The Comparison and Analysis of Two Extraction Methods for Polysaccharides in *Psidium guajava* L. Fruits

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ABSTRACT

Introduction: Fruit polysaccharides, essential health factors in foods, are widely present in edible fruits. Several methods for isolation of polysaccharide are available. Microwave Assisted Extraction (MAE) method is extensively used for separation of polysaccharides. Only little information is available about *Psidium guajava* L. Fruit Polysaccharide (PFP) and its extraction methods. **Objective**: In this study, two extraction methods of polysaccharides in *Psidium guajava* L. fruit were compared and the best method was ascertained. **Methods:** *Psidium guajava* L. polysaccharides were extracted using water extraction with conventional heating and microwave assisted extraction method. The factors that influenced water extraction with conventional heating included temperature, water to plant material ratio, extraction time and solvent to liquid ratio. The factors that influenced MAE of polysaccharides extraction included microwave power, irradiation time and solvent to liquid ratio. Yield is considered as the independent variable in both the methods. **Results:** By the result of the single factorial method and orthogonal experiments, the optimal extracting conditions were selected and then the best extraction method was ascertained. The optimum extraction conditions of water extraction with conventional heating include, temperature 90°C, water to plant material ratio 1:1, extraction time 1 hour 30 min and solvent to liquid ratio 2:1. The results showed that the extraction yield of microwave method was higher under the optimal conditions of irradiation time 20 min, microwave power 200 W and 3:1 solvent to liquid ratio than the water extraction with conventional heating.

Key words: Guava, Polysaccharides, Extraction, Optimization, Taguchi orthogonal, Comparison.

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INTRODUCTION

Psidium guajava L or common guava is a tropical/subtropical fruit plant of the world. It belongs to family Myrtaceae and originated from tropical America.[1] Guava contains wide-ranging of phytochemical including polysaccharides.[2,3] In addition, ascorbic acid, pectin and other mineral contents are more in guava fruits in comparison with other fruits. A major component of guava fruit is pectin, which is an anionic polysaccharide mainly composed of partially esterified *D*-galacturonic acid monomers linked by α-(1-4) bonds.[4]

Fruit polysaccharides, essential health factors in foods, are extensively present in edible fruits. In recent years, a close understanding of natural polysaccharides has promoted studies that deal with isolation and physical, chemical and structural characterization of polysaccharides[5-8] Only very few information is available about *Psidium guajava* L. Fruit Polysaccharide (PFP) and its extraction methods.

Most commonly used extraction methods for isolation of polysaccharide are water extraction, alcohol precipitation, acid or alkali extraction, ultra sound assisted, enzyme hydrolysis and Microwave Assisted Extraction (MAE). As the polysaccharides have lower solubility in high concentration of alcohol or ether,[9] it can be precipitated out when alcohol or ether added to an aqueous solution of polysaccharide.[10] Water extraction and alcohol precipitation are inexpensive and easy method but in contrast, they have higher extraction temperature and slow extraction rate. Ultrasound assisted extraction results tissue deformation and rupture, and release contents; in order to promote the dissolution of the active ingredients within the cell, but the high power will cause harm to the structure of polysaccharide and the material out of the cells induce the difficulty of separation.[11] Alkali, acid extraction method is suitable for selected polysaccharides.[12] Enzymatic method is expensive one.[13] Nowadays, Microwave Assisted Extraction method (MAE) is extensively used for separation of polysaccharides. MAE is easy, efficient method with low extraction time.

The present study aims to perform a comparative analysis of water extraction method with conventional heating and MAE of polysaccharides from *Psidium guajava* L. fruits.

MATERIALS AND METHODS

Fresh *Psidium guajava* L fruits were purchased from nearby garden in Coimbatore. The authentication of the plant was obtained from the Botanical Survey of India situated in the Tamil Nadu Agricultural University located in Coimbatore. All the other chemicals used in the study were of laboratory grade.

Water extraction of polysaccharides with conventional heating from *Psidium guajava* L. fruit

Half ripened fresh *Psidium guajava* L. fruits were cut, the skin and seeds were cleaned and grounded, exact quantity immersed in water for 2 hours. The pH of the solution was adjusted to pH 4.5 with tartaric acid solution (10%) and boiled under reflux for 1 hour with occasional stirring. After cooling excess fragments were removed by centrifugation process. The mucilage solution was precipitated with acetone. Extractions were performed at different conditions using single factor experiments. Then the polysaccharides thus obtained are filtered, air dried and dried in a hot air oven at a temperature not exceeding 50°C. The yield is calculated by the following formula (Equation 1).

Yield (%) = (Weight of the polysaccharide obtained / Weight of the fruit material) ×100 Equation 1.

The factors that influenced water extraction with conventional heating included temperature, water to plant material ratio, extraction time and solvent to liquid ratio. In single factor experiments, the *Psidium guajava* L. fruit polysaccharide was extracted using various temperature (70°C, 80°C, 90°C), water to plant material ratio (1:1, 2:1, 3:1), extraction time (30 min,1 hr, 1 hr 30 min) and solvent to liquid ratio (1:1, 2:1, 3:1).

Pectin from the *Psidium guajava* L fruits can be obtained from the same method explained above except that about 7-10 grams of citric acid or tartaric acid was added to fruit material and then it was subjected to heating then extracted with suitable solvent.[14]

Orthogonal experiment: Taguchi method is a new statistical method used to improve the quality of the manufactured goods. This method uses a special set of arrays known as orthogonal arrays. These standard arrays stipulate the way of conducting minimal number of experiments which could give the full information of all the factors that affect the performance parameter. This array assumes that there is no interaction between any two factors.[15] The L9 orthogonal array was adopted for the present study. The response variable in the data analysis is not the raw response or quality characteristic; it is the signal-to-noise ratio.[16]

The factors that influenced water soluble polysaccharides extraction included temperature, water to plant material ratio, extraction time and solvent to liquid ratio. In single factor experiments, the PFP was extracted using various temperature (70°C, 80°C, 90°C), water to plant material ratio (1:1, 2:1, 3:1), extraction time(30 min, 1 hr, 1 hr 30 min) and solvent to liquid ratio (1:1, 2:1, 3:1) as shown in Table 1. Signal to noise ratio can be calculated by the following formula.[17]

$SN_i=10\log (Y_i^2/S_i^2)$ (Equation 2).

Where SNi- Signal to noise ratio of the experimental data

Y_i - Mean of the three sets of experimental data

S_i-Standard deviation of the experimental data

MAE of *Psidium gujava* L fruit polysaccahride(PFP)

Except the boiling with reflux for 1 hour, the method is same as described above in water extraction method with conventional heating. In microwave assisted extrac-

Table 1: L9 (3^4) Orthogonal table of water extraction of *Psidium gujava* L fruit polysaccahride(PFP) with conventional heating

Level	Factors			
	A Temperature	B Water to plant material ratio	C Extraction time	D Solvent to liquid ratio
1	70^{0}C	1:1	30 min	1:1
2	80^{0}C	2:1	1hr	2:1
3	90^{0}C	3:1	1 hr 30 min	3:1

(Source: Original).

Table 2: L9 (3^4) Orthogonal table of Microwave Assisted Extraction(MAE) extraction of Psidium gujava L fruit polysaccahride(PFP)

Level	Factors		
	A Microwave Power	B Irradiation time	C Solvent to Liquid ratio
1	100 W	10 min	1:1
2	200 W	20 min	2:1
3	300 W	30 min	3:1

(Source: Original)

Table: 3 L9 (3^4) Orthogonal experiment results of water extraction method with conventional heating

S.no	Temperature (A)	Water to plant material ratio(B)	Extraction Time (C)	Solvent to Liquid ratio (D)	Extraction Yield (%)			Mean	S.D	SNi
					Trial 1	Trial 2	Trial 3			
1	A1	B1	C1	D1	0.7	0.5	0.4	0.53	0.15	10.86
2	A1	B2	C2	D2	0.4	0.6	0.5	0.50	0.10	13.98
3	A1	B3	C3	D3	0.6	0.6	0.7	0.63	0.06	20.80
4	A2	B1	C2	D3	0.8	1	0.9	0.80	0.10	18.06
5	A2	B2	C3	D1	0.8	0.7	0.6	0.70	0.10	16.90
6	A2	B3	C1	D2	0.4	0.3	0.5	0.40	0.10	12.04
7	A3	B1	C3	D2	1.5	1.4	1.6	1.50	0.10	23.52
8	A3	B2	C1	D3	1.2	1.1	1.2	1.17	0.06	26.11
9	A3	B3	C2	D1	0.8	1	1.1	0.97	0.15	16.03

(Source: Original)
S.D-Standard Deviation
SNi-Signal to Noise ratio.

Table 4: L9(3^4) Orthogonal response table of water extraction method with conventional heating

Response table for Signal to Noise ratio					
Level	Number	Temperature(A)	Water to plant material ratio (B)	Extraction time(C)	Solvent to liquid ratio(D)
1	K1i	15.21	17.48	16.34	14.60
2	K2i	18.58667	19.00	16.02	16.51333
3	K3i	21.89	16.29	20.40667	21.66
Δ		6.67	2.71	4.07	5.14
Rank(R)		1	4	3	2

(Source: Original).

Table: 5 L9(3^4) Orthogonal experiment results of MAE extraction method

S.no	Microwave Power	Irradiation Time	Solvent to liquid ratio	Extraction Yield (%)			Mean	S.D	SNi
				Trial1	Trial2	Trial 3			
1	A1	B1	C1	0.3	0.2	0.3	0.27	0.06	33.11
2	A1	B2	C2	0.6	0.6	0.7	0.63	0.06	31.01
3	A1	B3	C3	0.7	0.8	0.8	0.77	0.06	29.21
4	A2	B1	C2	0.8	1	0.9	0.83	0.10	37.97
5	A2	B2	C3	8.41	8.41	8.32	8.38	0.04	45.91
6	A2	B3	C1	6.95	7.4	7.2	7.18	0.18	31.83
7	A3	B1	C3	7.15	7.25	7.15	7.18	0.05	43.66
8	A3	B2	C1	6.8	6.9	6.7	6.80	0.08	38.41
9	A3	B3	C2	7.11	6.8	7.1	7.00	0.14	33.75

(Source: Original)
S.D-Standard Deviation
SNi-Signal to Noise ratio.

Table : 6 L9(3^4) Orthogonal response table of MAE extraction method

Response Table for Signal to Noise ratio				
Level	Number	Temperature (A)	Irradiation time (B)	Solvent to liquid ratio (C)
1	K1i	31.11	38.25	34.45
2	K2i	34.24	38.44	34.24
3	K3i	38.61	31.60	39.59
Δ		7.50	6.85	5.35
Rank (R)		1	2	3

(Source: Original).

Table: 7 Comparison of two extraction methods of polysaccharides

Method	Water extraction	Microwave extraction
Extraction temperature	90^0C	-
Water to plant material ratio	1:1	-
Extraction time	1hour 30 min	-
Microwave Power	-	200 W
Irradiation time	-	20 min
Solvent to liquid ratio	2:1	3:1

(Source: Original).

tion, heating was carried out using microwave oven where the material was kept in china dish. Small porcelain pieces were used to avoid pumping. Extractions were performed at different conditions using single factor experiments.

The factors that influenced MAE of polysaccharides extraction include microwave power, irradiation time and solvent to liquid ratio. The factors and their levels are given in Table 2.

RESULTS

Results of water extraction method with conventional heating

Based on the single factor experiments, the results of the orthogonal experiment is shown in Table 3, The results show that the extraction yield increased with the increase in temperature. Extraction yield was directly proportional to extraction time. When the solvent to water to plant material ratio reached at least 2:1 or above the extraction yield was the highest. No significant change in extraction yield was observed for solvent to liquid ratio above 1:2. From Table 3 and 4 the optimum conditions were determined by orthogonal test and extreme difference analysis (R=maxKi - minKi). According to the value R (R_A=6.67>R_D>R_C> R_B), the influence of temperature on the extraction yield of polysaccharides among the four factors was the biggest. These factors were sequenced by their influence on extraction yield for A>D>C>B. The optimum extraction conditions were $A_3B_1C_3D_2$ temperature 90^{o}C, water to plant material ratio 1:1, extraction time 1 hr 30 minutes and solvent to liquid to ratio 2:1, the extraction yield attained the optimum level.

Results of MAE extraction method

According to the single factor experiments of MAE method, the extraction yield was increased with increase in microwave power. Solvent liquid ratio 3:1 was the best ratio in single factor experiments. Based on the single factor experiments, the results of orthogonal experiment and extreme analysis were shown in Table 5 and 6. In Table 6, microwave power had the highest R value (7.50), the effects of the variables on extraction yields followed the order: microwave power>irradiation time>solvent to liquid ratio. According to the experimental values, the optimal combinations were $A_2B_2C_3$, microwave power 200 W, irradiation time 20 min and solvent-liquid ratio 3:1. The summary results of these two methods in optimal conditions are shown in Table 7. Extraction yield of microwave assisted method was higher than water extraction method with conventional heating.

DISCUSSION

Over the past decade, numerous studies have attempted to study extraction of polysaccharides and mucilage from several fruits. Huiping *et al* isolated polysaccharides by water extraction method by conventional heating with continuous stirring on a stir plate for 2 h at 100ºC from wolfberry, cherry, kiwi and cranberry fruits.[18] Hindustan *et al* isolated mucilage by water extraction method by boiling the fruit mixture for 30 minutes from Ficus fruit and formulated transdermal patches using the isolated mucilage.[19] Jia *et al* extracted five soluble polysaccharides using water and other different solvents by the conventional heating at room temperature for 4 hours from cell wall material of rabbit blue berry fruits. The sequential treatments yielded a total 36.02% soluble polysaccharides of the dry cell wall material.[20] Amina *et al* extracted polysaccharides by water extraction method by conventional heating at 80°C for 24 h and precipitation with 70% (v/v) ethanol.[21] Yu *et al* extracted and optimized polysaccharides from *Auricularia auricula* fruiting bodies (AAFB) using response surface methodology (RSM). The Box-Behnken experimental results showed the optimum extraction conditions as a liquid-solid ratio of 38.77 mL/g, a temperature of 93.98°C and a time of 3.41 h. Under these conditions, the maximal polysaccharide yield was 10.46 g/100 g.[22]

Shah (2010) *et al* optimized conventional and microwave assisted methods the extraction of mucilage from the fruits of *Trichosenthes dioica* plant. Microwave extraction at 320 W intensity and 20 min heating duration, 84.92% increase in the yield of mucilage while 52.06% and 38.09% increase in the yield at 640 W for 5 min and 160 W for 40 min respectively were obtained under microwave irradiation when compared to 1 h conventional heating method. The products obtained by both the methods were of similar nature both physically and chemically.[23] Brien (2010) *et al* extracted mucilage from the fruits of Lagenaria siceraria plant by conventional and microwave assisted methods. Microwave extraction at 320 W intensity and 20 min heating duration, 96.15% increase in the yield of mucilage while 93.95% and 91.20% increase in the yield at 160 W for 60 min and 480 W for 10 min respectively were obtained under microwave irradiation when compared to 1 h conventional heating method.[24] Yonggang *et al* (2010) optimized the Microwave-assisted extraction of water-soluble polysaccharides from Piteguo fruit by Response surface methodology a central composite design was used to optimize microwave power (400-600 W), extraction time (5-10 min) and ratio of water to material (30:1-50:1 mL/g) to obtain a high crude polysaccharides yield. The statistical analyses indicated that all of the three factors had significant effect on the extraction yield of crude polysaccharides ($p<0.001$). The results showed that the extraction ratio of crude polysaccharides was up to 7.86% under the optimum extraction conditions as follows: microwave power 550W, extraction time 9 min, water to material ratio 45 mL/g, which was consistent with the predicted models with the coefficients of determination (R^2) of 0.9809.[25] Biren *et al* (2011) isolated mucilage from *Ablmoschus esculentis* fruits both by water extraction heating and microwave method. In water extraction, the fruit material blend in water was boiled for 1 h under reflux and with bumping. In microwave method the blend was subjected to microwave irradiation at 160 W intensity and 40 min. 11.55 % increase in the yield of mucilage compared to the conventional heating method. The process parameters include microwave power, irradiation time as yield as independent variable.[26]

In recent years, extraction of polysaccharide from *Psidium guajava* L. fruit has been studied. Only hot water extraction from *Psidium guajava L* fruit is reported so far.[27] But the comparative research on the two methods water extraction with conventional heating and Microwave assisted extraction of *Psidium guajava* L. fruit polysaccharides have not been reported until now. So this study is attempted to isolate polysaccharides from *Psidium guajava* L. fruit using hot water and microwave and compare these two methods by the orthogonal experiment. Comparison is shown in Table 7. The best method which has got significance to the research and application of polysaccharides is ascertained.

Water extraction with conventional heating is an easy method and will not destroy the molecules of polysaccharides; it will have elevated temperature and long extraction time. Dehong *et al* reported that polysaccharides are obtained from *Psidium guajava* L. fruits at 80°C for 3 hours.[28] Mandal *et al* also reported about hot water extraction of polysaccharides from the same fruit.[27] In our study, according to the results of the orthogonal experiment in Table 3, it was found that, the optimal conditions of water extraction were 90°C temperature, 1:1 water to plant material ratio, extraction time 1 hour 30 minutes, 2:1 solvent to liquid ratio, and the extraction yield was 1.17%.

During microwave extraction, it is necessary to optimize the conditions. The results of the microwave orthogonal experiment were shown in Table 6, the optimal process conditions were microwave power 200 W, irradiation time 20 min, solvent to liquid ratio 3:1. The results of the microwave extraction indicate that the highest extraction yield is 8.38% under optimal conditions (200 W, 20 min, 3:1 ratio). The results indicate that there is linear rise in the polysaccharide yield from 100-200 W. This enhancement is owing to the fact that rise in microwave power will improve the solubility of the sample for the improved extraction efficiency.[29] The raise in microwave power increases dipole reactions which produces heat generation in the mixture as a result of power degeneration. After 200 W, there is slight decrease in the yield of polysaccharide.

When comparing the process variable of the water extraction with conventional heating with that of the available literature on extraction of mucilage or polysaccharides from other fruits, it is understood that the temperature in conventional heating 70°C-90°C time 30 min-1 h 30 min would give proper result, and is supported by the reports of Huiping *et al* (1 hr at 100°C), Hindustan (30 minutes), Shah *et al* (1 h). In the same way, when comparing the process variable of the water extraction with conventional heating with that of the reported studies on extraction of mucilage or polysaccharides from other fruits, it is understood that the process variables generally adopted were microwave power and irradiation time. Only very few articles discussed variables such as water to plant material ratio, liquid to solvent ratio. The process variables provided maximum yield by the reported study by Shah *et al* (320 W intensity and 20 min), Biren *et al* (320 W intensity and 20 min) supports the selection of the process variables of the present study as microwave power in the range of 100-300W and irradiation time 10-20 min.

CONCLUSION

In this study, water extraction with conventional heating and microwave assisted extraction methods of polysaccharides in *Psidium guajava* L. fruits were compared. By the result of the single factor method and orthogonal experiments, the optimal extracting conditions were selected and then the best extraction method was ascertained. Extraction time of microwave extraction time is too short and the extraction yield is higher. So by comparing the data and condition of two methods, it is concluded that microwave method is the better method. Further prospects for the experiment can be carried out by ultrasonic extraction of polysaccharides from *Psidium guajava* L fruit and even best of these three methods can be ascertained.

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CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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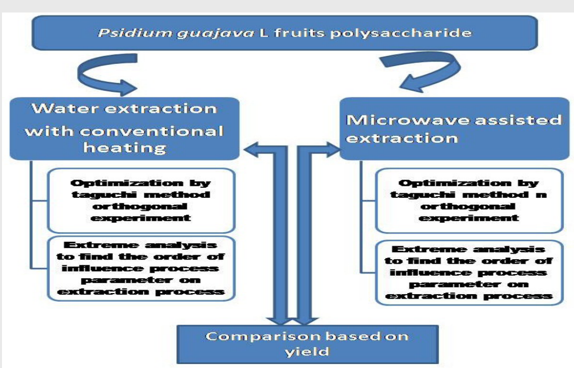
PICTORIAL ABSTRACT



SUMMARY

- Extraction of polysaccharides from *Psidium guajava* L fruits was carried out by water extraction with conventional heating and microwave assisted method.
- Optimization of polysaccharide extraction method was performed by single factor experiments and orthogonal experiment. Then extreme analysis was performed to know the order of parameters in which they influence the extraction process.
- Microwave assisted method was selected as a better method among these two extraction methods.
- The optimum extraction parameters in water extraction with conventional heating were identified as temperature 90^0C, water to plant material ratio 1:1, extraction time 1 hr 30 min and solvent to liquid to ratio 2:1, the optimal process conditions in Microwave assisted extraction were microwave power 200 W, irradiation time 20 min, solvent to liquid ratio 3:1. Highest yield of polysaccharide was obtained in the optimal extraction conditions for both the extraction methods
- Yield is higher in the microwave assisted extraction method than water extraction with conventional heating method.