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ORIGINAL PAPER

Application of Headspace Solid-Phase Microextraction and Gas Chromatography for the Analysis of Furfural in Crude Palm Oil

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Abstract Processing of vegetative material containing pentoses has been shown to result in the formation of furfural. Furfural exhibits a spectrophotometric absorption peak at 518 nm when complexed with aniline acetate. Headspace solid-phase microextraction (HS-SPME) method has been successfully used to confirm the presence of furfural in crude palm oil (CPO). Solid phase microextraction (SPME) fiber composed of divinylbenzene/Carboxen/polydimethylsiloxane (DVB/PDMS/CAR) was used to absorb the volatiles in the headspace of the oil. The isolated compounds from the fiber was desorbed and separated on a capillary polar column of a gas chromatograph. Response surface methodology (RSM) was used to optimize the SPME fiber condition for maximum absorption of furfural from CPO. The optimized temperature and time for furfural extraction onto the SPME fiber are 70 °C for 40 min. Oils obtained from the mill were found to contain between 2 and 13% furfural.

Keywords Furfural · Sterilization · Hemicelluloses · SPME · GC

Introduction

Malaysia processed about 88.5 million tons of fresh fruit bunches (FFB) of *Elaies guineensis* in 2008. Based on a 20% oil extraction rate (OER), the amount of crude palm oil (CPO) was equivalent to 17.7 million tons [1]. Processing of FFB for CPO involves sterilization to free the fruits from the bunch, mechanical screw pressing of the fruits to recover the crude oil from the sterilized fruits and purification of the pressed crude. The final product is CPO.

Sterilization remains the most important step in the palm oil extraction sequence. The high temperature/pressure sterilization terminates the formation of free fatty acid in the fruits, this should help detach all the fruits from the bunch and should cause separation of the kernel from the nut.

A fresh fruit bunch (FFB) consists of a central stalk carrying thousands of fruits. In addition to oil, cellulose and hemicelluloses are important structural components of the fruit bunch. Hemicelluloses are the cellular components that bind cells together and the fruits to the stalk. Hemicelluloses exist as random amorphous structures and are prone to degradation.

The high temperature sterilization processing of the palm oil mill causes changes in the structural deconformation of chemicals. Protein denatures, whilst carbohydrate caramelizes, hydrolyzes and/or dehydrates. Gandini and Belgacem [2], Cocchi et al. [3] and references therein reported that dehydration of xylose released three of its water molecules to eventually form the furfural. Ariffin et al. [4] have reported the presence of furfural in CPO, palm condensate and sludge oils. The techniques used for the determination were aniline acetate colorimetric and high performance liquid chromatography methods.

This paper will confirm the presence of furfural utilizing alternative headspace solid-phase microextraction (HS-SPME) methodology and optimizing the SPME conditions for the isolation of the volatile furfural.

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Materials and Methods

Materials

Crude palm oil, condensate oil and sludge oil, were obtained from a mill located in Malaysia. Refined palm oil fractions (refined palm olein and refined palm stearin) were obtained from a refinery. Fresh palm oil from FFB was obtained using Soxhlet extraction with hexane as the solvent. Standard furfural was purchased from Merck (Darmstadt, Germany).

Isolation of Volatiles from the Oil Using Headspace Solid-Phase Microextraction

Five hundred milligrams of the oil sample was placed in a 16-mL glass-vial and the vial was capped with a PTFE-faced silicone septum. The samples were equilibrated for 1 min to the absorption temperature while stirring thoroughly using a magnetic stirrer. A manual SPME sampler with a 2-cm StableFlex fiber assembly (Supelco, USA) coated with 50/30 μm divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS), was used to collect volatiles from oil headspace bottles [5]. Fiber was exposed at a constant depth into the headspace of the sample bottle and the absorption of the volatiles onto the fiber took place at 70 °C for 40 min.

Qualitative Characterization of the Isolated Volatiles

The SPME fiber from the Sect. "Isolation of Volatiles from the Oil Using Headspace Solid-Phase Microextraction" was placed immediately in the injection port of the Agilent 6890 series gas chromatography system (Agilent, USA) coupled with a Pegasus TOF Mass Spectrometer (LECO, USA). The fiber was desorbed in the injection port for 3.5 min. The injector temperature was 250 °C. The injector port was equipped with a glass liner designed for SPME measurements (0.75 mm i.d. splitless glass liner, Supelco). Injection took place in the splitless mode. The desorbed volatiles were separated on a DB-WAX column (30 m × 0.25 mm i.d., 0.25 μm film thickness) from J&W Scientific (USA). Pure helium gas was used as the carrier gas at a flow rate of 1 mL/min. The initial oven temperature was 50 °C and raised to a final temperature of 240 °C at a rate of 5 °C/min (held for 0.5 min). The detector temperature was 250 °C.

The ChromaTOF software was used to process the respective oil chromatogram and data from the mass spectrometer. Only compounds with a molecular weight between 50 and 300 were considered in this analysis following the molecular weight of furfural.

Optimization of the HS-SPME Method to Extract Furfural from CPO

Experimental Design

Response surface methodology (RSM) allows an evaluation of the effects of many explanatory variables and their interactions on the response variables [6]. RSM enables one to make a reduction in the number of experimental runs and yet provides sufficient information for statistically acceptable results for evaluating the effect of multiple explanatory variables, alone or in combination, on response variables [7–9]. RSM has been used widely in research particularly for the optimization of conditions and processes [10].

A central composite design was employed to study the response of SPME fiber in the extraction of furfural from CPO (Y). This design was selected because it requires four additional points outside the range which extend this model to a wider range of variation to include the highest and lowest value of the independent variables. The independent variables were absorption time (X_1) and absorption temperature (X_2), of the SPME fiber. The ranges for the independent variables were (low/high value): absorption time (min), 20/60, and absorption temperature (°C), 40/80. Six replicates run at the center of the design were performed to allow the estimation of pure error [7, 11]. The irrelevant extraneous factors varying the observed response were minimized by randomizing the order of the experiments [12]. Blocks are assumed to have no impact on the response surface analysis [13]. The extraction of furfural from CPO via SPME fiber at different absorption times and different absorption temperatures were generated by RSM, as indicated in Table 1. The responses were analyzed using Design-Expert version 7.0.0 software (Stat-Ease Inc., Minneapolis, USA).

Extraction and Analysis of Furfural

A similar extraction method to the one described in the Sect. "Isolation of Volatiles from the Oil Using Headspace Solid-Phase Microextraction" was employed in the extraction of furfural except that the absorption time and absorption temperature of the SPME fiber were pre-determined according to RSM.

Upon completion of the absorption, the SPME fiber was placed immediately in the injection port of the Hewlett Packard 6890 series gas chromatography system (Agilent, USA) equipped with a flame ionization detector (FID). The fiber was desorbed in the injection port for 3.5 min. The injector temperature was 250 °C. The injector port was equipped with a glass liner designed for SPME measurements (0.75 mm i.d. splitless glass liner, Supelco).

Table 1 Central composite design and experimental data for the extraction of furfural from CPO via HS-SPME–GC–FID

Run	Block	Independent variables		Response
		X_1 time (min)	X_2 temperature (°C)	Y peak area (pA s)
1	1	60.0	80.0	889.6
2 (C)	1	40.0	60.0	988.5
3 (C)	1	40.0	60.0	893.0
4 (C)	1	40.0	60.0	895.4
5	1	20.0	40.0	342.1
6	1	60.0	40.0	690.6
7	1	20.0	80.0	1003.7
8	2	68.3	60.0	842.9
9	2	40.0	31.7	325.6
10	2	11.7	60.0	440.3
11 (C)	2	40.0	60.0	834.0
12	2	40.0	88.3	775.6
13 (C)	2	40.0	60.0	872.9
14 (C)	2	40.0	60.0	926.8

C center point

Injection took place in the splitless mode. The desorbed volatiles were separated on a DB-WAX column (30 m × 0.25 mm i.d., 0.25 μm film thickness) from J&W Scientific (USA). Pure helium gas was used as the carrier gas at a flow rate of 1 mL/min. The initial oven temperature was 50 °C (held for 0.5 min), raised to 190 °C at a rate of 5 °C/min, and finally raised to 240 °C at a rate of 50 °C/min. The detector temperature was set at 250 °C. GC–FID was used for the optimization of the HS-SPME method and quantification of furfural from CPO.

Standard Curve for Furfural Analysis

Pure standard furfural (Merck, Germany) was used during the sample analysis as a positive control. The same method of extraction and analysis carried out for the sample (Sect. "Extraction and Analysis of Furfural") was used to produce a standard curve, which was used to identify and quantify the furfural in the samples.

This procedure was performed by the addition of a known concentration of furfural ranging from 0.00 (control) to 23.16 mg/kg to furfural-free refined palm olein. The peak obtained due to the addition of various concentrations of furfural into the refined palm olein was used to construct the standard curve, as shown in Fig. 1.

Validation of the Model

Optimum conditions for extracting the maximum amount of furfural in term of time and temperature were obtained using the predictive equations of RSM. The amount of furfural in the oil was determined after the extraction of furfural under optimum conditions. The experimental and predicted values were compared in order to determine the validity of the developed model.

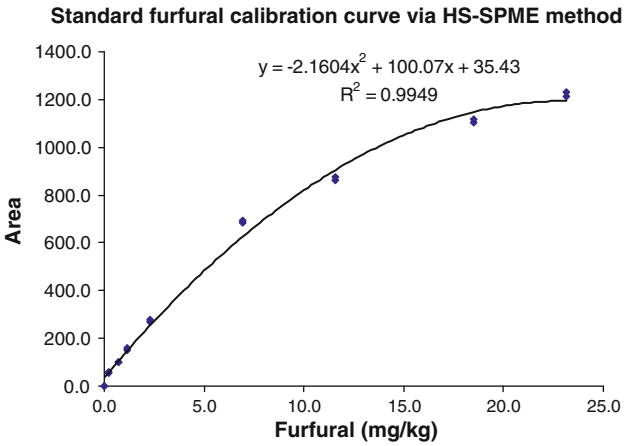


Fig. 1 Standard furfural calibration curve using the HS-SPME method

Means and standard deviations of furfural content were calculated with SPSS statistical software (Version 16.0, SPSS Inc., Chicago, IL, USA). SPSS was used to perform one-way ANOVA with a Tukey's post hoc test to the furfural content to detect statistically significant differences ($p < 0.05$) in the different types of oil from the mill.

Results and Discussion

Headspace Extraction and Identification of Volatiles Using GC–MS

The headspace of the oil sample was directly extracted onto the SPME fiber which was later analyzed using GC–MS. This analytical method allows one to make correct identification of compounds in the oil. Figure 2a shows the total ion chromatogram (TIC) of volatiles of CPO detected by HS-SPME–GC–MS. The highest peak in the TIC of volatiles of CPO appearing at 742 s matches the furfural compound in the library of the GC–MS. The TICs of volatiles of condensate oil and sludge oil also revealed the presence of furfural in the separation time between 730 and 745 s (Fig. 2b, c).

Refined palm olein and palm stearin are found to be furfural free (Fig. 3a). Fresh palm oil without undergoing the normal milling process also showed an absence of furfural. This shows that furfural is not a natural constituent in the palm fruit. Standard furfural extracted using the same method as the sample also showed a furfural peak in the region of 730–745 s (Fig. 3b).

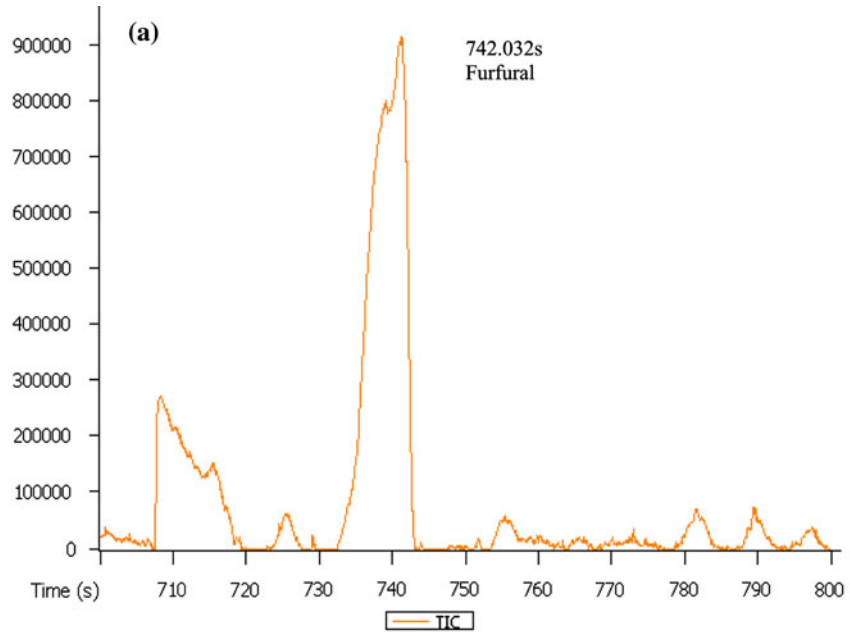
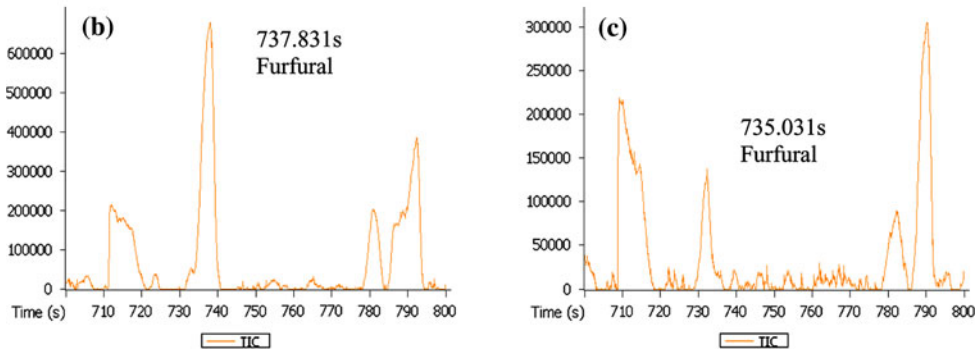


Fig. 2 TICs of volatiles of **a** CPO, **b** condensate oil, and **c** sludge oil, detected by SPME–GC–MS



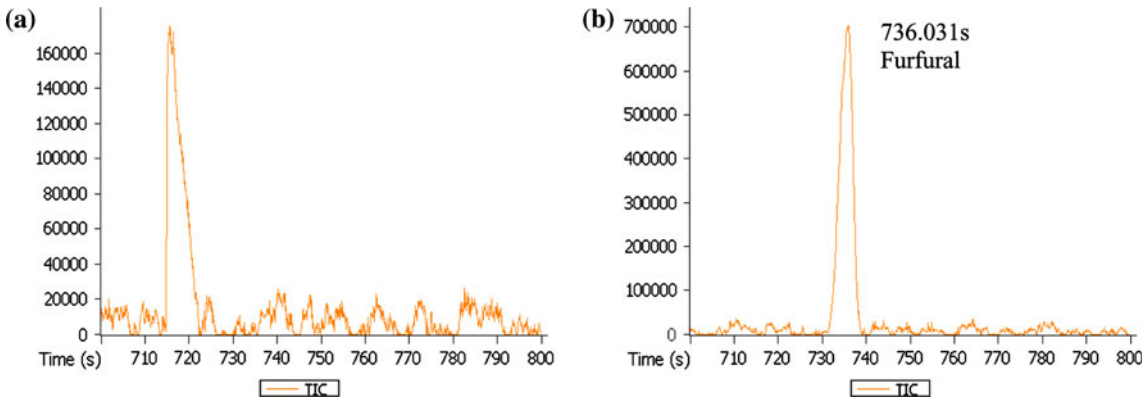


Fig. 3 TICs of volatiles of **a** furfural-free refined palm olein, and **b** standard furfural in refined palm olein, detected by SPME–GC–MS

The palm fruit bunch is rich in both oil and fibers. The hemicelluloses of the fibers are hydrolyzed and post-dehydrated to furfural during the sterilization process in the mill [4]. The mechanical and hot water extraction of oil in the mill may cause the entrapment of furfural in the CPO.

CPO is then refined to RBD palm oil which in turn is fractionated to palm olein (unsaturated acid rich oil) and stearin (saturated acid rich oil). Furfural is absent in palm olein and stearin. The deodorization process of CPO in the refinery may have removed the volatile furfural together

Table 2 ANOVA for response surface quadratic model

Source	SS	*df*	MS	*F* value	*p* value
Block	33,496.5029	1	33,496.5029		
Model	616,813.2059	5	123,362.6412	23.2318	0.0003
X_1-time	80,754.2455	1	80,754.2455	15.2077	0.0059
X_2-temperature	280,124.6666	1	280,124.6666	52.7534	0.0002
X_1 X_2	53,499.6900	1	53,499.6900	10.0751	0.0156
X_1^2	68,387.8482	1	68,387.8482	12.8789	0.0089
X_2^2	148,344.6482	1	148,344.6482	27.9364	0.0011
Residual	37,170.5484	7	5,310.0783		
Lack of fit (model error)	26,895.9218	3	8,965.3073	3.4903	0.1293
Pure error (replicate error)	10,274.6267	4	2,568.6567		
Corrected total	687,480.2571	13			
R^2	0.9432				

df degrees of freedom, *SS* sum of squares, *MS* mean square

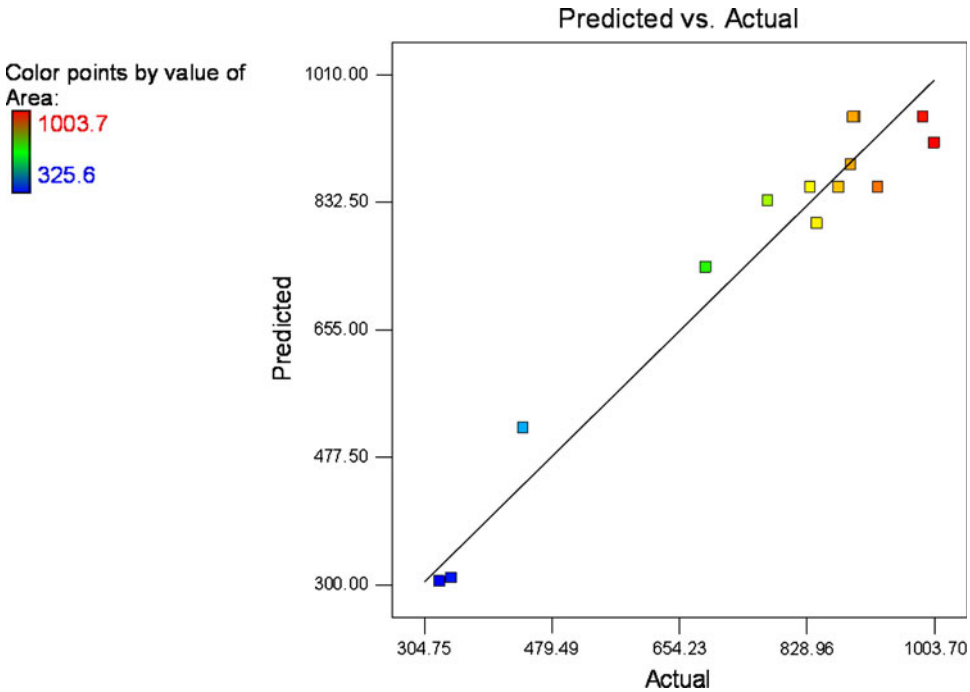


Fig. 4 Plot showing relationships between observed values and values predicted by the model

with other oxidative products and free fatty acid initially present in the oil.

Condensate oil, collected from the sterilizer discharges, contains furfural. This shows that the sterilization process is the initial point where furfural is formed and trapped in the oil. Sludge oil, the final mill discharges, also contains furfural and this may due to the affinity of furfural to oil.

Optimization of SPME Fiber for the Extraction of Furfural by RSM

In order to quantify the furfural content in the oils, the SPME fiber conditions, absorption time and temperature, to extract furfural need to be optimized. Optimization is important because the HS-SPME method is based on the principle of equilibrium. SPME fiber will reach equilibrium with a compound at a certain time and temperature. The maximum absorption of furfural onto the SPME fiber is optimized as it represents the equilibrium point of the compound with the fiber. RSM was applied to the headspace solid-phase microextraction of furfural from CPO, with two absorption parameters: time and temperature. Table 1 lists the peak area of furfural in each of the 14 experimental sets generated by the RSM software and the peak area ranged from as low as 325.6 to as high as 1,003.7.

The six center points in Table 1 produce four measures of variation known as pure error. There are four measures of variation due to the use of a block as the optimization cannot be performed within the same day. Pure error represents the replication error and it is used in the evaluation

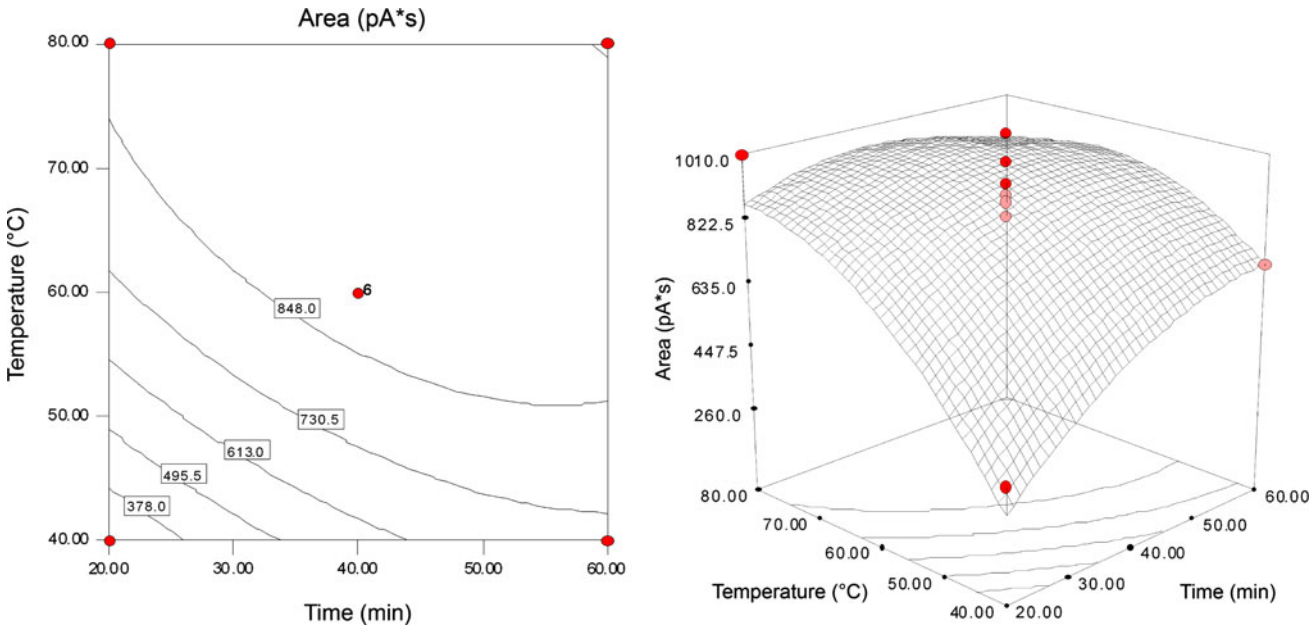


Fig. 5 Contour plot and response surface for the effects of absorption time and temperature on the peak area of furfural

Table 3 Average furfural content of various oils in Mill A

Types of oil	Furfural concentration (mg/kg)
CPO	12.93 ± 0.24^{a}
Condensate oil	4.69 ± 0.38^{b}
Sludge oil	2.50 ± 0.35^{c}

Results are means ± standard error ($n = 3$). Values with different letters are significantly different ($p < 0.05$)

for lack of fit. The fits of the RSM model was evaluated by coefficients of determination (R^2) and a test for lack of fit from ANOVA (Table 2). The quadratic model is significant with a p-value of 0.0003 ($p < 0.05$) and the model has no lack of fit at 95% confidence level. The R^2 value of 0.9432 (Table 2) and the observed values versus predicted values plot (Fig. 4) shows that the model is statistically acceptable. According to Joglekar and May [14], the R^2 should be at least 0.80 for a good fit of a model.

In this study, the response and explanatory variables were fitted to each other by RSM. A good fit was obtained and there were no outliers observed for the quadratic model:

$$\text{Peak area of furfural} = -2214.98 + 41.62X_1 + 63.44X_2 - 0.29X_1X_2 - 0.24X_1^2 - 0.35X_2^2$$

Figure 5 illustrates the relationship between the explanatory and response variables in a three-dimensional representation of the response surface and two-dimensional contour plot generated by the model. It can be observed that the peak area of furfural increases in a quadratic manner with the extension of time and increment of temperature. It is noted that there is an effect of interaction between the absorption time and the temperature on the peak area of furfural.

A numerical optimization was also carried using the Design-Expert software to determine the optimum absorption parameters (time and temperature) of the SPME fiber for the maximum extraction of furfural. Results showed that the optimum absorption condition to yield maximum furfural extraction is 43 min at 72 °C. The condition was rounded to the nearest tenth to provide a viable option to extract furfural at 40 min and 70 °C yielding a peak area of 959.9 pA s. Therefore the latter set of conditions was chosen for the extraction of furfural from CPO, condensate oil and sludge oil.

The model was verified by performing three extractions from CPO using the selected conditions and a one-sample t test was performed at 0.05 significance level using SPSS software to determine the validation of the experimental values as compare to the predicted value from RSM. The result from the t test ($p = 0.303$) suggests that the predicted value and experimental values are identical. Hence, the RSM model fitted successfully.

Furfural Content of Various Oils in the Mill

The ANOVA result of the quantification of furfural via the HS-SPME-GC method shows that CPO significantly contains ($p < 0.05$) the highest amount of furfural, followed by condensate oil and sludge oil (Table 3). The variation in furfural content in different oils from the mills may be due

to the nature of the processing. Condensate oil and sludge oil are regarded as mill discharges and are kept in an open environment. The uncontrolled environment for the oils and the volatility of furfural may lead to a lower content of furfural. While the intended product of the mill, CPO, is kept in a storage tank with a well-controlled environment to avoid a reduction in its quality. The storage provision may at the same time preserve the furfural content in CPO.

Conclusion

The HS-SPME–GC–MS analysis confirmed the presence of furfural in CPO, condensate oil and sludge oil. The sterilization process of FFB resulted in the formation of furfural which is eventually trapped in the final CPO of the palm oil mill. The presence of furfural in the condensate oil supports that the formation of furfural is initiated during the sterilization process. Even the final mill discharge, sludge oil, contains furfural which means that furfural has a rather strong affinity to oil. The optimum conditions for the absorption of furfural onto the SPME fiber were determined by RSM in order to quantify the furfural content in various oils from the mill. The extraction of furfural should be carried out at 70 °C for 40 min. The quantification of furfural in various oils in the mill reveals that most of the furfural derived from hemicelluloses is trapped in the crude palm oil.

References

1. Malaysian Palm Oil Board (2009) Production of crude palm oil in 2008. http://econ.mpob.gov.my/stat/web_report1.php?val=200804, 2009
2. Gandini A, Belgacem MN (1997) Furans in polymer chemistry. Prog Polym Sci 22:1203–1379
3. Cocchi M, Ferrari G, Manzini D, Marchetti A, Sighinolfi S (2006) Study of the monosaccharides and furfurals evolution during the preparation of cooked grape musts for Aceto Balsamico Tradizionale production. J Food Eng 79:1438–1444
4. Ariffin AA, Mat Soom R, Boo HC, Loi CC, Chai YH, Abdulkarim SM (2009) Detection and determination of furfural in crude palm oil. J Food Agric Environ 7(2):136–138
5. Giordano L, Calabrese R, Davoli E, Rotilio D (2003) Quantitative analysis of 2-furfural and 5-methyl furfural in different Italian vinegars by headspace solid-phase microextraction coupled to gas chromatography-mass spectrometry using isotope dilution. J Chromatogr A 1017:141–149
6. Box GEP, Wilson KB (1951) On the experimental attainment of optimum conditions. J R Stat Soc Ser 13:1–45
7. Chu BS, Quek SY, Baharin BS (2003) Optimization of enzymatic hydrolysis for concentration of vitamin E in palm fatty acid distillate. Food Chem 80:295–302
8. Huang KH, Akoh CC (1996) Optimization and scale-up of enzymatic synthesis of structured lipids using RSM. J Food Sci 61:137–141
9. Xu X, Skands ARH, Høy CE, Mu H, Balchen S, Alder-Nissen J (1998) Production of specific-structured lipids by enzymatic interesterification: elucidation of acyl migration by response surface design. J Am Oil Chem Soc 75:1179–1186
10. Junqueira RM, Castro IA, Areas JAG, Silva ACC, Scholz MBS, Mendes S (2007) Application of response surface methodology for the optimization of oxidants in wheat flour. Food Chem 101:131–139
11. Montgomery DC (2001) Design and analysis of experiments, 5th edn. Wiley, New York
12. Liyana-Pathirana C, Shahidi F (2005) Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chem 93:47–56
13. Mirhosseini H, Tan CP, Hamid NSA, Yusof S (2008) Effect of arabic gum, xanthan gum and orange oil on flavour release from diluted orange beverage emulsion. Food Chem 107:1161–1172
14. Joglekar AM, May AT (1987) Product excellence through design of experiments. Cereal Foods World 32:857–868