600 ppm granules extracts, respectively.
T2: ghee treated with 200, 400 and 600 ppm granules extracts, respectively.
T3: ghee treated with 200, 400 and 600 ppm granules extracts, respectively.
Means with different letters within the same column differ significantly at P< 0.01–0.05.

Table 3. Effect of fruits peel pellets at different concentration on the PV (meq.O2 /kg fat) of ghee during storage

Treatments	Storage period (months)		
	0	3	6
C	0.013a± 0.001	0.13a± 0.001	0.015a± 0.002
T1	0.013a± 0.001	0.013d± 0.001	1.015ef± 0.002
T2	0.014a± 0.0201	0.013d± 0.001	1.015f± 0.002
T3	0.011a± 0.001	0.014d± 0.002	1.013f± 0.003
Total	0.013	0.020	0.0021

C: control without antioxidants.
T1: ghee treated with 200, 400 and 600 ppm pallets extracts, respectively.
T2: ghee treated with 200, 400 and 600 ppm pallets extracts, respectively.
T3: ghee treated with 200, 400 and 600 ppm pallets extracts, respectively.
Means with different letters within the same column differ significantly at P< 0.01–0.05.

Table 4. Effect of fruits peel powder at different concentration on the PV (meq.O2 /kg fat) of ghee during storage

Treatments	Storage period (months)		
	0	3	6
C	0.013a± 0.001	0.13a± 0.001	0.015a± 0.002
T1	0.013a± 0.001	0.013d± 0.001	1.015ef± 0.002
T2	0.014a± 0.0201	0.013d± 0.001	1.015f± 0.002
T3	0.011a± 0.001	0.014d± 0.002	1.013f± 0.003
Total	0.013	0.020	0.0021

C: control without antioxidants.
T1: ghee treated with 200, 400 and 600 ppm powder extracts, respectively.
T2: ghee treated with 200, 400 and 600 ppm powder extracts, respectively.
T3: ghee treated with 200, 400 and 600 ppm powder extracts, respectively.
Means with different letters within the same column differ significantly at P< 0.01–0.05.

The second attribute is based on enzymatic and non enzymatic antioxidants.In fruits peel, Vitamin C, Vitamin E and Carotenoids are present. In this fruits peel analysis the how much quantity of Vitamin C are found. The testing of Vitamin C is in Rfrac (Regional Food Analysis & Research Center) laboratory, lucknow.

Table 5. Ghee with fruits peel powder, granules and pellets

S.No.	Parameter	Results	Test method
1.	Rancidity	Absent	FSSAI Manul

In ghee fruits peel powder, granules and pallets, rancidity are not present in the storage of 6 months.

Determination of Vitamin C in fruits peel by IS: 5886:1970 (RA 2010)

Purification

Preparation of column: Connect the chromatographic tube to a Witt's or equivalent filtering apparatus with a beaker (150 ml) as receiver or to a suction flask of 250 ml capacity, through a rubber stopper. Loosely plug the lower end of the chromatographic tube with glass wool or cotton and turn on the suction. Add adsorption mixture through a funnel in small amounts to a height of 10 to 12 cm and pack the column by pressing down with a cork-stopper, just fitting the tube and attached to a glass rod. Place on the top of the column 2 to 3 cm layer of anhydrous sodium sulphate.

Tool: The testing method used- IS: 5886:1970(RA 2010).

Antioxidant activity (Inhibition) %
(A control-A sample) = (A control) multiply by 100

Where,
(A control)- is the absorbance of the control reaction and
(A sample)- is the absorbance in the presence of extract.

Rancidity and shelf life: The products were in excellent condition when originally analyzed, but, when the study was undertaken, had been maintained in storage under the conditions mentioned earlier for 3-4 years. The characteristics were determined in the usual way: acidities are expressed as acid values (mg. KOH/g. fat); peroxide values as ml. 0-002w-Na2S203/g. fat; and refractive indices in terms of Butyro refractometer degrees at 400.

Stability of ghee enriched with food processing wastes: The butter, used for preparing ghee in the present study, was made from pasteurized and un-ripened buffaloes' cream. The butter was converted into ghee by boiling. Ghee samples were divided into four portions and treated as follows:

Portion (1) was kept without additives and was considered to be as a negative control (C). Portions (2) were treated with 200 ppm of PS ethanolic extract respectively, (T1). Portions (3& 4) were treated with 200 & 400 of OP & AP ethanolic extract respectively, (T2 & T3). All samples were incubated in an oven at 63 ± 1C to accelerate the oxidation for 6 months. Samples were analyzed every three days for peroxide value (PV), acid value (AV), and 2-thiobarbituric acid (TBA) value.

Conclusion

Food rich in bioactive compounds has become an important approach for more consumers, to achieve their desires to reduce the risk of a specific disease or a health problem and to treat minor illnesses. These are also important for improved utilization of food and agricultural products. The levels of actives in the wastes are usually found to be higher than the actual products.Granules, pallets and powder gave good antioxidant activity during accelerated oxidative incubation of ghee. The oxidative stability of ghee is rich in fruits peel powder. They keep store at long time at home.

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