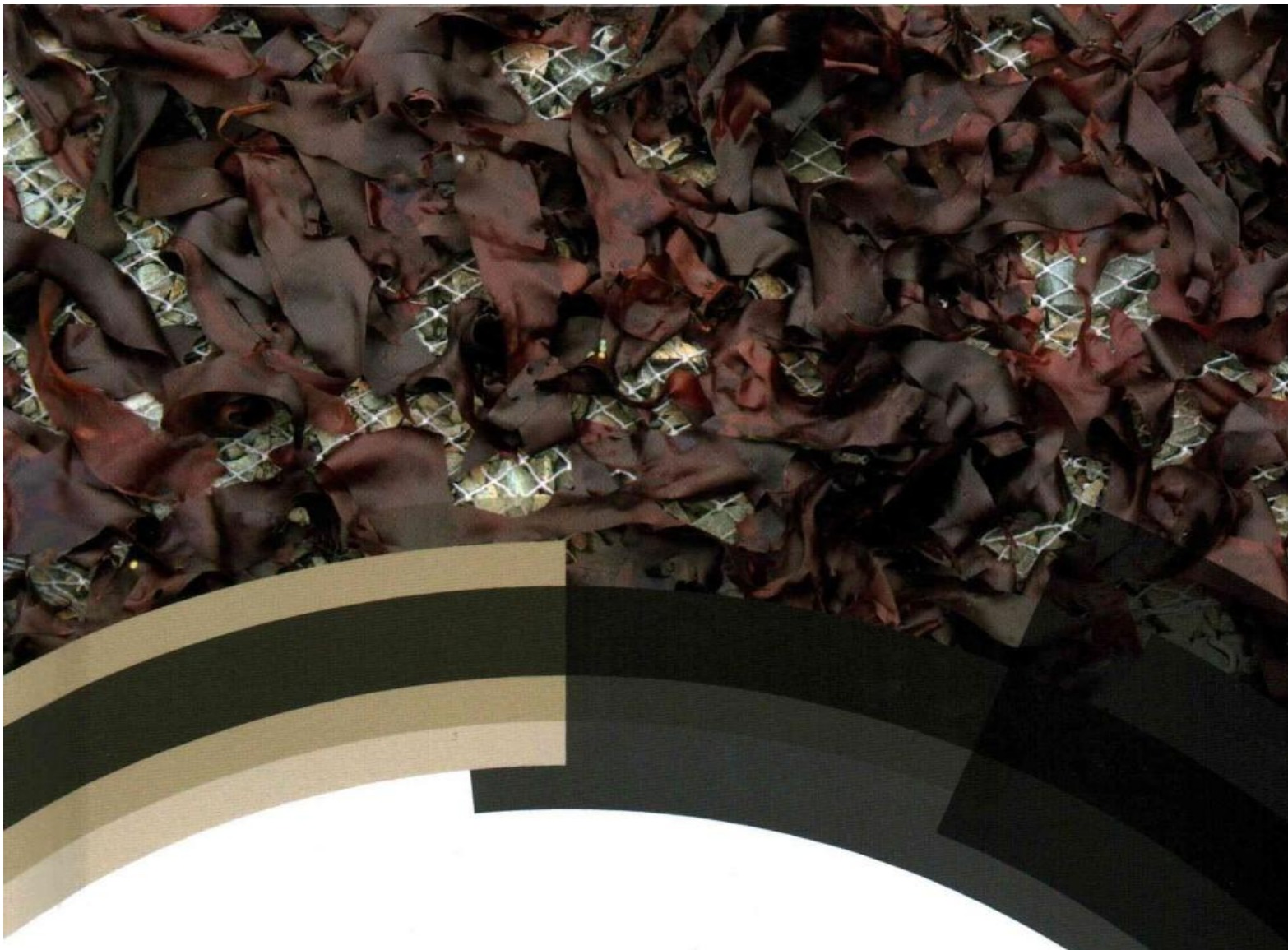

Gums and Stabilisers for the Food Industry 17

The Changing Face of Food Manufacture: The Role of Hydrocolloids

Edited by Peter A. Williams and Glyn O. Phillips

ROYAL SOCIETY OF CHEMISTRY

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The Changing Face of Food Manufacture: The Role of Hydrocolloids

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Preface

It is a pleasure and a privilege once again to introduce this collection of selected and reviewed papers from the 17th International Gums and Stabilisers for the Food Industry Conference held once again at its traditional home at Glyndwr University, Wales, following its "sabbatical" to Wageningen, the Netherlands for the 16th Conference. These volumes have now appeared continuously every two years since their inception in 1982 and have established their position as the most widely referenced publications in the food hydrocolloids sector and as a partner publication to the journal "*Food Hydrocolloids"*.

Significantly proteins have now taken their place tidily alongside polysaccharides within the food hydrocolloids classification. The papers in Chapters 1 and 4, in particular, underline this partnership. Peter Wilde outlines clearly the complex field of food proteins. Subsequent papers show how modification and protein-polysaccharide interactions can produce new structures and better emulsification properties. Gelatin, of course, has been a major ingredient in the food industry and the review by Douglas Goff gives us a great deal of new information about its properties and applications. The papers on emulsions, foams and films demonstrate major changes in the technologies now used and even in the nomenclature. The term "oxygen cocktails" is certainly new to me!

The growth of Chinese activity in this field, particularly with regard to natural bioactive polysaccharides is reflected not only in the growing input from this country but also in the increasing amount of collaboration between East and West. The new materials from biological sources such as *Ganoderma atrum*, *Amorphophallus Muelleri, Pomelo* pectin, *Okra, Brachystegia Eurycoma etc* offer linguistic and structural challenges. However, they do bridge the traditional food hydrocolloids with these new exotic areas.

As in all previous volumes emphasis again is placed on the traditional rheological properties of hydrocolloids. There is convenient connection between this field and the health field in the interesting paper which describes methods to simulate the behaviour of the human tongue to obtain sensory information.

Above all it is the health benefits of natural hydrocolloids which are the most appealing aspect today. The papers on fat replacement, *in vitro* digestion of dietary fibres, controlling lipid digestion, controlling human digestion, the synergistic gastric roles of polysaccharide mixtures, the management of dysphagia etc. show how widespread is the role of food hydrocolloids in improving human health.

It is a pleasure to note how we all enjoyed the new accommodation and conference facilities at Glyndwr University. We have become a family after all these years with new and exciting colleagues joining the old stagers like myself in bringing new subjects and inspiration into the proceedings. I must thank all concerned, particularly, of course, my friend Professor Peter Williams, who with our colleague Haydn Hughes undertake most of the organisation and management of this publication and the conference. I thank also the

Chairman of the Food Hydrocolloids Trust Dr Graham Sworn, the Trustees and the Committee Members for their invaluable work in planning and attracting such wonderful international participation.

"Diolch yn fawr i bawb" - which is the Welsh for "Very many thanks to all"

Glyn O. Phillips

Chairman, Organisation Committee

Gums and Stabilisers for the Food Industry Conferences

This series of international conferences was initiated at the North East Wales Institute (now Glyndwr University) in Wrexham, UK in 1981 and has been held biennially since then. It is organised under the auspices of the Food Hydrocolloids Trust and the proceedings of all of the conferences have been published and details can be found below:

Prog. Fd. Nutr. Sci., "*Gums and Stabilisers for the Food Industry*" (Eds. G. O. Phillips, D.J. Wedlock and P. A. Williams). Pergamon Press Ltd, Oxford, Vol 6 (1982).

"*Gums and Stabilisers for the Food Industry 2*" (Eds., G. O. Phillips, D. J. Wedlock and P. A. Williams), Pergamon Press Ltd., Oxford (1984).

"*Gums and Stabilisers for the Food Industry 3*" (Eds., G. O. Phillips, D. J. Wedlock and P.A. Williams), Elsevier Applied Science Publishers (1986).

"*Gums and Stabilisers for the Food Industry 4*" (Eds., G. O. Phillips, D. J. Wedlock and P. A. Williams), IRL Press (1988).

"*Gums and Stabilisers for the Food Industry 5*" (G. O. Phillips, D. J. Wedlock and P. A. Williams), Oxford University Press Ltd. (1990).

"*Gums and Stabilisers for the Food Industry 6*" (eds G.O. Phillips, P.A. Williams and D.J. Wedlock), Oxford University Press Ltd (1992).

"*Gums and Stabilisers for the Food Industry 7*" (eds G.O. Phillips. P.A. Williams and D.J. Wedlock), Oxford University Press (1994).

"*Gums and Stabilisers for the Food Industry 8*" (eds G.O. Phillips, P.A. Williams, and D.J. Wedlock), Oxford University Press (1996).

"*Gums and Stabilisers for the Food Industry 9*" (eds. P.A. Williams and G.O. Phillips), Royal Society of Chemistry, Cambridge UK (1998).

"*Gums and Stabilisers for the Food Industry 10*" (eds P.A. Williams and G.O. Phillips), Royal Society of Chemistry, Cambridge UK (2000).

"*Gums and Stabilisers for the Food Industry 11*" (eds P.A. Williams, P. A. and G.O. Phillips), Royal Society of Chemistry, Cambridge UK (2002).

"*Gums and Stabilisers for the Food Industry 12*" (eds P.A. Williams and G.O. Phillips), Royal Society of Chemistry, Cambridge UK (2004).

"*Gums and Stabilisers for the Food Industry 13*" (eds P.A. Williams, and G.O. Phillips), Royal Society of Chemistry, Cambridge UK (2006).

"*Gums and Stabilisers for the Food Industry 14*" (eds P.A. Williams, and G.O. Phillips), Royal Society of Chemistry, Cambridge UK (2008).

"*Gums and Stabilisers for the Food Industry 15*" (eds P.A. Williams, and G.O. Phillips), Royal Society of Chemistry, Cambridge UK (2009).

"*Gums and Stabilisers for the Food Industry 16*" (eds P.A. Williams, and G.O. Phillips), Royal Society of Chemistry, Cambridge UK, (2012)

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Contents

PROPERTIES AND APPLICATIONS OF FOOD PROTEINS

OPTIMIZATION OF ULTRASOUND-ASSISTED EXTRACTION OF KONJAC FLOUR FROM *AMORPHOPHALLUS MUELLERI* BLUME

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1 INTRODUCTION

Amorphophallus muelleri Blume is one of the most abundant *Amorphophallus* species in Indonesia forest. This native plant is specifically being cultivated for chips production and it is exported to Japan, Hongkong, China and other countries.[1] Konjac flour from *Amorphophallus konjac* K. Koch is a vital source of glucomannan.[2] It is a polysaccharide used in food, cosmetics and the pharmaceutical industry. *Amorphophallus konjac* is well known to be one family of *Amorphophallus muelleri*. In India, during the scarcity, people collected many types of wild roots and tubers to supplement their meager food, including corms of elephant foot yam or *Amorphophallus muelleri*.[3] With regard to quality, *Amorphophallus muelleri* corms had high amount of calcium oxalate, which is responsible for the acridity compared to other *Amorphophallus species*.[4] In Indonesia, corms of *Amorphophallus muelleri* are processed into chips or sold as fresh tubers for the food industry.[1]

Crude konjac flour (CKF) is a term for konjac flour, in which the chips are milled to a 40-60 mesh powder, then wind blown to separate the lighter starch from the heavier konjac flour through a cyclone. Usually three cylces are performed consecutively to reduce tachiko or t*obico* (dust from the konjac tuber).[5, 2] It has been reported that the consistent palatibility problems, due to 2.1% calcium oxalate, hindered the use of CKF for food consumption.[6, 7] Other problems associated with utilizing CKF in the food industry are the low glucomannan content (37.78 – 45.89%) and low viscosity, dark color and high calcium oxalate.[8]

Our laboratory has made several attemps to purify CKF.[6, 7, 8, 9, 10] In our previous experiments, dipping CKF in 40% ethanol solution, followed by 60% and finally at 80%, for 4 hours each time, showed the best method of purifying konjac flour (PKF). This technique resulted in a lower calcium oxalate, higher glucomannan content and viscosity compared to untreated flour (CKF). However, the degree of whiteness of PKF remained nearly the same to CKF.[12, 13]

The use of a maceration technique to purify konjac flour by dipping in ethanol solution at room temperature. [12, 13, 14, 15, 16] The effect of ultrasound on purifying konjac flour was

publically noted. [12, 13, 17, 18] The application of ultrasound seems to be very promising to obtain a high yield and activity, as it was concluded from the studies of protein, medicinal compounds, tea etc.[19] Ultrasound has been reported to improve the extraction of bioactives from roots and tubers,[20] apple pomace, [21] oil seed tobacco, [22] and citrus peel extract.[23]
KGM is becoming an important industrial polysaccharide[24] and has received much attention recently.[25, 26, 27, 28, 29, 30] Nevertheless, studies on the optimization process using response surface methodology (RSM) with the application of ultrasound on CKF extracted from *Amorphophallus muelleri*, to the best of our knowledge, has been unexplored.
Development of this novel bioprocess relies on the advancements in optimization of the process which is tedious and time consuming, because of the effects of multivariable process parameters.[31] For such proposes, RSM was employed to optimize purification process conditions which could bring improvement to the existing product design. RSM is a collection of statistical techniques for designing experiments, building models, evaluating the effects of independent variables on one or more measured dependent responses. It is advantageous over conventional methods available and it includes less experiment numbers.[32] RSM has been reported to find out the optimal extraction condition from *Zizyohus jujuba* cv jinsixiaozao,[33] *Inga idulis* leaves,[34] and almond powder.[35] This paper reports the optimization of the ultrasound-assisted extractionof KGM, using Response Surface Methodology (RSM) from *Amorphophallus muelleri* Blume.

2 MATERIAL AND METHODS

Konjac tubers with outer diameters between 19 and 25 cm, weight 3 kg±0,2, were collected from a konjac farmer at Sumberbenda Village, Saradan district, Madiun Regency, Indonesia. All the chemicals used were analytical grade, while the water was glass distilled. Commercial konjac flour (KGM, Made in USA) was bought through on line trading.

2.1 Sample Preparation

Clean and fresh *Amorphophallus muelleri* Blume was sliced to ±0.5-1 cm thickness and dried for ±11 hours in a cabinet dryer (±60 ^{0}C). The chips were ground with a stamp-mill to pass through 30 mesh screens and it was air classified by using a cyclone three times and filtered to pass through 80 mesh screens. The powder was called crude konjac flour (CKF). The ultrasound treatment of CKF was then carried out as described in the experimental design below.

2.2 Experimental Design and Statistical analysis

RSM was used to obtain the optimal ultrasonically assisted extraction condition of crude konjac flour (CKF) from *Amorphophallus muelleri* Blume to obtain PKF with high glucomannan content, high viscosity, low calcium oxalate and clear white colour of konjac flour. The extraction experiment was carried out according to a central composite design with 2 factors and 5 levels. Two independent variables selected for this paper were extraction time (min, X1) and solvent/flour ratio (Table 1). For each factor an experimental range was based on the results of preliminary experiments. Four responses namely, glucomannan, viscosity, calcium oxalate and degree of whiteness, were chosen as

Table 1: *Independent variables and their levels in the response surface design*

Independent variables	Factor level				
	-1.414	-1	0	1	1.414
Time (min, X_1)	10.86	15	25	35	39.14
Solven/flour ratio (ml/g, X_2)	5.17:1	6:1	8:1	10:1	10.83:1

dependent variables. The complete design consisted of 13 experimental points including 5 centre points, 4 factorial points and 4 axials points.
Data from the central composite design were analyzed by multiple regressions to fit into the empirical second order polynomial model, as shown in the following equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1,j=2}^{k-1,k} \beta_{i,j} X_i X_j + \varepsilon \quad (1)$$

Where, β_o ;β_i ;β_{ii} and $\beta_{i,j}$ are regression coefficients in the intercept, linear, quadratic and interaction terms, respectively; X_i ; X_{ii} ; $X_{i,j}$ are the independent variables; Y are responses and ε is a random error component.
Software of Design Expert 7.1.0 Version Trials (State-Ease, Inc., Minnepolis MN, USA) was used to obtain the coefficients of the quadratics polynomial model. The quality of the fitted model was expressed by Lac of fit value, R^2 value and Adjusted R^2 value and its statistical significance was checked by an F-test.

2.3 Ultrasound procedure

The irradiation was carried out at 300 dB and 60 kHz frequency.The immersion stainless steel transducer was a horn type with the length of 6 cm and diameter of 1.8 cm. The ultrasonic extraction of crude konjac flour (CKF) with Brown Sonic Type 2000 ultrsound instrument was conducted in three stages of extraction processes. Stage 1, CKF was dispersed at solvent/flour ratios (ml/g) of 5.17:1, 6:1, 8:1, 10:1, 10.83:1 in 40% ethanol and the ultrasound treatment was conducted at extraction times of 10.86, 15, 25, 35 and 39.14 minutes. CKF slurry was sieved through a filter paper to separate residue and supernatant. Stage 2, the residue was again extracted in 60% ethanol, at the extraction time and solvent/flour ratio as described at stage 1. Finally, the extraction process was repeated in 80% ethanol with processing conditions as stated in stage 1 (Figure 1 and Table 3). The residue was then dried at 40^0C in an oven for 12 hours. Each sample and control (commercial KGM) was stored in airtight containers until it was used for further analysis.

Figure 1 shows the procedure scheme of ultrasound-assisted extraction of CKF of *Amorphophallus muelleri*.

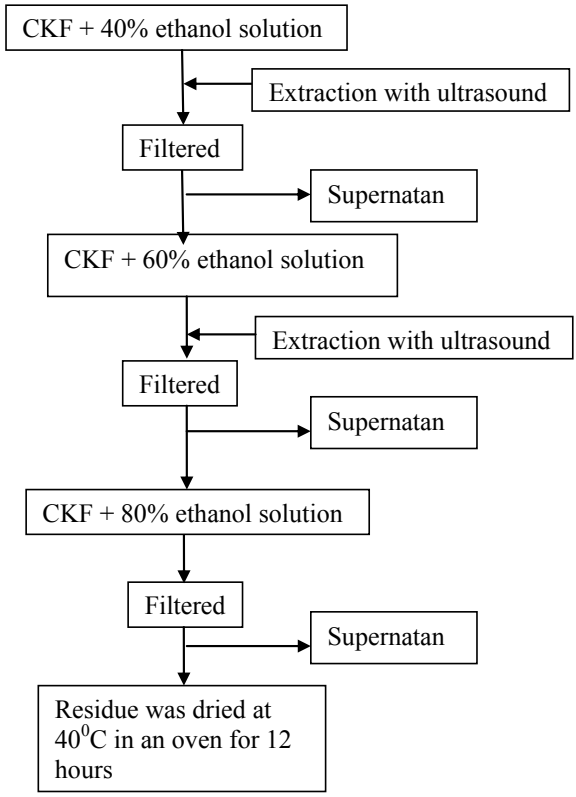


Figure 1: *Scheme for the extraction of CKF from* Amorphophallus muelleri *by ultrasound-assisted extraction procedure*

The optimal process conditions of ultrasound-assisted extraction of purified konjac flour and commercial KGM were analyzed for proximate analysis as well as four responses chosen including: glucomannan, viscosity, calcium oxalate and degree of whiteness.

2.4 Analysis of samples

Water content was determined by weight difference after drying the samples, as described by.[36] Ash content was determined gravimetrically.[36] Fat content was determined using a Soxhlet apparatus according to[37] Protein content was calculated from the nitrogen content (N% X 6.25) analyzed by the Kjeldahl method. Starch content was determined by spectrophotometric method as described by.[37] Glucomannan content was determined using 3.5 dinitrosalicylic acid reagent (Sigma-Aldrich) and measured spectrophotometrically at 550 nm as described by.[38] Viscosity of 1% konjac flour solution was determined using spindle needle 1 at 60 rpm of Brookfield Viscometer (Brookfield LD IV) at room

temperature according to.[38] Calcium oxalate content was determined using the method originally developed by.[39] Degree of whiteness was determined using a color reader (Minolta CR-100) according to the procedure noted by.[40]

3. RESULTS AND DISCUSSION

3.1 Choosing the fitted model for purifying crude konjac flour

The results of analysis variance, lack of fit and the adequacy of the model are summarized in Table 2. The data showed a good fit with equation (1), which was statistically acceptable at $P<0.05$ level and adequate with a satisfactory R^2 value (R^2 for glucomannan, viscosity and calcium oxalate were 0.8556, 0.9771 and 0.9311, respectively). Adjusted R^2 values for glucomannan, viscosity and calcium oxalate were 0.7525, 0.9607 and 0.8819, respectively, indicated that the model for an independent variable on those three responses was a quadratic model. The fitted quadratic model was observed on antioxidant activity of polysaccharides from *Tremella mesenterica* when R^2 value and adjusted R^2 value were 0.9918 and 0.9812, respectively.[41] The fitted quadratic model was also reported by [42], when R^2 value and adjusted R^2 value of optimization of the ultrasound-assisted synthesis of allyl 1-naphyl ether using RSM, were 0.949 and 0.818, respectively.

Furthermore, results of the probability F value for responses (glucomannan, viscosity and calcium oxalate) were significant ($p<0.05$). There are only 0.19%, 0.01% and 0.97% chances, respectively, that the "Model F-value" for glucomannan, viscosity and calcium oxalate could occur due to noise. On the other hand, probability F value model quadratic for the degree of whiteness was non significant ($p>0.05$), and there is 67.35% chances that noise could occur for the "Model F-value". The result of the sequential model sum of the

Table 2: *P value of response from different source of variability*

Source of variability	P value of Response Prob >F			
	Glucomannan	**Viscosity**	**Calcium oxalate**	**Degree of whiteness**
Model (Quadratic)	0.0019	0.0001	0.0097	0.6735
Time (A)	0.1139	<0.0001	<0.0001	0.0084
S/F ratio (B)	0.1156	0.0025	0.0093	0.7311
AB	0.8805	0.0037	1.000	
A^2	0.0009	<0.0001	0.9667	
B^2	0.0297	0.0001	0.0033	
Lack of Fit	0.2471	0.0510	0.1460	0.0571
R^2	0.8556	0.9771	0.9311	0.5201
Adjusted R^2	0.7525	0.9607	0.8819	0.4241

square of the degree of whiteness for the probability of F-value suggested the model linear fit for the effect of independent variables on the degree of whiteness with p value<0.05 (0.0255) or 2.55% (data not shown at Table 2).[43] also found the model linear fit on responses of flavanol, total phenol and anthocyanins activity from the fruit pulp of *Euterpe edulis* using RSM.

The lack of fit value (Table 2), was over 5% with the model quadratic for responses such as: glucomannan, viscosity, calcium oxalate, and the model linear for the response of the degree of whiteness. The model was appropriate, when the lack of fit test has p value>0.05.[44]

3.2 Influence of process independent variables on responses

The principle of ultrasonic-assisted extraction is that ultrasonic waves hit on the vegetal material cells, break the cells and release any impurities on the surface of the cells as well as the cells' contents into the extraction medium.[45] The ultrasonic waves increase the power and speed of the extraction medium into the material cells and improve the efficacy, extraction time and yield. [46, 47, 48] The effect of extraction conditions assisted by ultrasound on responses of PKF is presented in Table 3. As shown in Table 3, a remarkable increase of the glucomannan and viscosity were observed, when CKF was purified with the extraction time of 25 minutes and solvent/flour ratio at 8 ml/g. Beyond that time, the range and solvent/sample ratio had little effect on the glucomannan and viscosity of PKF. A significant decrease of calcium oxalate was observed over the extraction time range (15 – 39.14 min), calcium oxalate reaching the minimum level of 0.02% at 39.14 min and solvent/flour ratio 8 ml/g. When the extraction time is short, a lower response on the degree of whiteness was observed, and the longer the extraction time was beneficial to the purification process for removing all impurities on the surface of glucomannan granules. Therefore the maximum score of the degree of whiteness was observed at the extraction time 39.14 min.

Table 3: *Results of response surface analysis of the variation of independent variable (glucomannan, viscosity, calcium oxalate and degree of whiteness) of PKF from* Amorphophallus muelleri *affected by time and solvent/flour ratio*

No.[a]	Time (min)	Solvent/flour ratio (ml/g)	Responses			
			Glucomannan (%)	Viscosity (cPs)	Calcium oxalate (%)	Degree of whiteness
1	25.00	5.17	72.18	10300	0.09	60.37
2	35.00	10.00	70.27	9000	0.03	59.30
3	15.00	6.00	75.31	11000	0.09	58.52
4	10.86	8.00	74.18	13000	0.07	58.50
5	25.00	8.00	87.41	14000	0.04	58.97
6	25.00	8.00	86.77	14000	0.05	59.61
7	25.00	8.00	85.97	14000	0.05	59.48
8	25.00	8.00	86.90	14000	0.04	59.72
9	15.00	10.00	74.61	13500	0.08	58.66
10	39.14	8.00	67.70	8500	0.02	63.85
11	25.00	10.83	85.71	13000	0.05	60.04
12	25.00	8.00	79.97	13500	0.05	60.46
13	35.00	6.00	69.81	10000	0.04	60.04

a. Experiments were conducted in a random order.

3.3 Response surface optimization of ultrasonic extraction condition

Responses surface methodology using Design Expert 7.1.0. Version Trial resulted in a predicted second-order polynomial model quadratic for Glucomannan, viscosity and calcium oxalate, respectively, as follows:

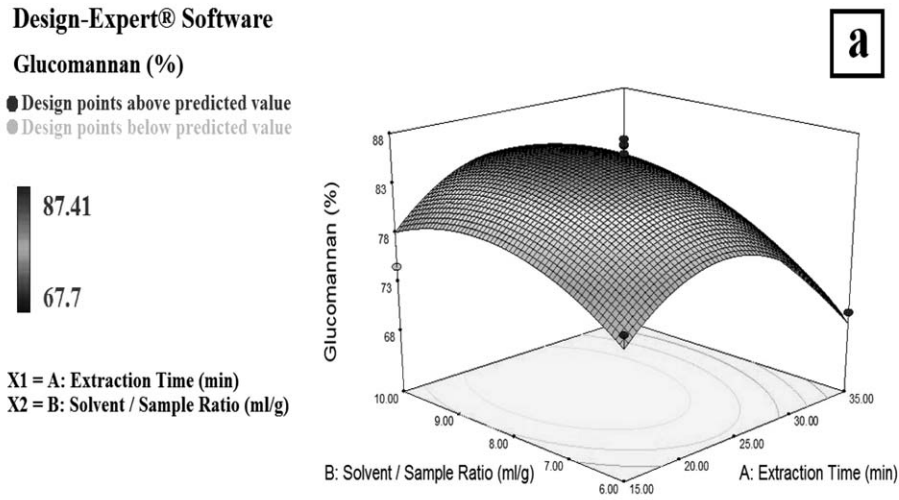
$$Y_1 = -25{,}66278 + 3{,}56776\ X_1 + 16{,}17889\ X_2 - 0{,}078426\ X_1^2 - 0{,}96003\ X_2^2 + 0{,}014500\ X_1X_2 \quad (2)$$

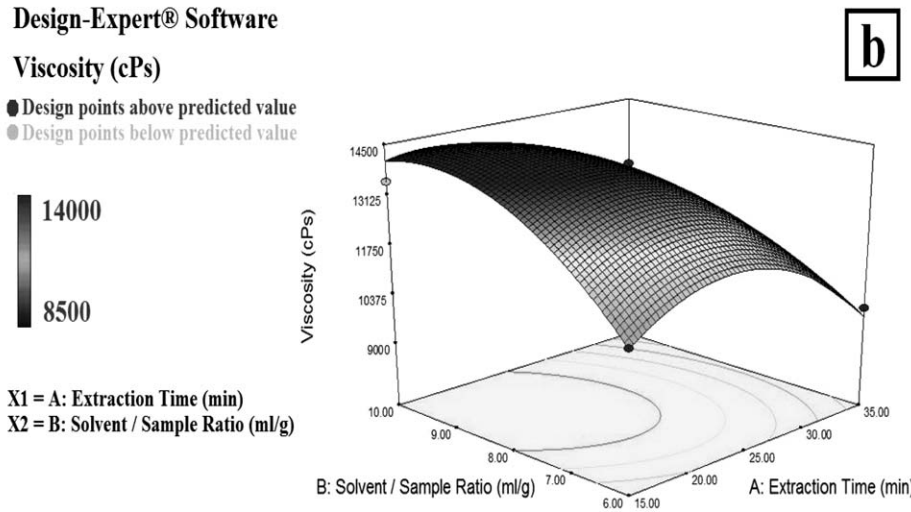
$$Y_2 = -23453{,}26299 + 1029{,}82549\ X_1 + 6251{,}14854\ X_2 - 43{,}75000\ X_1X_2 - 16{,}56250\ X_1^2 - 301{,}56250\ X_2^2 \quad (3)$$

$$Y_3 = 0.34041 - 0.00219638\ X_1 - 0.055286\ X_2 + 1{,}71304E\text{-}018\ X_1X_2 + 1.25E\text{-}06\ X_1^2 + 0.00315625\ X_2^2 \quad (4)$$

$$Y_4 = 57{,}29368 + 0{,}12159\ X_1 - 0{,}065659\ X_2 \quad (5)$$

Where: Y_1 = response of glucomannan, Y_2 = response of viscosity, Y_3 = response of calcium oxalate, Y_4 = response of degree of whiteness, X_1 = extraction time (min), and X_2 = solvent/flour ratio.





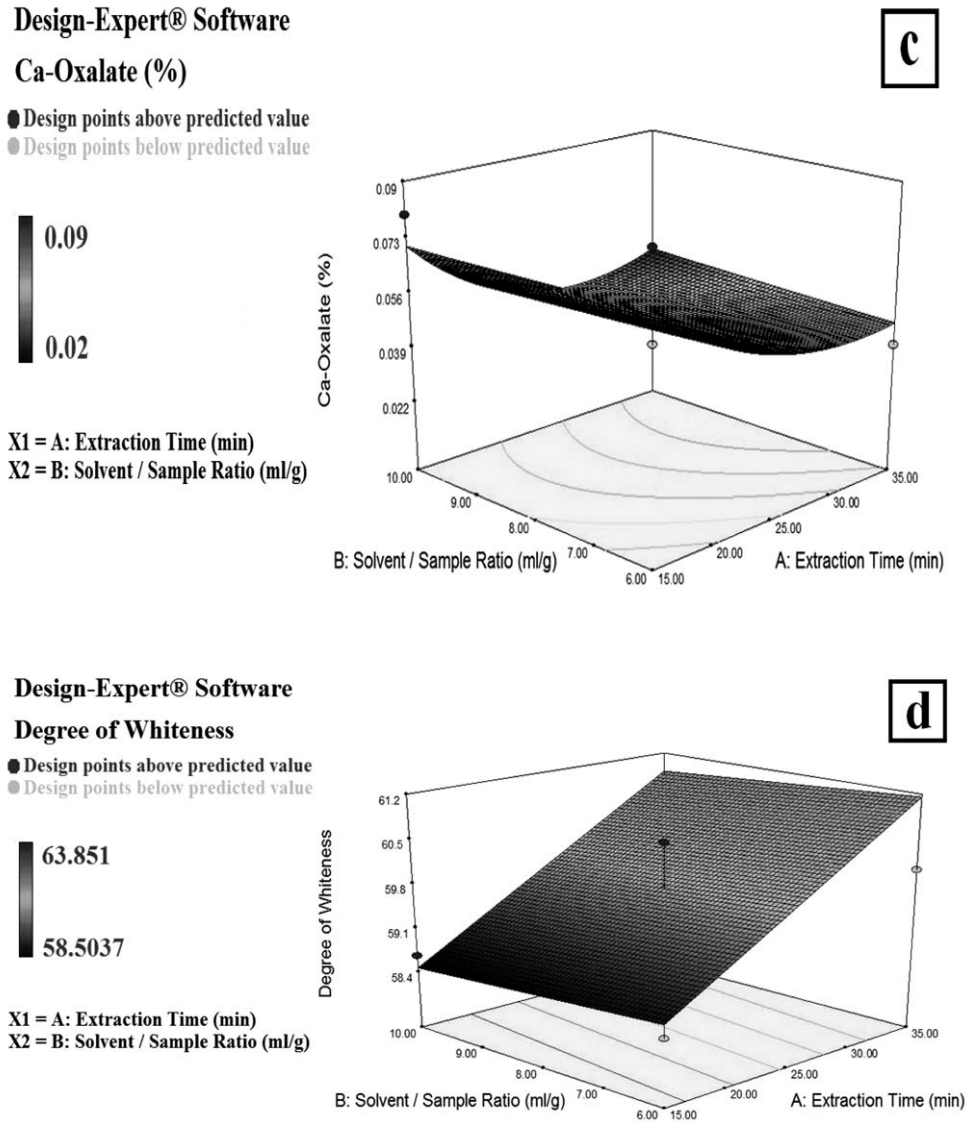


Figure 2: *Response surface plots of the glucomannan (a), viscosity (b), calcium oxalate (c) and degree of whiteness (d) affected by extraction time and solvent/flour ratio.*

To determine optimal levels, the surface plots of the glucomannan content, viscosity, calcium oxalate and the degree of whiteness affected by extraction time and solvent/flour ratio were constructed according to Eq. (2), (3), (4) and (5), respectively.

Figure 2a shows the glucomannan content increased slightly as the extraction time increased, with the optimal extraction time around 25 minutes, and started to decrease as the extraction time continued. The yield of xyloglucan from apple pomace decreased as the extraction time increased.[49] Meanwhile, the glucomannan content decreased as the solvent/flour ratio decreased from 10 ml/g to 8 ml/g and reached a minimum at a solvent/flour ratio of 6 ml/g. Generally, the larger the volume of solvent used to extract a vegetal material, the higher the yield obtained from the extraction process. [50] This theory was corroborated that the more extraction solvent used, the higher the total soluble solid dissolved in the extraction solvent. [51]

A plot of viscosity against extraction time and solvent/flour ratio shows a model quadratic of the optimal extraction condition at around 25 min. and 8 ml/g solvent/flour ratio (Fig 2b). A similar pattern can be seen in the surface plot of calcium oxalate against extraction time and solvent/flour ratio (Fig. 2c). The yield and purity of polysaccharides from *Zizyphus jujube cv. Jinsixiaozao* showing a model quadratic with increasing the yield and purity in water/solid ratio and temperature ratio. [33]

Figure 2d represents the response surface and contour plot of the degree of whiteness. According to this plot, the fitted model of interaction between the extraction time and solvent/flour ratio was linear. Fig. 2d suggests that the solvent/flour ratio had no effect on the degree of whiteness. However, the longer the extraction time the higher the degree of whiteness score, with the optimal condition of the extraction time at around 25 minutes and 8 ml/g solvent/flour ratio. This data was in accordance with Hossain *et al*. who examined the effects of amplitude and extraction time on carnosol and apigenin-7-O-glucoside from marjoram (*Origanum majorana*) using RSM.[52] They reported that the fitted model for optimizing ultrasound-assisted extraction of antioxidant compounds from marjoram (*Origanum majorana*) using RSM was linear.

3.4 Verification

The optimal condition obtained using RSM was as follows: the predicted extraction time, 25.10 minutes and solvent/flour ratio, 8.65:1 ml/g resulted in glucomannan content, viscosity, calcium oxalate and the degree of whiteness of 85.47%, 13970.7 cPs, 0.044% and 59.78, respectively. A verification run conducted in duplicate, confirmed the optimal condition. The real experiment showed that the glucomannan content, viscosity, calcium oxalate and the degree of whiteness were as follows: 84.37%, 13750.0 cPs, 0.045% and 60.38, respectively. The differences in responses between the predicted results and the real experiments were less than 5%. The strong correlation between the real and the predicted results confirm that the response model was adequate to reflect the expected optimization. The differences between the predicted results and the real, if it was not over 5%, indicated that the response model was quite accurate.[53]

3.5 The composition analysis of purified konjac flour and KGM

In general composition of commercial KGM, in terms of quality, is better than the quality of the optimal PKF obtained by ultrasound-assisted extraction using RSM (Table 4).

Table 4: *Chemical compositions of the optimal purified konjac flour treatment and commercial KGM.*

Parameters	PKF Ultrasound treatment	KGM
Glucomannan (%)	84.37±1.79	92.51±1.27
Viscosity (cPs)	13750±52.92	14000±36.06
Calcium Oxalate (%)	0.064±0.004	0.08±0.012
Degree of Whiteness	60.38±0.675*	62.86±0.481*
Moisture (%)	8.99±0.58	8.25±0.23
Ash (%)	0.32±0.025	0.37±0.026
Protein (%)	0.26±0.021	0.63±0.030
Fat (%)	0.53±0.031	0.79±0.055
Starch (%)	0.53±0.046	0.27±0.04

Note: * score 100 for degree of whiteness means 100% white.
Calculated from triplicate data

Glucomannan of KGM was higher than the optima PKF, but its viscosity was relatively indistinguishable. The amount of calcium oxalate of the optima PKF was roughly 8 times higher than the control (commercial KGM), but the degree of whiteness was rather low. Proximate data of both samples indicated they were somewhat identical, except the starch content in PKF was twice that in KGM, although, the protein content of PKF was slightly lower than the protein content in KGM. This analytical data showed that the optimization process of PKF on ultrasonically assisted extraction using RSM, affected positively the quality of PKF. This remarkable finding highlights that treating olive and olive paste using ultrasound for 8 min significantly improved the extractability of virgin olive oil.[54] These results are in agreement with a previous study.[55]

Results for Table 4 conforms the statement of Feng *et al*., that ultrasonic processing is establishing itself as a significant food-processing technology with the capability of large commercial scale-up and good payback on capital investment.[56]

4. CONCLUSION

The ultrasound-assisted extraction with three stages of ethanol washing for crude konjac glucomannan flour from *Amorphophallus muelleri* was performed with two variables (the extraction time and solvent/flour ratio) based on the RSM. The optimal condition of extracting crude konjac glucomannan flour was at the extraction time, 25 min. and 6 sec. and solvent/flour ratio 8.65 ml/g. Under this optimal condition the glucomannan, viscosity, calcium oxalate and the degree of whiteness were at optimum level. The real experiment confirmed the optimal condition of the extraction process with p value <0.05. The experimental results showed that the extraction time and solvent/flour ratio at the centre and factorial treatments of central composite design were significant to increase the glucomannan content and viscosity of PKF. The calcium oxalate content was affected by the extraction time and solvent/flour ratio. The degree of whiteness was significantly affected by the extraction time, but it was not affected with the solvent/flour ratio. Proximate analysis and four other parameters (glucomannan, viscosity, calcium oxalate and degree of whiteness) of the optimal ultrasound treatments of PKF were virtually the

same as those of commercial KGM. Sonication during the CKF extraction process needs to be explored on a larger scale.

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