Optimisation of Extrusion for Enhancing the Nutritive Value of Palm Kernel Cake Using Response Surface Methodology

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The palm kernel cake (PKC) inclusion level in poultry diets is limited by the high indigestible polysaccharides content. Hence, PKC was subjected to an extrusion treatment to reduce the content of these components. The effects of extrusion on the total non-starch polysaccharides (T-NSP), fibre, monosaccharides, and mannooligosaccharides contents were evaluated according to the response surface methodology (RSM) with various temperatures, screw speeds, hopper speeds, and moisture contents. The optimum conditions observed according to the RSM were a temperature of 178 °C, screw speed of 100 rpm, hopper speed of 5 Hz, and moisture content of 75%. The T-NSP content was significantly reduced ($p<0.05$), from 63.3 ± 1.85% to 57.6 ± 0.89%, and the crude fibre content decreased ($p<0.05$) from 16.7 ± 0.68% to 13.5 ± 0.99%. The mannose, glucose, and fructose contents of the PKC increased ($p<0.05$) 2.9-, 1.9-, and 1.4-fold, respectively. The 1,4-β-D-mannobiose, 1,4-β-D-mannotriose, 1,4-β-D-mannotetraose, and 1,4-β-D-mannopentaose increased ($p<0.05$) 3.7-, 3.8-, 3.5-, and 32.8-fold, respectively. This study showed that extrusion enhanced the nutritive value of PKC.

Keywords: Palm kernel cake; Twin-screw extrusion; Response surface methodology; Non-starch polysaccharides; Sugars

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INTRODUCTION

Palm kernel cake (PKC), a by-product of the oil palm industry, can be used as a feed ingredient for poultry, with a recommended inclusion rate of 15% to 20% in poultry diets (Zahari and Alimon 2004). The inclusion rate is constrained by the high non-starch polysaccharide (NSP), or high dietary fibre content of PKC (Sundu *et al*. 2006). The NSPs represent a group of heterogeneous compounds of non-α-glucan polysaccharides from plant cell walls with varying degrees of water solubility and sizes. The NSPs refer to all carbohydrate fractions and types of dietary fibre (Englyst and Hudson 1987). Pectic substances, hemicelluloses, celluloses and gums (guar), inulin, fructans, and mucilages are all examples of NSPs. They are typically made up of long polymeric carbohydrate chains containing up to several hundred thousand monomers (Căpriţă *et al*. 2010). Omar and Hamdan (1998) reported that PKC contains at least 60% of NSP. The NSP of PKC is mostly linear, insoluble, and highly crystalline β-mannans, with some galactose substitution, that accounts for 78% of the total NSP. The other components include 12% cellulose, 3% (4-O-methyl)-glucuronoxylans, and 3% arabinoglycans (Daud and Jarvis 1992; Düsterhöft *et al*. 1992). The

anti-nutritive properties of NSPs could interfere with the digestion and absorption of nutrients (de Vries *et al*. 2012). It was reported by Le Gall *et al*. (2009) that the digestibility of protein, fat, and starch was reduced in pigs fed rye aleurone flour bread rich in NSPs. The rigid nature of the aleurone cell walls encapsulated the nutrients.

A number of studies have been conducted to enhance the nutritive value of PKC using solid-state fermentation with various fungal species. It was reported by Wong *et al*. (2011) that solid-state fermentation of PKC by *Aspergillus flavus* increased the mannose content 5.9-fold as a result of the depolymerisation of β-mannans. The cellulose level of PKC was reduced from 28.3% to 12.1% through fermentation with *Trichoderma longibrachiatum*, and the hemicellulose content decreased from 37.0% to 19.0% when fermented with *A. niger* (Iluyemi *et al*. 2006). A study conducted by Lateef *et al*. (2008) also showed the positive effect of fungal treatment, where a reduction of 44.5% of crude fibre in PKC was observed after 5 d of fermentation using *Rhizopus stolonifer* LAU 07.

These studies indicated that although biological treatment of PKC with solid-state fermentation can improve the nutritive value of PKC, the process is time-consuming and dependent on controlled conditions to prevent contamination by other microorganisms. Fungal metabolic activity may also produce anti-nutritive metabolites, such as mycotoxins that can reduce the growth of fish when fed fungal-treated PKC (Lim *et al*. 2001; Ng 2004).

In lieu of increasing the cost of importing feed ingredients for poultry, local feed materials, such as PKC, should become a major component of the feed formulation. The abundance of PKC in Malaysia, with a production rate of more than three million tonnes per annum (Malaysian Palm Oil Board 2014), would ensure a reliable and continuous source of the feed ingredient for a sustainable poultry industry. Problems arising from the high fibre content in PKC have to be resolved if PKC is to be included at a higher inclusion rate in poultry diets. Hence, physical treatments, such as extrusion, should be considered as an alternative method to treat PKC. The extrusion method is based on a hydrothermal process with high shear force, which has the ability to disrupt the components of feedstuffs to produce nutritious food and animal feeds (de Vries *et al*. 2012; Kumar *et al*. 2015; Colovic *et al*. 2016) within a shorter period of time. Therefore, this study focused on the physical treatment of PKC by extrusion to reduce the levels of indigestible polysaccharides. The interaction of dependent variables (extrusion temperature, screw speed, hopper speed, and PKC moisture content) on the total NSP (T-NSP), monosaccharides, and mannooligosaccharides (MOS) contents were evaluated by response surface methodology (RSM) to optimise the extrusion conditions.

EXPERIMENTAL

Sample Preparation

The PKC was obtained from a commercial palm oil mill in Kapar, Selangor, Malaysia. The moisture content of the PKC was adjusted by adding different levels of distilled water to 7 kg of PKC with a particle size less than or equal to 1.5 mm, and was stirred with a mechanical stirrer for 10 min. The PKC was then subjected to extrusion.

Extruder

Extrusion was carried out using a co-rotating twin-screw extruder with a screw diameter of 50 mm and length to diameter ratio of 24. A diagram of the extruder is shown in Fig. 1. It was custom made by the company *Dr. Cropp BioF2 Sdn Bhd*, Malaysia, for the treatment of PKC. The extruder was equipped with six thermocouples to measure the

temperature of the barrel and inside the barrel, and three heaters to heat the barrel. Two nozzles with 13-mm diameter holes were fitted at the end of the barrel, for collecting the extrudates. The extruded PKC was cooled to room temperature, then immediately stored in polyethylene bags at -20 °C. The PKC was oven dried at 60 °C before further chemical analyses.

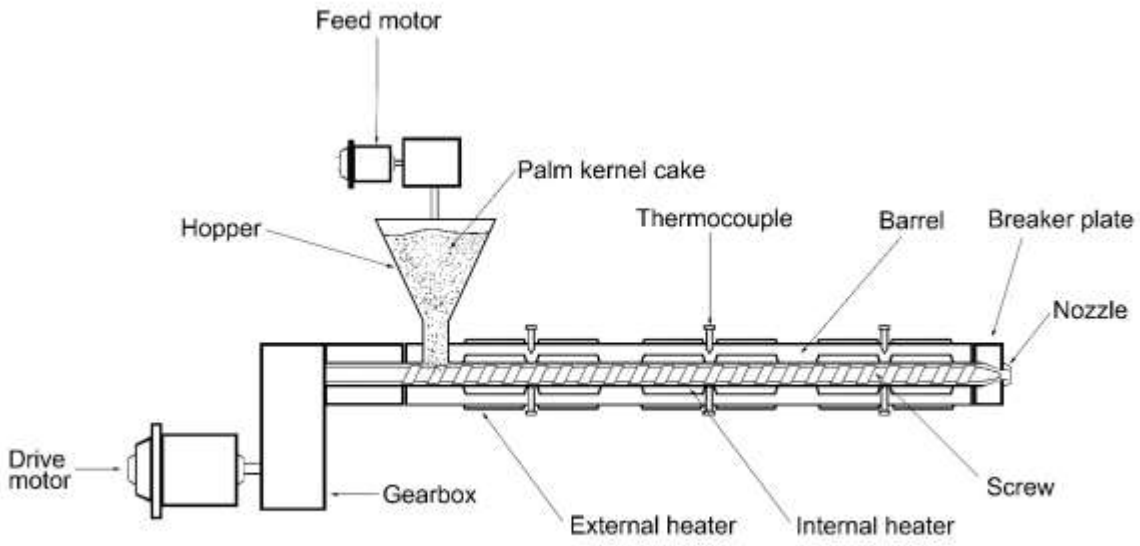

Fig. 1. Simple diagram of extruder

Extrusion Experimental Design by RSM

Response surface methodology (RSM) based on the Box-Behnken design was used as the experimental design for the extrusion process with four independent variables (Ferreira *et al*. 2007). Table 1 shows the coded and actual variables of the four independent variables consisting of extrusion temperature (X_1, °C), screw speed (X_2, rpm), hopper speed (X_3, Hz), and PKC moisture content (X_4, %).

The T-NSP, monosaccharides (mannose, glucose, and fructose), and MOS (1,4-β-D-mannobiose, 1,4-β-D-mannotriose, 1,4-β-D-mannotetraose, and 1,4-β-D-mannopentaose) contents were taken as the dependent variables of the extrudates. The experimental design, which included 29 runs, was developed using Design Expert 7.0.0 software (Stat-Ease, Inc., USA). Regression analysis was performed to analyse the results of the dependent variables, which was fitted to empirical second-order polynomial models.

Proximate Analyses

The moisture content of the PKC was determined according to the standard procedures of the Association of Official Agricultural Chemists (2000), while crude fibre was determined by using a FiberTec 2010™ (FOSS, Hillerød, Denmark). The gross energy was determined by using an IKA® Calorimeter System C2000 (Staufen, Germany), standardised with benzoic acid.

Analysis of T-NSP

The T-NSP contents were determined according to the enzymatic calorimetric method described by Englyst *et al*. (1994). The PKC sample was ground to pass through a 0.5-mm mesh sieve.

The digestion of starch was carried out by using a heat-stable amylase from *Bacillus licheniformis* (Termamyl®, Sigma-Aldrich, Inc., Missouri, USA), pancreatin from bovine pancreas (Sigma-Aldrich, Inc.), and pullulanase from *Klebsiella pneumonia* (Sigma-Aldrich, Inc.). Pectinase from *A. niger* (Sigma-Aldrich, Inc.) was used to complete the hydrolysis of uronic acid-containing polymers. The amount of T-NSP was determined by referring to a standard curve prepared from a standard sugar solution consisting of arabinose, glucose, and galacturonic acid. The absorbance was obtained by using a Rayto RT-2100C Microplate Reader (Shenzhen, China).

Analysis of Monosaccharides and MOS

The analyses of the monosaccharides and MOS were conducted according to Chen *et al*. (2015). The extract was prepared by dissolving 1 g of sample in 5 mL of water, which was mixed well. Two millilitres of the mixture were centrifuged at 14,000 g for 5 min. One millilitre of the supernatant was mixed with 1 mL of acetonitrile. The mixture was vortexed and centrifuged at 14,000 g for 5 min. The supernatant was filtered using a 0.22-µm syringe filter and transferred to a 2-mL high-performance liquid chromatography (HPLC) vial. The monosaccharides were separated on a COSMOSIL sugar-D packed column (4.6 mmI.D. x 250 mm, Kyoto, Japan) using a RI detector at 30 °C. The eluent was a mixture of 20% HPLC-grade water and 80% acetonitrile, with a flow rate of 0.8 mL/min. D-mannose, D-glucose, and D-fructose were used as reference standards.

For the MOS analysis, the eluent was a mixture of 35% HPLC-grade water and 65% acetonitrile, with a flow rate of 0.7 mL/min. The standards were 1,4-β-D-mannobiose, 1,4-β-D-mannotriose, 1,4-β-D-mannotetraose, and 1,4-β-D-mannopentaose.

Determination of Sucrose, Inulin, and Fructan in PKC

The sucrose, inulin, and fructan contents were determined in the PKC samples based on the method given by the Fructan Assay Kit (Megazyme, Wicklow, Ireland) with some modifications. Two grams of ground (0.5-mm screen) PKC were placed in a 200-mL beaker containing 80 mL of hot distilled water (~80 °C) and heated on a magnetic hot plate stirrer at approximately 80 °C for 15 min until the sample was thoroughly mixed. The sample was cooled to room temperature and transferred to a 100-mL volumetric flask. The volume was made up to 100 mL with distilled water and was mixed thoroughly. The mixture was centrifuged at 14,000 g for 5 min.

The supernatant was filtered using a 0.22-µm syringe filter and transferred into HPLC vials for analysis. The HPLC was fitted with a RI detector and Rezex RPM-Monosaccharides Pb+ column (300 mm × 7.8 mm, Torrance, USA) set at 75 °C. The eluent used was an ultra-filtered deionised water with a flow rate of 0.6 mL/min. The pure standards used as references were sucrose, inulin, and fructan.

Fourier Transform Infrared (FTIR) Spectroscopy

The spectra of the PKC were obtained by using a Nicolet 6700 FT-IR spectrometer (Waltham, USA) based on the method described by Barsberg *et al*. (2011). The PKC discs were prepared by mixing 1 mg of dried PKC with 100 mg of potassium bromide, which was followed by pressing the mixture at 10 MPa for 5 min. The Fourier transform infrared (FTIR)

spectra were measured with 100 scans from 4000 to 400 cm^{-1} at a temperature of 30 °C. The infrared (IR) band positions (frequencies) of mannan and cellulose were determined using 1,4-β-D-mannan (Ivory nut, purity >98%, Megazymes) and cellulose (microcrystalline powder, Sigma-Aldrich) as references.

Determination of Hydration Properties

The water retention capacity (WRC) and swelling capacity (SC) were determined according to the methods described by Robertson *et al*. (2000). For the determination of the WRC, 3 g of PKC were added to 30 mL of distilled water in a 50-mL centrifuge tube. The mixture was vortexed and left for 18 h at room temperature. The mixture was centrifuged for 20 min at 3,000 g, and the supernatant was discarded. The pellet was weighed, and the WRC was expressed as g of water retained per g of dry PKC.

The SC was determined by adding 10 g of PKC to 50 mL of distilled water in a 50-mL measuring cylinder. The mixture was vortexed to remove air bubbles and was left undisturbed at room temperature for 18 h on a level surface to allow the sample to settle. The final volume occupied by the PKC was determined. The SC was expressed as the volume occupied by PKC per g of dry PKC.

Statistical Analysis

All experiments were done in triplicates. The Design Expert 7.0.0 software was used for the analysis of variance (ANOVA) of experimental data. The t-test was performed by using a Minitab software version 16.2.4 (Minitab Pty Ltd, Sydney, Australia). Means were considered statistically significant when $p<0.05$.

RESULTS AND DISCUSSION

Table 1 shows the experimental and predicted results of the 29 extrusion runs for the T-NSP and monosaccharides contents in the PKC extrudates. The experimental data showed that the amount of T-NSP of the PKC extrudates was in the range of 58.9% to 62.1% under different extrusion conditions. The amount of mannose, glucose, and fructose were in the range of 12.2 to 54.9 mg/100 g, 134.3 to 307.7 mg/100 g, and 125.6 to 289.2 mg/100 g, respectively.

Table 2 shows the ANOVA for the optimisation of PKC extrusion conditions for T-NSP and monosaccharides mannose, glucose, and fructose. The models showed an insignificant lack of fit ($p>0.05$), which indicated the models could be used to predict values for the T-NSP and monosaccharides derived during extrusion of PKC.

If a model has a significant lack of fit, it is not a good indicator of the response and should not be used for prediction. Based on the analysis, the predictive models developed for T-NSP and monosaccharides *via* RSM were acceptable ($p<0.0001$; $p<0.0001$) with coefficient of determination (R^2) values of 0.9573, 0.8561, 0.9427 and 0.9948, for T-NSP, mannose, glucose, and fructose, respectively.

These models could be adequately used as predictor models, although the adjusted R^2 value for mannose (28.78%) was not explainable. Yağcı and Göğüş (2008) also considered their RSM models to be adequate as predictor models, when the coefficient of determinations of regression equations ranged from 0.710 to 0.802 with significant probability values ($p< 0.0001$) and a non-significant lack of fit.

Table 1. Experimental Design for Extrusion of PKC with the Respective Uncoded Factors and Response Functions for T-NSP and Monosaccharides

Treatment	Uncoded variables				Responses							
	X_1 (°C)	X_2 (rpm)	X_3 (Hz)	X_4 (%)	T-NSP (%)		Mannose (mg/g)		Glucose (mg/g)		Fructose (mg/g)	
					Experimental	Predicted	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	80	100	7.5	55	61.38	61.32	0.300	0.346	1.659	1.825	1.740	1.775
2	180	100	7.5	55	59.45	59.35	0.485	0.489	3.033	3.017	2.696	2.665
3	80	300	7.5	55	62.05	62.12	0.260	0.293	1.343	1.380	1.256	1.264
4	180	300	7.5	55	59.94	59.97	0.206	0.198	2.425	2.280	1.617	1.559
5	130	200	5	35	59.93	59.88	0.179	0.238	2.447	2.425	2.362	2.313
6	130	200	10	35	60.58	60.32	0.350	0.330	2.034	2.181	2.341	2.354
7	130	200	5	75	59.99	60.22	0.204	0.262	2.489	2.363	2.189	2.153
8	130	200	10	75	60.04	60.05	0.255	0.234	2.353	2.396	2.480	2.506
9	80	200	7.5	35	61.33	61.32	0.312	0.306	1.679	1.599	1.548	1.536
10	180	200	7.5	35	60.07	59.99	0.287	0.251	2.322	2.407	2.112	2.144
11	80	200	7.5	75	61.99	62.07	0.173	0.191	1.591	1.438	1.612	1.548
12	180	200	7.5	75	59.27	59.29	0.306	0.294	2.710	2.722	2.144	2.124
13	130	100	5	55	59.74	59.59	0.549	0.503	2.857	2.838	2.591	2.568
14	130	300	5	55	60.60	60.55	0.122	0.139	2.139	2.118	1.854	1.855
15	130	100	10	55	59.92	59.98	0.378	0.343	2.650	2.603	2.892	2.859
16	130	300	10	55	60.28	60.44	0.336	0.363	2.190	2.141	1.964	1.955
17	80	200	5	55	61.62	61.57	0.335	0.255	1.522	1.607	1.656	1.699
18	180	200	5	55	59.32	59.37	0.301	0.293	2.541	2.643	2.266	2.330
19	80	200	10	55	61.60	61.56	0.312	0.301	1.546	1.491	1.942	1.933
20	180	200	10	55	59.58	59.65	0.251	0.311	2.585	2.547	2.476	2.488
21	130	100	7.5	35	58.90	59.21	0.402	0.429	3.077	2.946	2.754	2.761
22	130	300	7.5	35	61.22	61.31	0.245	0.222	1.767	1.768	1.303	1.312
23	130	100	7.5	75	60.69	60.63	0.353	0.357	2.389	2.435	2.071	2.117
24	130	300	7.5	75	60.23	59.94	0.267	0.221	2.252	2.430	1.900	1.948
25	130	200	7.5	55	60.67	60.28	0.245	0.231	2.543	2.376	1.532	1.529
26	130	200	7.5	55	59.95	60.28	0.202	0.231	2.522	2.376	1.503	1.529
27	130	200	7.5	55	60.52	60.28	0.201	0.231	2.141	2.376	1.505	1.529
28	130	200	7.5	55	59.96	60.28	0.253	0.231	2.442	2.376	1.549	1.529
29	130	200	7.5	55	60.28	60.28	0.253	0.231	2.232	2.376	1.554	1.529

Where:
X_1, Extruder temperature (°C); X_2, Screw speed (rpm); X_3, Hopper speed (Hz) and; X_4, PKC moisture content (%).

Table 2. ANOVA for the Optimisation of PKC Extrusion Conditions for T-NSP and Monosaccharides

Response	T-NSP	Mannose	Glucose	Fructose
Source	Prob > F			
Model	< 0.0001	0.0010	< 0.0001	< 0.0001
X_1	< 0.0001	0.4105	< 0.0001	< 0.0001
X_2	0.0002	< 0.0001	< 0.0001	< 0.0001
X_3	0.3482	0.2770	0.2547	< 0.0001
X_4	0.8355	0.2219	0.4075	0.8841
$X_1 X_2$	0.7195	0.0287	0.3616	< 0.0001
X_1X_3	0.5618	0.7870	0.9494	0.4290
X_1X_4	0.0095	0.1292	0.1465	0.7367
X_2X_3	0.3172	0.0015	0.4187	0.0599
X_2X_4	< 0.0001	0.4807	0.0020	< 0.0001
X_3X_4	0.2316	0.2410	0.3861	0.0048
X^2_1	0.0008	0.1833	0.0002	0.0270
X^2_2	0.9351	0.0018	0.4129	< 0.0001
X^2_3	0.1497	0.1140	0.9715	< 0.0001
X^2_4	0.8815	0.8961	0.6003	< 0.0001
Lack of Fit	0.8974	0.0886	0.7444	0.0689
Pure Error	4	4	4	4
Cor Total	28	28	28	28
R^2	0.9573	0.8561	0.9427	0.9948
Adj. R^2	0.9147	0.7122	0.8854	0.9897

The following second-order polynomial equations showed the relationship between the independent and dependent variables of the extrusion experiment:

$$\text{T-NSP (\%)} = 60.28 - 1.03X_1 + 0.35X_2 + 0.07X_3 + 0.02X_4 - 0.04X_1X_2 + 0.07X_1X_3 - 0.36X_1X_4 - 0.13X_2X_3 - 0.70X_2X_4 - 0.15X_3X_4 + 0.41X^2_1 + 0.01X^2_2 - 0.14X^2_3 - 0.01X^2_4 \quad (1)$$

$$\text{Mannose (mg/100 g)} = 23.08 + 1.20X_1 - 8.59X_2 + 1.60X_3 - 1.81X_4 - 5.98X_1X_2 - 0.67X_1X_3 + 3.95X_1X_4 + 9.63X_2X_3 + 1.78X_2X_4 - 3.00X_3X_4 + 2.69X^2_1 + 7.38X^2_2 + 3.24X^2_3 + 0.26X^2_4 \quad (2)$$

$$\text{Glucose (mg/100 g)} = 237.60 + 52.30X_1 - 29.57X_2 - 5.31X_3 + 3.82X_4 - 7.40X_1X_2 + 0.5X_1X_3 + 11.90X_1X_4 + 6.45X_2X_3 + 29.32X_2X_4 + 6.92X_3X_4 + 30.18X^2_1 + 5.13X^2_2 - 0.22X^2_3 - 2.36X^2_4 \quad (3)$$

$$\text{Fructose (mg/100 g)} = 152.86 + 29.64X_1 - 40.42X_2 + 9.81X_3 - 0.20X_4 - 14.87X_1X_2 - 1.90X_1X_3 - 0.80X_1X_4 - 4.77X_2X_3 + 32.00X_2X_4 + 7.80X_3X_4 + 4.52X^2_1 + 24.19X^2_2 + 53.87X^2_3 + 26.41X^2_4 \quad (4)$$

Table 3. Experimental Design for Extrusion of PKC with the Respective Uncoded Factors and Response Functions for MOS

Treatment	Uncoded variables				Responses							
	X_1 (°C)	X_2 (rpm)	X_3 (Hz)	X_4 (%)	1,4-β-D-mannobiose (mg/g)		1,4-β-D-mannotriose (mg/g)		1,4-β-D-mannotetraose (mg/g)		1,4-β-D-mannopentaose (mg/g)	
					Experimental	Predicted	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	80	100	7.5	55	0.670	0.599	0.087	0.082	0.011	0.011	0.002	0.002
2	180	100	7.5	55	0.636	0.625	0.098	0.095	0.019	0.017	0.008	0.009
3	80	300	7.5	55	0.407	0.345	0.052	0.047	0.015	0.014	0.001	-0.003
4	180	300	7.5	55	0.525	0.523	0.083	0.080	0.012	0.010	0.006	0.003
5	130	200	5	35	0.643	0.680	0.090	0.085	0.014	0.015	0.007	0.010
6	130	200	10	35	0.541	0.561	0.096	0.089	0.016	0.016	0.009	0.010
7	130	200	5	75	1.043	0.950	0.128	0.126	0.027	0.024	0.029	0.025
8	130	200	10	75	1.023	0.913	0.103	0.100	0.025	0.021	0.025	0.019
9	80	200	7.5	35	0.308	0.325	0.049	0.053	0.011	0.012	0.001	0.000
10	180	200	7.5	35	0.608	0.569	0.081	0.078	0.010	0.009	0.008	0.003
11	80	200	7.5	75	0.658	0.779	0.069	0.080	0.012	0.015	0.002	0.009
12	180	200	7.5	75	0.674	0.738	0.098	0.102	0.019	0.020	0.016	0.019
13	130	100	5	55	0.886	0.889	0.117	0.122	0.022	0.023	0.023	0.020
14	130	300	5	55	0.549	0.582	0.086	0.084	0.014	0.014	0.007	0.006
15	130	100	10	55	0.633	0.682	0.088	0.098	0.013	0.015	0.005	0.008
16	130	300	10	55	0.554	0.633	0.083	0.086	0.019	0.020	0.006	0.011
17	80	200	5	55	0.583	0.595	0.081	0.080	0.016	0.015	0.001	0.002
18	180	200	5	55	0.645	0.654	0.094	0.099	0.013	0.016	0.006	0.010
19	80	200	10	55	0.491	0.474	0.069	0.065	0.016	0.014	0.002	0.000
20	180	200	10	55	0.638	0.618	0.091	0.092	0.012	0.014	0.004	0.005
21	130	100	7.5	35	0.612	0.614	0.091	0.093	0.016	0.016	0.008	0.008
22	130	300	7.5	35	0.539	0.502	0.056	0.065	0.011	0.011	0.007	0.009
23	130	100	7.5	75	0.962	0.991	0.123	0.115	0.020	0.021	0.027	0.026
24	130	300	7.5	75	0.758	0.748	0.096	0.094	0.020	0.021	0.014	0.015
25	130	200	7.5	55	0.576	0.543	0.072	0.077	0.013	0.013	0.007	0.009
26	130	200	7.5	55	0.503	0.543	0.076	0.077	0.013	0.013	0.011	0.009
27	130	200	7.5	55	0.524	0.543	0.078	0.077	0.011	0.013	0.008	0.009
28	130	200	7.5	55	0.532	0.543	0.081	0.077	0.014	0.013	0.009	0.009
29	130	200	7.5	55	0.582	0.543	0.079	0.077	0.012	0.013	0.012	0.009

Where: X_1, Extruder temperature (°C); X_2, Screw speed (rpm); X_3, Hopper speed (Hz) and; X_4, PKC moisture content (%).

Experimental Results of MOS

The experimental and predicted results of the 29 extrusion runs on the amount of MOS in the PKC extrudates are shown in Table 3. The amounts of 1,4-β-D-mannobiose, 1,4-β-D-mannotriose, 1,4-β-D-mannotetraose, and 1,4-β-D-mannopentaose were in the range of 30.8 to 104.3 mg/100 g, 4.9 to 12.8 mg/100 g, 1.0 to 2.7 mg/100 g, and 0.1 to 2.9 mg/100 g, respectively.

Table 4 shows the ANOVA for the optimisation of PKC extrusion conditions for MOS. The models showed an insignificant lack of fit ($p>0.05$), which indicated that the models could be used to predict values for MOS derived during extrusion of PKC. Based on the analysis, the predictive models developed for MOS *via* RSM were acceptable ($p<0.0.001$; $p<0.0012$) with coefficient of determination (R^2) values of 0.9109, 0.9261, 0.8518 and 0.8510, for 1,4-β-D-mannobiose, 1,4-β-D-mannotriose, 1,4-β-D-mannotetraose, and 1,4-β-D-mannopentaose, respectively. These models could be adequately used as predictor models although the adjusted R^2 value for 1,4-β-D-mannotetraose, and 1,4-β-D-mannopentaose were slightly low (0.7020 to 0.7035).

Table 4. ANOVA for the Optimisation of PKC Extrusion Conditions for MOS

Response	1,4-β-D-mannobiose	1,4-β-D-mannotriose	1,4-β-D-mannotetraose	1,4-β-D-mannopentaose
Source	Prob > F			
Model	< 0.0001	< 0.0001	0.0012	0.0012
X_1	0.0257	< 0.0001	0.6350	0.0188
X_2	0.0006	< 0.0001	0.2452	0.0468
X_3	0.0752	0.0176	0.5539	0.1561
X_4	< 0.0001	< 0.0001	< 0.0001	0.0002
$X_1 X_2$	0.2989	0.1799	0.0366	0.9077
X_1X_3	0.5559	0.5355	0.8366	0.7286
X_1X_4	0.0634	0.8354	0.1150	0.4226
X_2X_3	0.0884	0.0878	0.0107	0.0645
X_2X_4	0.3682	0.5813	0.3113	0.1786
X_3X_4	0.5698	0.0461	0.4148	0.4905
X^2_1	0.0323	0.0177	0.1288	0.0010
X^2_2	0.1241	0.0481	0.0834	0.8982
X^2_3	0.0016	0.0002	0.0017	0.3928
X^2_4	0.0005	0.0084	0.0083	0.0085
Lack of Fit	0.0570	0.0556	0.0540	0.0584
Pure Error	4	4	4	4
Cor Total	28	28	28	28
R^2	0.9109	0.9261	0.8518	0.8510
Adj. R^2	0.8217	0.8522	0.7035	0.7020

The relationship of the extrusion parameters and response variables of the experiment are shown below in the form of second-order polynomial equations:

$$\text{1,4-β-D-mannobiose (mg/100 g)} = 54.34 + 5.08X_1 - 8.89X_2 - 3.91X_3 + 15.56X_4 + 3.80X_1X_2 + 2.13X_1X_3 - 7.10X_1X_4 + 6.45X_2X_3 - 3.28X_2X_4 + 2.05X_3X_4 - 6.57X^2_1 + 4.53X^2_2 + 10.75X^2_3 + 12.50X^2_4 \quad (5)$$

$$\text{1,4-β-D-mannotriose (mg/100 g)} = 7.72 + 1.15X_1 - 1.23X_2 - 0.55X_3 + 1.28X_4 + 0.50X_1X_2 + 0.22X_1X_3 - 0.075X_1X_4 + 0.65X_2X_3 + 0.20X_2X_4 -$$

$$0.78X_3X_4 - 0.75X^2_1 + 0.60X^2_2 + 1.43X^2_3 + 0.85X^2_4 \quad (6)$$

$$\text{1,4-β-D-mannotetraose (mg/100 g)} = 1.26 + 0.033X_1 - 0.083X_2 - 0.042X_3 + 0.038X_4 - 0.028X_1X_2 - 0.025X_1X_3 + 0.20X_1X_4 + 0.35X_2X_3 + 0.12X_2X_4 - 0.10X_3X_4 - 0.15X^2_1 + 0.17X^2_2 + 0.36X^2_3 + 0.29X^2_4 \quad (7)$$

$$\text{1,4-β-D-mannopentaose (mg/100 g)} = 0.94 + 0.33X_1 - 0.27X_2 - 0.18X_3 + 0.61X_4 - 0.025X_1X_2 - 0.075X_1X_3 + 0.17X_1X_4 + 0.43X_2X_3 - 0.30X_2X_4 - 0.15X_3X_4 - 0.69X^2_1 + 0.022X^2_2 + 0.15X^2_3 + 0.51X^2_4 \quad (8)$$

Effect of Extrusion on the T-NSP of PKC

The T-NSP of PKC was affected by the extrusion process. The level of T-NSP was reduced ($p<0.05$) when the temperature was increased and the moisture content values decreased. Low screw and hopper speeds were observed to be more effective than high screw and hopper speeds for the depolymerisation of the T-NSP. The reduction of the T-NSP observed could have been due to the enhanced depolymerisation of polysaccharides into their monomers as the PKC stayed longer in the barrel at low screw and hopper speeds.

Effect of Extrusion Variables on the Monosaccharides of PKC

The effect of the extrusion parameters was significant ($p<0.05$), as shown by the 3-fold increase in the level of mannose in the PKC. The response surface plots, as shown in Figs. 2 to 7, illustrate that the extrusion process resulted in the highest value of mannose content when the extrusion temperature and moisture levels were high and the screw and hopper speeds were low. As shown in Figs. 2, 5 and 6, the levels of mannose were inversely proportional to the screw speed. The residence time of the PKC in the barrel increased as the screw speed became slow, and this allowed more time for hydration, thermal degradation, and mechanical separation of the lignin and cellulosic components (Karunanithy and Muthukumarappan 2013). At this combination of extrusion conditions, the hopper speed must not be too low. When the screw and hopper speeds were too slow, *e.g.*, less than 100 rpm and 3 Hz, respectively, the water present in the PKC was squeezed out from the barrel and outlet. On the other hand, it is not recommended to run extrusion at a very high hopper speed, *e.g.*, 13 Hz, combined with a low screw speed and moisture content. Under these conditions, the developed pressure was too much, and caused the PKC to harden into a compact solid structure in the barrel. The hardened PKC would jam the barrel, which affected the screw speed as more PKC was deposited and clumped together at the end of the screw near the outlet.

The level of mannose produced, shown in Fig. 3, reached the highest level at high moisture contents and extrusion temperatures. As the temperature was increased, the evaporation of water in the PKC increased. As a result, more friction developed inside the barrel, which resulted in more disturbances to the feed materials (Karunanithy and Muthukumarappan 2013). At a low temperature and high moisture content, the level of mannose was at its lowest value. Under these conditions, the effect of temperature was negligible. With a high moisture content, most of the heat from the heater might have been used for moisture removal from the PKC (Karunanithy and Muthukumarappan 2011). Also, under these conditions, the effect of extrusion on the polysaccharides was low because the shearing effect of the screw was the only factor that contributed to the breakdown of the polysaccharides.

As shown in Fig. 4, when the screw speed and moisture content were kept at the zero level, with either a low or high extrusion temperature, the level of mannose was observed to be higher at higher hopper speeds. This was probably because at high hopper speeds, the increase

in pressure which developed during the extrusion, enhanced the breakdown of the polymers. The effect of the hopper speed seemed to be higher at high temperatures than at low temperatures. This was due to the combined effect of high temperature and high pressure that increased the extrusion effect compared to the effect of temperature or pressure alone.

The effect of the moisture content, shown in Fig. 7, became more significant as the hopper speed increased. The results showed that at high hopper speeds, the increase of the moisture content reduced the level of mannose. This was because the combination of a high hopper speed and moisture content reduced the rate at which the temperature increased in the barrel, which resulted in the reduction of the effect from the extrusion temperature on the PKC.

The effects of the independent variables (moisture content, temperature, screw speed, and hopper speed) on the glucose and fructose contents were similar to those on the mannose content.

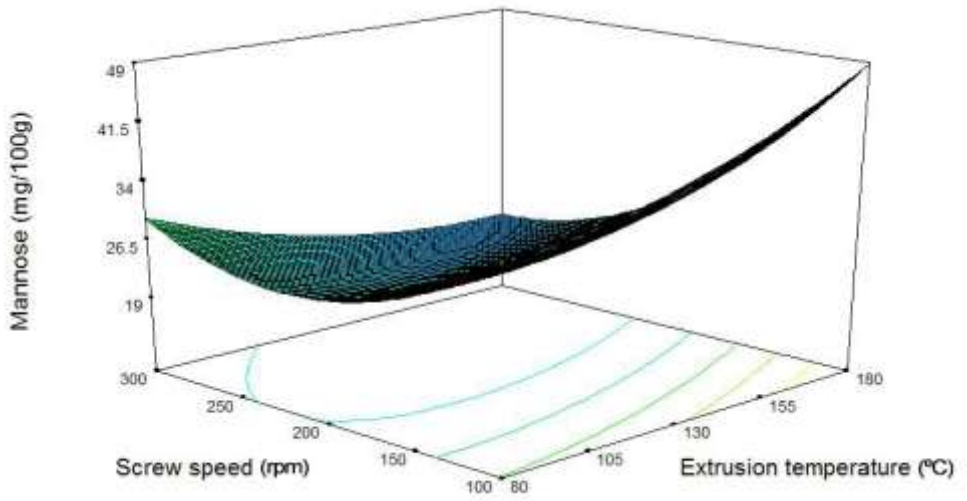

Fig. 2. Effects of extrusion temperature and screw speed on the mannose content of the extruded PKC with a moisture content of 55% and hopper speed of 7.5 Hz

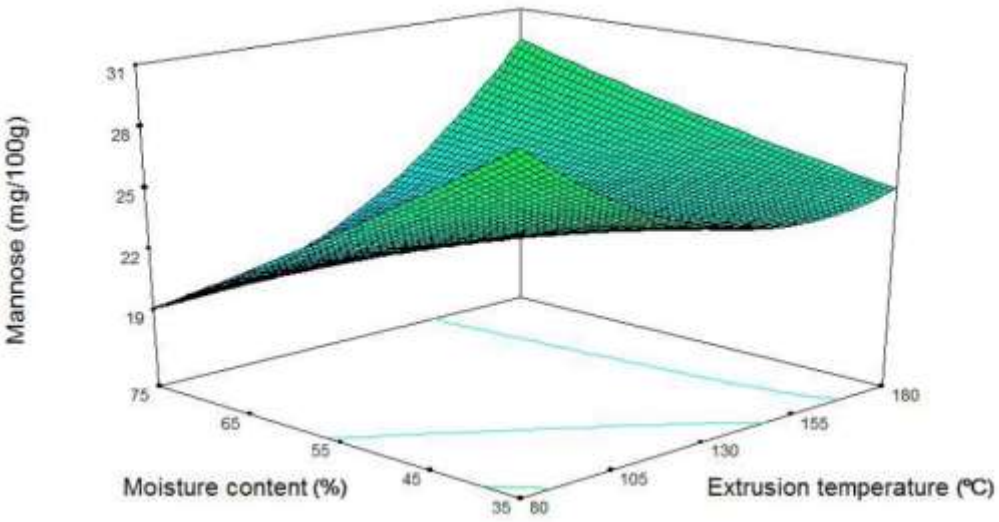

Fig. 3. Effects of extrusion temperature and moisture on the mannose content of the extruded PKC with a screw speed of 200 rpm and hopper speed of 7.5 Hz

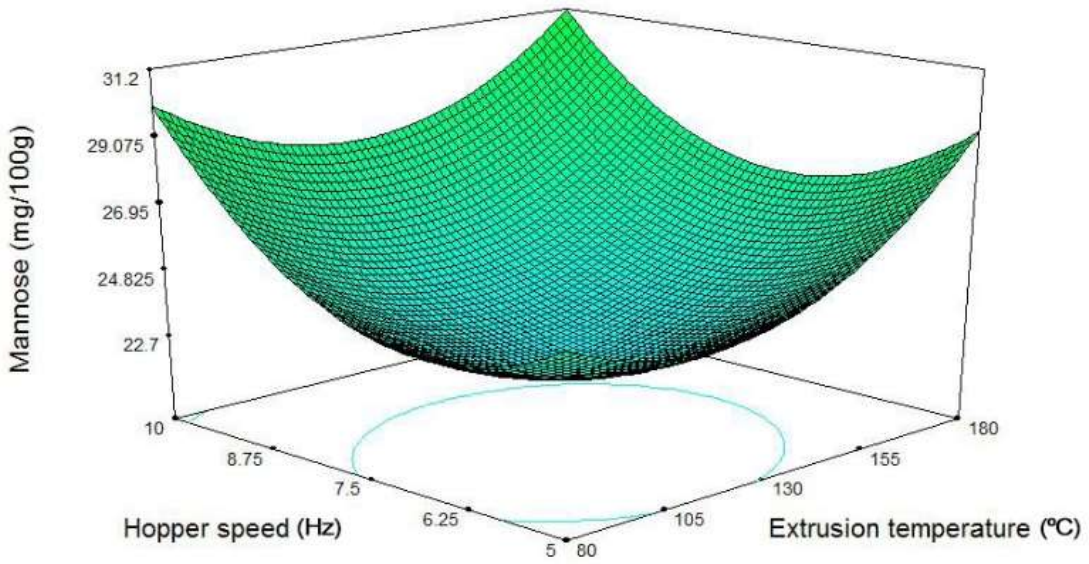

Fig. 4. Effects of extrusion temperature and hopper speed on the mannose content of the extruded PKC with a moisture content of 55% and screw speed of 200 rpm

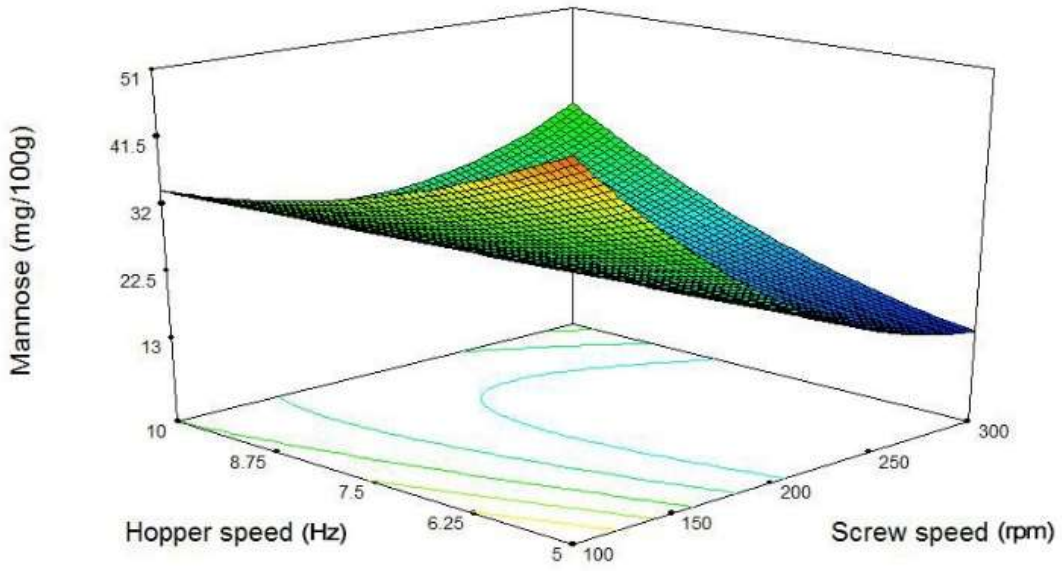

Fig. 5. Effects of screw speed and hopper speed on the mannose content of the extruded PKC with a temperature of 130 °C and moisture content of 55%

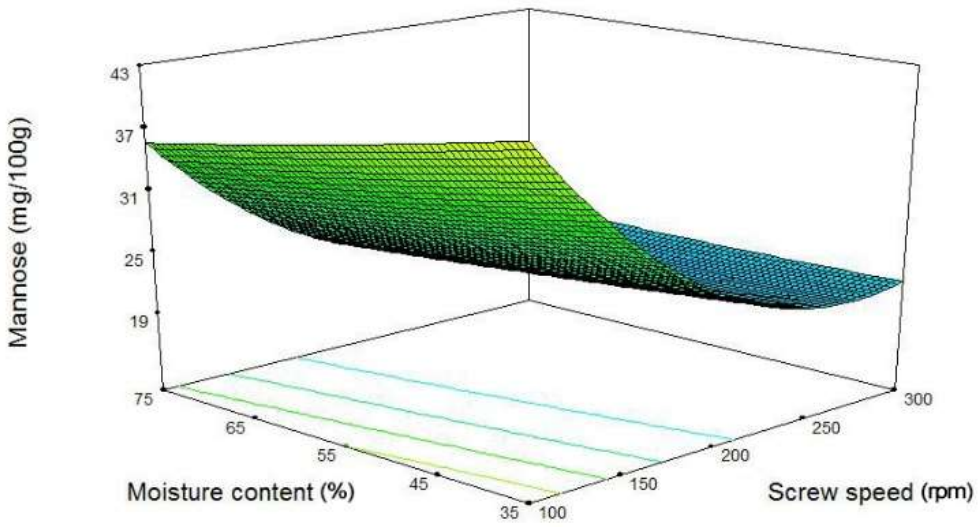

Fig. 6. Effects of screw speed and moisture on the mannose content of the extruded PKC with a temperature of 130 °C and hopper speed of 7.5 Hz

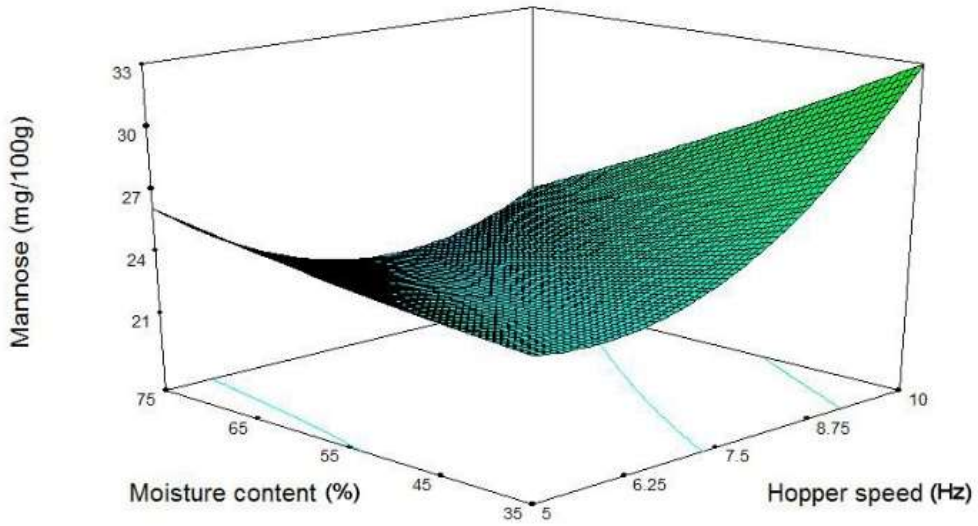

Fig. 7. Effects of hopper speed and moisture on the mannose content of the extruded PKC with a temperature of 130 °C and screw speed of 200 rpm

Effects of Extrusion Variables on the MOS of PKC

There was a significant increase ($p<0.05$) in the contents of all MOS of the extruded PKC. The increase in the MOS might have been caused by the breaking of the bonds between the polysaccharides and glycosidic linkages within the polysaccharides because of the severe extrusion process under high temperature, pressure, and shear force (de Vries *et al*. 2012). The results showed that the changes in the contents of the MOS under different extrusion

conditions were similar. Based on the RSM, the extrusion conditions of high temperature and moisture content, and low screw and hopper speeds gave the best results with regards to increasing the levels of all MOS (1,4-β-D-mannobiose, 1,4-β-D-mannotriose, 1,4-β-D-mannotetraose and 1,4-β-D-mannopentaose).

The β-(1-4) linkage of the highly crystalline linear mannan is extremely difficult to cleave, unless it is totally hydrated, and its crystalline structure somehow disturbed (Choct 2001). Hence, the extrusion of PKC for efficient MOS formation, as shown by the present study, is recommended to be carried out at a high moisture content. The extrusion of the high moisture PKC must be conducted at a high temperature under low screw speed to allow for sufficient development of the autoclaving effect to break the mannan polymer to MOS. Also, the hopper speed must be at a low level to prevent blockage and hardening of the PKC inside the barrel.

Effect of Extrusion on the Vibrational Signatures of Mannan and Cellulose in the PKC

The FTIR spectra of both the untreated and extruded PKC were normalised at a wavelength of 4000 cm^{-1}, where they are devoid of distinct IR bands. The changes in the band intensities and band positional shifts of both spectra were analysed based on a previous study on PKC by Barsberg *et al*. (2011). Figure 8 shows the reduction of the intensity of three characteristic fingerprint bands in mannan at 806, 871, and 939 cm^{-1} after the extrusion treatment. This indicated that the degradation and removal of mannan occurred *via* extrusion. The depolymerisation of the mannan polymers was also observed by mannan band positional shifts, which was positive from 1180 to 1182 cm^{-1}.

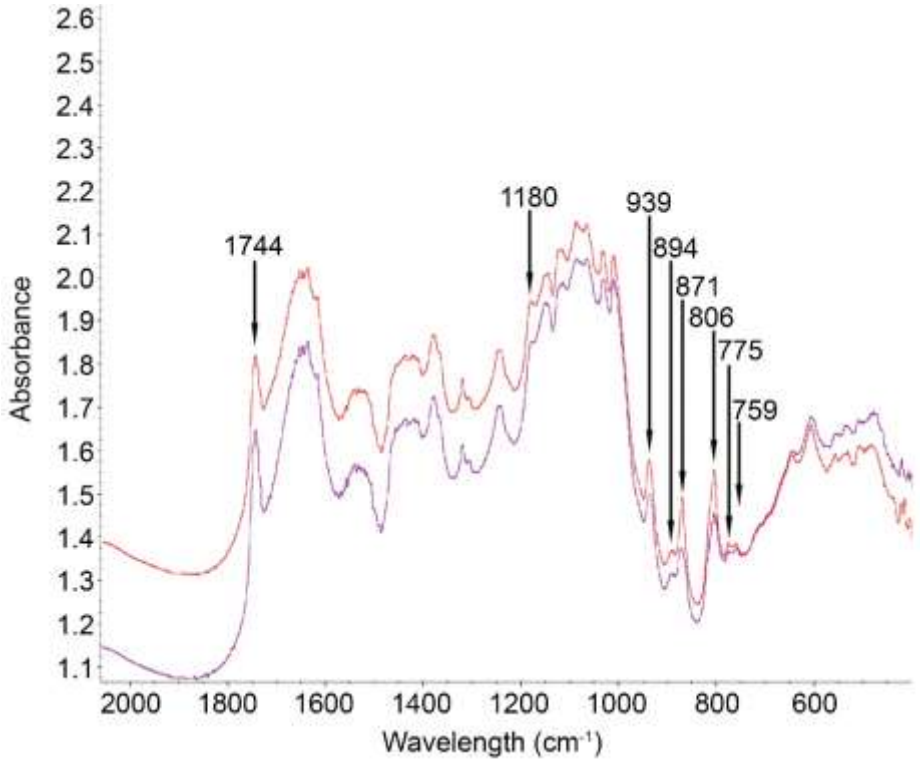

Fig. 8. Normalised FTIR spectra of the untreated PKC (red) and extruded PKC (blue) samples. The arrows indicate the wavelengths of the peaks.

This occurred because the decreasing degree of polymerisation (DP) led to a higher frequency of the IR in normal modes corresponding to this vibration (Barsberg *et al*. 2011). The reduction of the band at 1744 cm^{-1} that represented the reduction of carbonyl containing structures was closely associated with mannan reduction.

The effect of extrusion on the cellulose band could be observed by the clear positive positional shifts from 894 to 896 cm^{-1}. However, with regards to cellulose reduction, the strongest cellulose bands (894 cm^{-1}, untreated PKC; 896 cm^{-1}, extruded PKC) appeared weak, as similarly observed by Barsberg *et al*. (2011).

Optimised Extrusion Conditions

The amounts of T-NSP, monosaccharides, and MOS of the extruded PKC play a major role in determining the nutritive value of PKC. The extruded PKC should contain low T-NSP, high monosaccharides and high MOS levels. Hence, the conditions of the extrusion were set to produce a low amount of T-NSP and high amounts of both monosaccharides and MOS. Numerical optimisation methods were used in the present study by selecting the desired goals for each independent and dependent variable.

The results indicated that the optimised extrusion conditions were an extrusion temperature of 178 °C, screw speed of 100 rpm, hopper speed of 5 Hz, and PKC moisture content of 75%. The predicted and experimental values of the responses under the optimised conditions are shown in Table 5. The results showed that the experimental values were not biased towards the predicted values determined from the predictive models, and the model developed for each response was adequate and could be used for establishing the optimum conditions for twin-screw extrusion of PKC.

Effect of Extrusion on the Components and Hydration Properties of the Untreated PKC and Extruded PKC

The amount of mannose, glucose, fructose, and sucrose were significantly higher ($p<0.05$) in the extruded PKC compared to that of the untreated PKC (Table 5). The increase in glucose was probably due to the hydrolysis of cellulose, which could occur at high temperatures (180 to 220 °C) in the presence of water (Kupiainen *et al*. 2012). At normal temperatures the polysaccharides stay in a stable crystalline form, but at high temperatures the monosaccharide unit exists in an open-chain form (less stable) rather than a ring form, which makes it more susceptible to hydrolysis (Nattorp *et al*. 1999). The increase of fructose and sucrose in the extruded PKC was probably due to the release of entrapped molecules in the glucan matrix. The presence of sucrose in palm kernel and PKC had been reported by Kok *et al*. (2011) and Lawal *et al*. (2010), respectively. Inulin and fructan (fructose-based oligosaccharides) were not detected in the PKC by the HPLC analysis. The MOS (1,4-β-D-mannobiose, 1,4-β-D-mannotriose, 1,4-β-D-mannotetraose, and 1,4-β-D-mannopentaose) contents of extruded PKC significantly increased ($p<0.05$) compared to untreated PKC. The percentage of crude fibre significantly decreased ($p<0.05$) from 16.7 ± 0.68% to 13.5 ± 0.99%, and the T-NSP percentage decreased ($p<0.05$) from 63.3 ± 1.85% to 57.6 ± 0.89%. The gross energy values were not affected by extrusion.

The hydration properties, such as the SC and WRC, are major parameters determined in many fibrous feeds, such as copra and coconut meals (Raghavendra *et al*. 2004; Sundu *et al.* 2009), as they are nutritionally relevant. As shown in Table 5, the extruded PKC had a significantly lower ($p<0.05$) WRC and significantly higher ($p<0.05$) SC when compared to the untreated PKC. The decrease in the WRC of the extruded PKC was due to the reduced ability of the extruded PKC to incorporate water within its matrix, as a result of disruption in the fibre

structures. As the feedstock passed through the extruder barrel, high shear was exerted by the screw, resulting in high pressure and temperatures (Lamsal *et al.* 2010) that caused defibration, fibrillation, and shortening of the fibres (de Vrije *et al.* 2002). The effect of the mechanical shear by the twin screw at high temperatures would result in the reduction of the particle size of the PKC. The increase in the SC of the extruded PKC was due to the higher ability of small particles to trap water.

Table 5. Components of the Untreated PKC and Extruded PKC and Hydration Properties

	Untreated PKC	Extruded PKC (Experimental)	Extruded PKC (Predicted)
Mannose (mg/100 g)	20.80 ± 0.94^{b}	60.64 ± 0.34^{a}	63.83
Glucose (mg/100 g)	149.60 ± 0.44^{b}	284.43 ± 0.63^{a}	288.58
Fructose (mg/100 g)	212.40 ± 1.04^{b}	294.34 ± 0.53^{a}	291.18
Sucrose (mg/100 g)	180.15 ± 2.42^{b}	272.03 ± 7.09^{a}	-
Inulin (mg/100 g)	Not detected	Not detected	-
Fructan (mg/100 g)	Not detected	Not detected	-
1,4-β-D-mannobiose (mg/100 g)	28.97 ± 0.32^{b}	106.64 ± 0.46^{a}	104.30
1,4-β-D-mannotriose (mg/100 g)	3.75 ± 0.54^{b}	14.34 ± 0.86^{a}	14.53
1,4-β-D-mannotetraose (mg/100 g)	0.88 ± 0.64^{b}	3.07 ± 0.08^{a}	3.28
1,4-β-D-mannopentaose (mg/100 g)	0.12 ± 0.06^{b}	3.94 ± 0.09^{a}	3.48
T-NSP (%)	63.3 ± 1.85^{a}	57.6 ± 0.89^{b}	59.5
Crude fibre (%)	16.7 ± 0.68^{a}	13.5 ± 0.99^{b}	-
Gross energy (MJ/kg)	17.75 ± 0.28	17.63 ± 0.09	-
WRC (g/g DM)	3.84 ± 0.17^{a}	3.34 ± 0.18^{b}	-
SC (mL/g DM)	2.97 ± 0.17^{b}	3.43 ± 0.21^{a}	-

The means ± the standard deviation (n = 3) with different superscripts within rows are significantly different ($p<0.05$).
WRC: Water retention capacity
SC: Swelling capacity

CONCLUSIONS

1. The present study demonstrated that twin screw extrusion is a potential technological process to reduce the anti-nutritive factors and improve the nutritive value of palm kernel cake (PKC).
2. The optimum conditions were temperature of 178 °C, screw speed of 100 rpm, hopper speed of 5 Hz, and moisture content of 75%.
3. The treatment increased ($p<0.05$) the mannose, glucose, fructose, and sucrose contents, and decreased ($p<0.05$) the crude fibre and T-NSP contents of the PKC.
4. The increase in the mannose content was accompanied by an increase ($p<0.05$) in the 1,4-β-D-mannobiose, 1,4-β-D-mannotriose, 1,4-β-D-mannotetraose, and 1,4-β-D-mannopentaose contents.
5. The water retention capacity (WRC) was significantly decreased ($p<0.05$), while swelling capacity (SC) was significantly increased ($p<0.05$) for the extruded PKC.

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