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Effect of microwave pretreatment on extraction yield of pomegranate seed (cv. Bhagwa) oil

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Abstract
Present experiment was conducted to study the effect of microwave pretreatment on recovery and quality of pomegranate seed oil. Samples were pretreated in microwave oven at microwave power levels (MWP) of 360, 540, 720 and 900 W for pretreatment time (PT) of 30, 60, 90, and 120 seconds and extracted for 3, 4 and 5 h extraction time (ET). The extraction yield was increased with increase in microwave power applied, pretreatment time as well as extraction time. Light microscopy was carried out to study the effect of microwave radiation on the microstructural changes in cell structure pomegranate seeds. It was observed that, the extraction yield from the microwave treated samples was higher as compared to control samples. The microwave pretreatment was also found to be effective in reducing the extraction time. Light microscopy showed the rupture of cells and loosening of oil bodies. The optimum conditions for microwave pretreatment were 720 Watt, pretreatment time of 60 s and extraction time of 4 h.

Keywords: pomegranate seeds, oil, extraction, microwave, light microscopy, lipid bodies

Introduction
Pomegranate (*Punica granatum* L.) is important horticultural crop of India. The area under pomegranate cultivation in India is 1.81 Lakh ha with production of 17.89 Lakh tons in year 2014-15 [1]. The Maharashtra is the largest producer of pomegranate in India followed by Karnataka, Gujarat, Andhra Pradesh, and Rajasthan. The increased production of pomegranate has led to glut in the markets and corresponding fall in price. The processing of pomegranate in to various value added products will provide sustained demand in market and thus will improve the profit of pomegranate producers. The increased production of fruits had led to start of new pomegranate processing and value addition industry.

The edible part of pomegranate is called as aril. The pomegranate fruit contains approximately 40% juice, 10% seeds and 50% peel. The pomegranate is primarily processed for extraction of juice and ready to serve beverage. The juice industry has seeds as major co-product. Pomegranate seeds are excellent sources of dietary fiber. The soft seeded varieties of pomegranate contain seed oil to the tune of 25-26% (V/W). The pomegranate seed oil contains more than 70% of conjugated linoleic acid. The Pomegranate seed oil contains Punica acid (65.3%), politic acid (4.8%), stearic acid (2.3%), oleic acid (6.3%) and linoleic acid (6.6%).

Conjugated fatty acids are important because they inhibit eicosanoid metabolism at several points in the synthesis of prostaglandins from arachidonic acid. This makes them significant natural anti-inflammatory agents. The supplementation of pomegranate seed oil along with tamoxifen to breast cancer patients reduces the serum tumor marker levels [2]. Pomegranate seed oil facilitates skin repair by promoting regeneration of epidermis. Pomegranate seed oil contains other significant bioactive compounds. For example, Pomegranate Seed Oil is the richest known plant source of a steroidal estrogen, stone. Other important compounds found in Pomegranate seed oils include gamma-tocopherol, a rare and potent form of Vitamin E and the phytosterols: beta-sit sterol, stigma sterol and campestral. It has been linked to improve heart health and also indicates to protect against cancer [3; 4] and atherosclerosis [5].

Conventional vegetable oil extraction is carried out by pressing or solvent extraction. Solvent oil extraction is usually used for seeds that contain low amounts of fat (<20%), such as soybeans [6]. Three main disadvantages of conventional Soxhlet extraction include first, the extraction time is long; second a large amount of solvent is used; in addition, n-hexane, the

main component of commercial hexane, is listed as No. 1 on the list of 189 hazardous air pollutants by the US Environmental Protection Agency parameters lead to wide criticism of the conventional Soxhlet extraction method [7]. Since efficiency of conventional extraction for oil seeds with low fat/oil content are not deniable, utilization of microwave pretreatment enable to improve conventional extraction through decreased solvent consumption and time of processing.

Microwave pretreatment of oil seeds before oil extraction is preferred choice owing to improvement in oil recovery as well as for retention nutritional values. Several researchers have demonstrated this in different oil seeds such as soybean [8], rapeseed oil [9], palm oil [10], pumpkin seeds [11], Chilean hazelnuts [12]. Microwave pretreatment for oil extraction has many advantages e.g. improvement of extracted oil yield and quality, direct extraction capability, lower energy consumption, faster processing time and reduced solvent contents [13].

Limited information is available in the literature associated to application of microwave radiation as a pretreatment for pomegranate seed and its effect on extraction yield and quality of oil. The objective of the present study was to probe the influence of microwave radiation pretreatment power and time upon recovery of oils and extraction time.

Materials and Method

Pomegranate seeds

Pomegranate fruits were harvested from ICAR-NRCP farm, Solapur. The harvested fruits were graded based on size and visual appeal. The top grade fruits were marketed for table purpose consumption while remaining fruits after removal of rotten fruits were used for extraction of arils using modified CIPHET pomegranate aril extractor (Make: Padmatech, India). The juice is extracted from arils using the hydraulic juice press (Make: Johnston Automation Co.). The marc was then soaked in water for 48 hours and subjected to fruit pulper. The clean seeds were dried in tray dryer at 40 °C up to 6% db. The dried seeds were stored in desiccator until used for oil extraction.

Microwave pretreatment

For each microwave pretreatment experiment 15 g of pomegranate seed were arranged in a single layer in pyrex petri dish. The sample was placed on the external border of the turntable plate of the microwave variable power oven (Make: LG Electronics India Pvt Ltd., India; Model: MC2844SPB). Sample was microwave-treated at a frequency of 2,450 MHz. The seeds were pretreated at 360, 540, 720, and 900W microwave power for 30, 60, 90 and 120 seconds. The samples without pretreatment were served as control.

Soxhlet extraction

Pretreated and control seeds were crushed using mortar and pestle. Crushed pomegranate seeds (10 g) were used for soxhlet extraction. The extraction for all treatment combination was carried out for 3, 4 and 5 h with soxhlet extractor using petroleum benzene (Himedia, India) as a solvent at 60 °C [14].

Microscopic seed examination

Light microscopy was carried out to study the effect of microwave radiation on the microstructural changes in pomegranate seeds. Pomegranate seed kernels (i.e. dehulled seeds) microwave pretreated at optimum conditions was microscopically examined to determine the physical effects of the treatments on the kernel cotyledon/cell structure. The cut section of seed kernel for microscopic imaging was prepared using modified method followed by [15]. Wherein, the kernel samples were cut with sharp razor blade. Cut section was placed on clean and dry glass slide. The ethanol, histoclear, single distilled water and toluidine blue (as stain) were added at 1 minute intervals each. The slides were flooded with water, and as soon as the water douches the first section, DePex was put on a coverslip, placed over sections and allowed to dry overnight. A Nikon (Eclipse 90 i, Kawasaki, Japan) light microscope equipped with a Nikkon (DS- Ri 1 model, Kawasaki, Japan) photographic camera was used to view and record representative images.

Statistical analysis

The oil extraction experiment was conducted in triplicate, and the results were reported. Statistical significance of the differences between mean values was assessed by GLM procedure of SAS with Tukeys multiple comparison test to compare the means.

Results and Discussion

Effect of microwave pretreatment and extraction time on oil yield extraction

Table 1 shows the effect of microwave pretreatment and extraction time on oil yield from pomegranate seeds. Oil yield was affected by all the three factors significantly. It was found from the results that, extraction yield was increased with increase in the extraction time. Oil yield for 3, 4 and 5 h in case of control samples was 18.00, 19.38 and 20.72% respectively which indicates the higher yield for longer extraction time. Increase in extraction yield with increasing the time was evident for garden cress seed oil [16] and Chilean hazelnuts [12]. Elkhaleefas and Shigidi1(2015) [17] examined the impact of the residence time between the sesame seeds and n-hexane and reported that, more the time seeds were given in contact with the solvent, higher was the extraction yield obtained. This was true for 24 hours, afterwards no more extraction had been noticed.

Table 1: Oil extraction yield from pomegranate seed obtained by different microwave pretreatment

MWP (W)	PT (s)	ET (h)		
		3	4	5
900	30	21.97^{lm}	23.07^{ijkl}	23.17^{hijk}
	60	26.40^{bc}	28.30^{a}	28.30^{a}
	90	26.67^{b}	28.37^{a}	28.40^{a}
	120	26.87^{b}	28.53^{a}	28.60^{a}
720	30	21.87^{m}	23.00^{ijkl}	23.53^{ghij}
	60	26.57^{b}	28.23^{a}	28.27^{a}
	90	26.63^{b}	28.33^{a}	28.37^{a}
	120	26.87^{b}	28.50^{a}	28.53^{a}
540	30	21.50^{mn}	22.00^{lm}	22.53^{jklm}
	60	24.00^{fghi}	25.03^{def}	25.13^{de}
	90	24.23^{efgh}	25.20^{de}	25.33^{cde}
	120	24.50^{degf}	25.37^{cd}	25.43^{cd}
360	30	18.47^{qr}	20.30^{op}	20.53^{no}
	60	19.37^{pq}	21.97^{lm}	22.30^{klm}
	90	19.63^{op}	22.20^{klm}	22.37^{klm}
	120	19.90^{op}	22.47^{jklm}	22.53^{jklm}
Control		18.00^{r}	19.98^{op}	20.72^{no}

Values followed by different letters are significantly different. (Where, MWL: Microwave Power Level; PT: Pretreatment time and ET: Extraction time)

Oil extraction yield was also observed to be increased with increasing the microwave power as well as treatment time. Oil yield was higher at higher microwave power for longer treatment time. Effect of microwave treatment on yield was significant (Table 1). The higher yield obtained at 900, 720 W microwave power level and treatment time of 60, 90, 120 minutes was non-significant (at par) when observed for 4 and 5 h extraction time. Whereas, yield obtained on other combinations was significantly lower. Pretreatment time and temperatures affects the oxidation of oil as reported [18]. Higher microwave power for longer treatment time was found to increase the acid value and peroxide value of pomegranate seed oil (data not shown). Farag, *et al.* (1992) [19] reported acceleration of cottonseed oil oxidation during microwave heating observed by an increase in PV, due to the presence of reactive radicals that might be formed by exposure to microwaves. It has been reported that reactive free radicals might be formed by exposure to microwave energy, and various chemical reactions are said to be induced by microwave energy [12]. The results revealed that, the optimum conditions for solvent extraction of pomegranate seed oil were respectively. 720 W, 60 s and 4 h microwave power level, pretreatment time and extraction time

Effect of microwave power on cell structure of pomegranate seeds

Light microscopy of pomegranate seed kernel is depicted in Fig.1. Loosened cell walls and expanded lipid bodies were observed after the microwave treatment. Microwave radiation in oil seeds helps for higher extraction yield due to increase in mass transfer coefficient as cell membrane was ruptured. In addition, permanent pores are generated as result, enabling the oil to move through the permeable cell walls. Microwave pretreatment increases oil extraction yield and increasing the treatment time also had positive effect on oil extraction yield.

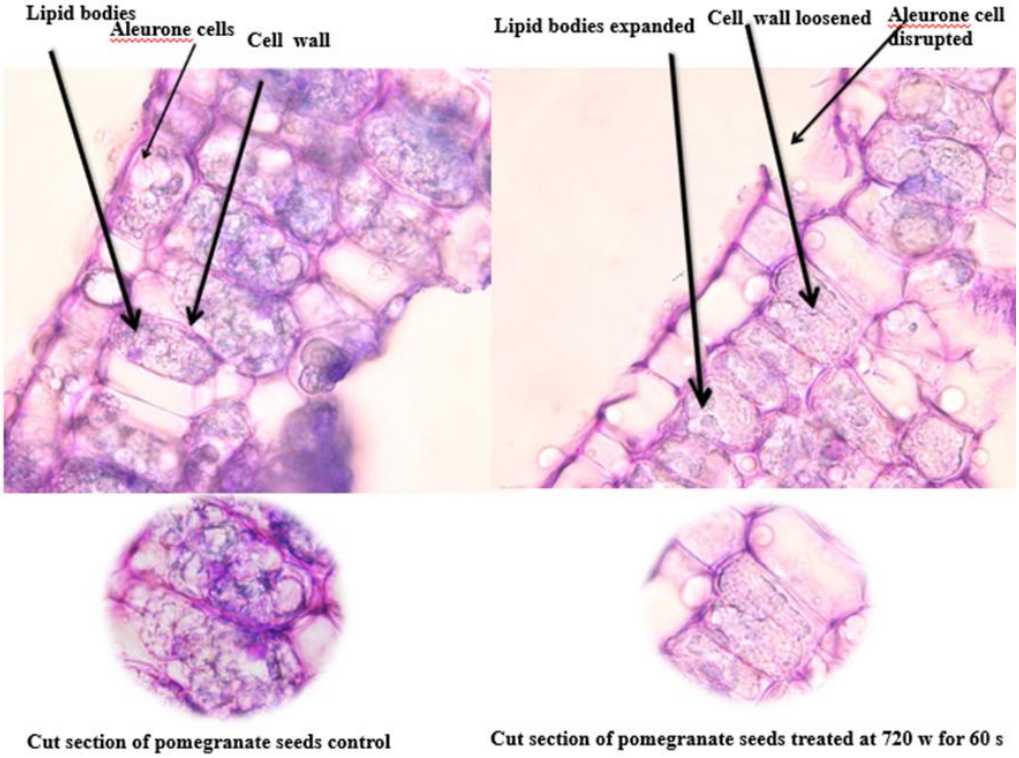

Fig 1: Light microscopy of pomegranate seed

Conclusion

The microwave level, pretreatment time, and extraction time has positive effect on yield. The optimum conditions for pomegranate seed oil extraction by soxhlet 720 W, 60 s and 4 h microwave power level, pretreatment time and extraction time respectively.

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