Optimization of Cocoa liquor, *Allanblackia floribunda* fat and *African elemi* oil blend for formulation of chocolate based on physicochemical properties

Chukwu Susan Philbianna[1],*Obinna-Echem P C[1], Mepba H.D[1], Okwechime, U. Justice[1],
[1]Department of Food Science and Technology, Rivers State University, Nkpolu-Oroworokwo, Port Harcourt, Rivers State, Nigeria
*Correspondence author: phoebesusan15@yahoo.com
DOI: 10.56201/rjfsqc.v10.no6.2024.pg48.59

Abstract
The objective of this study was to optimize the physicochemical properties cocoa liquor, Allanblackia floribunda fat and African elemi oil blend for developing dark chocolate using RSM combining with I-optimal Design to replace chocolate from cocoa butter and partially hydrogenated vegetable oils to increase nutritional health benefits. Sixty percentage (60%) dark chocolate formulations based on complete randomized I-optimal Design was developed and physicochemical properties (moisture, melting point, hardness, viscosity, free fatty acid and peroxide value) of the blends were evaluated as responses. Regression models, response surface models, numerical optimization based on desirability function to determine an optimum formulation, contour plots of response surface models to visualize an optimum region were developed. Results of the physicochemical properties of individual oils showed melting point, viscosity, peroxide value, free fatty acid and iodine value of 35.5 ℃, 37 ℃ and 30.68 ℃, 0.40 Cst, 0.41 Cst and 0.36 Cst, 0.70, 0.81 and 1.13 meq/kg oil, 0.40%, 0.51% and 0.57%, 37.4, 39.04 and 76.78 g/100, respectively, for cocoa liquor, Allanblackia floribunda and African elemi oil. The regression models were able to predict the physicochemical properties of the dark chocolate as a function of factors with an accuracy of 86-99% depending on the responses. The optimum condition selected after numerical optimization revealed that the mixture of cocoa liquor (61.1%), Allanblackia floribunda (35%) and African elemi (38%) with desirability index of 0.705 was the optimum formulation which improved the dark chocolate. Physicochemical properties of the formulated dark chocolate showed moisture content of 1.18%, melting pt (35.5℃), hardness (15.9 Nmm2) viscosity (3.54 Cst), FFA (0.08 %) and PV (1.28 meq/kg) whereas the control sample recorded 1.12%, 36.55℃, 16.16 Nmm^{2,} 2.95 Cst, 0.06 %, and 1.05 meg/kg for moisture content, melting pt, hardness, viscosity, FFA and PV, respectively. The formulated dark chocolate compared favourably with control sample; hence, this formulation can be used for commercial production of value-added dark chocolate.

Keywords*: Cocoa liquor; Allanblackia floribunda fat; African elemi oil, dark chocolate; physicochemical properties*

1. Introduction

Chocolate is a product from the combination of cocoa liquor, cocoa powder and cocoa butter, with other constituents (Urbańska & Kowalska, 2019; Deus *et al*., 2021), besides its good taste, chocolate have some health benefits in blood circulation, High cocoa content in chocolates offer phenolic compounds known to have antioxidant properties that can reduce the risk of heart disease (Nascimento *et al*., 2020). Chocolate is solid at room temperature but completely melts in the mouth at body temperature. The characteristic of chocolate that allows it to melt at body temperature is due to the fatty acid composition containing triglycerides SOP (stearate, oleic acid and palmitic acid with melting range of 32-34℃ (Ostrowska-Ligęza *et al*., 2019).
Cocoa butter (CB) is an essential ingredient in the chocolate production. It is also called "chocolate fat" is obtained by pressing of mature cocoa beans (*Theobroma cacao*). The great symmetric mono unsaturated triacylglycerols (SMUT) content is a vital component in chocolate production that is responsible for key characteristics such as melting point, hardness, chocolate shine, contraction during DE-molding shrinkage and stability to fat bloom. Cocoa butter is the continuous fat phase in chocolate, which helps in the spreading of other ingredients (Suri and Basu, 2022). The melting temperature of CB is around 35℃ close to the human body temperature (Wang *et al*., 2006) while final solid fat content is between 65% and 75% (Glicerina *et al*., 2016; Ostrowska-Ligęza *et al*., 2019). The major fatty acids present in CB is dominated by saturated-monounsaturated-saturated TAGs (ester derived from glycerol and three fatty acids); palmitate-oleate-stearate (POS, 36-42%), stearate- oleate-stearate (SOS, 23-29%) and palmitate-oleate-palmitate (POP, 13-19%) (5). This characteristic affects the melting behaviour of CB which is hard and brittle at room temperature but melts completely in the human mouth (Jin *et al*., 2021).
Allanblackia floribunda is a fruit tree of Clusiaceae family or Guttiferae. It is a plant which is in abundance in the forests of Central Africa and West Africa. The fat obtained from the seed, known as 'Allanblackia fat' is used in food preparation; the seeds contain a fat that is solid at ambient temperatures similar to cocoa butter. The kernel, which makes up about 60% of the seed, contains about 72% fat. The fatty acid composition of the fat is stearic acid approximately: 45 - 58% and oleic acid 40 - 51%. Its composition and relatively high melting point (35°c) makes the fat a valuable raw material that can be used without transformation to improve the consistency of cocoa butter substitutes and similar products (Obinna-Echem *et al*., 2023).
African elemi (*Canarium schweinfurthii bursaraceae*) fruit is a perennial plant which belongs to the Burseraceae family, widely distributed in the East central and West Africa, In Nigeria, the plant is found in parts of middle belt, South east and South West regions of the country. The pulp has 38.9% oil 32.24 % stearic acid and 30.24 % oleic acid (Kabari *et al* 2020) the seed-kernel is oily and edible too. They contain several fatty acids including oleic (36 %), linoleic (28 %), palmitic (26 %), stearic (37 %) (Kin-Kabari *et al*., 2018).
Many leading chocolate producers such as Hershey uses cocoa butter alternatives (CBAs) derived from Shea butter, palm oil or other sources to replace partially the cocoa butter. Other researchers have also carried out production of cocoa butter replacers (CBRs), cocoa butter equivalents (CBEs) and cocoa butter substitutes (CBSs) from various natural sources. All of these fats are obtained from a natural plant such as palm kernel oil (PKO), palm olein (PO), mango seed fat, soy oil, rapeseed oil, cotton oil, groundnut oil and coconut oil.Lauric CBS is economical vegetable fat to

replace CB and have physical properties resembling those of CB. On the other hand, lauric unfortunately, chocolate made of fully CBS lacks the pleasant flavor that is typical of pure chocolate made of fully CB. However, such CBS base compound chocolate presents outstanding problems associated with a poor tolerance between CBS and CB and not healthy, hence, this study was aimed at optimization of Cocoa liquor, *Allanblackia floribunda* fat and *African elemi* oil blend for formulation of dark chocolate based on physicochemical properties.

2. Materials and Methods

The study was conducted at Divi Laboratories of Food analysis section, Elelewoh, Port Harcourt, Rivers State.

2.1. Materials

The materials used in the study were cocoa liquor, *Allanblackia floribunda* fat and African elemi oil. Cocoa (*Theobroma cacao*) was obtained from Oyigbo-Afam Market, Oyigbo Local Government, Rivers State, Nigeria. *Allanblackia floribunda* was obtained from RSSDA Mgodu-Afam in Oyigbo local government area, Rivers State. African elemi was purchased from Aba Main Market Ariaria, Abia State.

All chemicals and reagents were of food grade and analytical grade, manufactured by BDH Limited, Poole, England supplied by Annacity and sons Nigeria limited, Port Harcourt, Rivers State.

2.2. Methods

2.2.1. Experimental design for the dark chocolate formulation

2.2.2. Experiments

Complete randomized I-optimal Design, no blocking with 16 randomized experimental runs, was employed and sixteen runs generated. The mixture components (Cocoa liquor, *A. floribunda* fat and African elemi) were coded low and high as shown in the Mixture Component Table 1. The formulation design constraints were: cocoa liquor (68.9%), *A. floribunda* (45.7%) and African elemi (5%). Dependent variables such as moisture, melting point, Hardness, viscosity, free fatty acid and peroxide value of the blends were evaluated as responses. The different blends generated from the runs and the formulated dark chocolate were analyzed and evaluated for the physicochemical properties using standard procedures. All experiments were performed in duplicates. Statistical design, analyses and optimization were performed in response surface methodology using Design Expert (Stat-Ease, 2019). The design matrix for the formulation experiments is presented in Table 2

Table 1: Factors and Levels for complete randomized I-optimal design

Component	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	CL		Mixture	0.5	0.689765	+0 ↔ 0.5	+1 ↔ 0.69	0.5889	0.0591
B	ASO		Mixture	0.28	0.457395	+0 ↔ 0.28	+1 ↔ 0.47	0.3713	0.0586
C	AE		Mixture	0.03	0.05	+0 ↔ 0.03	+0.105263 ↔ 0.05	0.0398	0.0081
			Total =	1.0000		L_Pseudo Coding			

CL, ASO, AE represents factors: Cocoa liquor, *A. floribunda* fat and African elemi

2.3.Physicochemical analysis

2.3.1. Moisture content

The moisture content was determined using an oven drying method as described in AOAC, (2019). About 5 grams of the sample was dried at 105℃ for 2 h to a constant weight. The moisture content was calculated from the weight difference between the initial and dried sample and was expressed as a percentage of the initial weight using following equation. Two replications were performed for the samples.

$$Moisture\ content\ (\%) = \frac{\text{Initial weight of sample} - \text{Dried weight of sample}}{\text{weight of sample}} \times 100$$

2.3.2. Melting Point

The melting point of the blends was determined according to the AOCS, (2017) Method No. Cc 3-25. A capillary tube filled with a 1 cm high column of the sample was tied to a thermometer with the aid of a rubber band and chilled for 2 h before being immersed in a beaker with cold water filled to half of a 500 mL beaker. The water was stirred and heated slowly. The temperature was recorded when the blend in the tube started to rise due to hydrostatic pressure. The temperature at the moment was taken as the melting point.

2.3.3. Chocolate hardness

This was measured using Brookfield texture analyzer with probe no. 39 attached with diameter of 2 mm. The measurement was conducted using compression test with 3 mm mark value. The measurement was recorded as load value in gram, and then converted to tension in N/mm^2. The conversion is expressed using equation below;

$$\sigma = \frac{load \times a}{\pi \mathrm{r}2}$$

2.3.4. Viscosity

Kinematic viscosity was measured using a calibrated Cannon- Ubbelohde Viscometer: (Cannon Instrument Co. PA, USA) from procedure of the American Oil Chemist Society (AOCS, 2017) The viscometer was charged with the oil sample and suspended vertically in a constant temperature bath. The temperature of the system was maintained at 40℃. The efflux time of the oil through the capillary bulb was measured and recorded in seconds. viscosity was calculated using the following formula:

V = Kc x t f (2)
Where; V = Kinematic viscosity (mm^2 /s^2)
Kc = Capillary factor/ Viscometer constant (mm^2 /s^2)
t f = Sample efflux time (s)
mm^2 /s = cSt (centistokes)

2.3.5. Free Fatty Acid

One gram (1g) of oil was introduced into a 250 cm^3 conical flask, to this was added 100 mL neutralized ethanol followed by the addition of phenolphthalein indicator. Titration was with NaOH solution (0.1 M) until a pink color developed. As described by Official Methods of Analysis of AOAC, (2019). Free fatty acid was evaluated as shown below:

Free fatty acid (%) = $\frac{\text{T x M x Mw}}{w}$

Where;
T = Titre value
M = Molarity of the titrant
W = Weight of oil used
Mw = Molecular weight of dominant fatty acid

2.3.6. Iodine value

The iodine number was determined by the volumetric titration method (AOCS, 2017). Iodine numbers are expressed as grams of iodine absorbed per 100gram sample. A sample weight of 0.15gram was put into the Erlenmeyer and then 25mL carbon tetrachloride ($CCL_{4)}$ was added followed by the addition of and 25 mL Wijs solution, covered and stirred until homogeneous. The mixture was incubated in a dark room for 1 h. After that, the mixture was added 20mL of KI 10% and 150mL distilled water. The solution was mixed and titrated with 0.1N thiosulfate solution until a purple-purplish colour almost disappears, 10% 2 mL starch indicator was added and titration continued until colourless. The iodine number was calculated as follows.

$$Iodine\ Value\ (g\ /\ 100\ g\ sample) = \frac{(V2-V1)\times N\times 12.69}{W}$$

Where:
N = normality of sodium thiosulfate
V2 = volume of thiosulfate used in the blank determination (mL),
V1 = volume of thiosulfate used in sample determination,

W = test weight (gram).

3.0. Results and Discussion

The results of the physicochemical properties of Cocoa liquor, *Allanblackia floribunda* oil, and African elemi oil are shown in Table 1.

Table 1: Physicochemical properties of Cocoa liquor, *Allanblackia floribunda* oil, and African elemi oil

Sample	Moisture%	Melting point ℃	Viscosity (Cst)	FFA %	Peroxide value (meq/kg)	Iodine value g/100g
Cocoa butter	$0.82^{b}\pm0.07$	$35.5^{b}\pm0.07$	$0.36^{c}\pm0.00$	$0.40^{c}\pm0.02$	$0.70^{c}\pm0.35$	$37.4^{c}\pm0.14$
Allanblackia floribunda	$0.61^{c}\pm0.01$	$37.00^{a}\pm1.41$	$0.40^{ab}\pm0.00$	$0.51^{b}\pm0.01$	$0.81^{b}\pm0.01$	$39.04^{b}\pm0.03$
African elemi oil	$1.11^{a}\pm0.11$	$30.68^{c}\pm0.01$	$0.41^{a}\pm0.02$	$0.57^{a}\pm0.01$	$1.13^{a}\pm0.00$	$76.78^{a}\pm0.02$

Values are means ±standard deviation of duplicate determination. Means that do not share a letter are significantly different(P<0.05)

Moisture, Melting point and Viscosity varied from 0.61-1.11%, 30.68-37.0℃ and 0.36-0.41cst respectively, for Cocoa liquor, *Allanblackia floribunda* oil, and African elemi oil. Similarly, FFA, peroxide value and iodine value ranged from 0.40- 0.51%, 0.70- 1.13 meq/kg and 37.4-76.78 g/100g for Cocoa liquor, *Allanblackia floribunda* oil, and African elemi oil separately.

Table 2 shows the mixture combination and experimental values of responses for optimization of the properties of Cocoa liquor, *Allanblackia floribunda* oil, and African elemi fruit pulp oil in chocolate formulation

Table 2: The experimental design and obtained values of the responses

Run	Moisture %	Melting pt °C	Hardness Nmm^2	Viscosity Cst	FFA %	PVmeqKOH
1	1.12	37	16.16	3	0.06	0.6
2	2.21	35	16.09	3	0.05	0.92
3	2.2	37	16.14	3.1	0.06	1.15
4	2.15	35	16.11	3.1	0.05	2.06
5	2.14	37	16.08	3.1	0.07	1.15
6	2.13	35	16.13	3.2	0.05	2.35
7	1.12	37	16.16	3	0.06	0.6
8	1.12	37	16.16	3	0.06	1.6
9	2.11	38	16.15	3.2	0.07	2.82
10	2.13	38	16.15	3	0.06	2.06

11	2.12	38	16.15	3.2	0.07	2.03
12	1.12	37	16.16	2.8	0.06	0.6
13	2.12	37	16.19	2.9	0.05	0.58
14	2.14	36	16.05	3.4	0.06	2.86
15	2.12	37	16.16	2.8	0.05	0.58
16	2.15	35	16.07	2.8	0.07	0.55

KEY
FFA = Free fatty acid
PV = Peroxide Value

Moisture content ranged from 1.12-2.21%, melting point 35-38 ℃ hardness 16.05-16.19 Nmm^2, and viscosity 2.8 – 3.4 Cst. Similarly, free fatty acid content and peroxide value ranged from 0.05-0.07% and 0.00-2.86 meqKOH, respectively.

3.1. Optimization and verification

The optimum formulations were selected and used for calculating the predicted values of response variables using the prediction equations derived by RSM. Verification of the optimum formulation for producing dark chocolate was performed. The chocolate produced was experimentally analyzed and the results were statistically compared to the predicted values of the mathematical model.

Table 3: Analysis of variance (ANOVA) for the regression models of moisture content, Melting pt, Hardness, Viscosity, FFA, PV of dark chocolate

Model Source	Sum of Squares	DF	Mean Square	F-value	p-value	R^2	Adjusted R^2	Predicted R^2	Adequate Precision	CV %
Moisture %	3.15	8	0.3937	1063	<0.00	0.9992	0.9982	0.9236	75.13	1.02
Melting pt ℃	17.71	8	2.21	382.3	<0.00	0.9977	0.9951	0.9382	55.2755	0.20
Hardness Nmm^2	0.0212	6	0.0035	11.93	0.000	0.8883	0.8138	0.6252	11.1780	0.10
Viscosity Cst	0.3813	8	0.0477	9.20	0.004	0.9132	0.8140	0.7902	11.0434	2.37
FFA %	0.0008	6	0.0001	9.90	0.001	0.8684	0.7806	0.6109	9.0993	6.09
PVmeqKOH	9.83	8	1.23	9.85	0.003	0.9184	0.8252	0.7068	8.7355	25.0

P<0.05

The fitted regression model in terms of coded factors;

$$\text{Moisture content} = +2.13 *A+2.13*B+33.51*C-0.0701*AB -34.47*AC-33.00 *BC-162.41 *A^2BC-180.24 *AB^2C+1413.02 *ABC^2$$

$$\text{Melting pt}= +37.87*A+36.15*B-541.40*C+4.17*AB +617.37*AC+632.29*BC-311.25*A^2BC-301.89*AB^2C+2243.95*ABC^2$$

Hardness = +16.15*A+16.21*B+6.57*C-0.1446*AB+9.35*AC+8.73*BC-+6.14*AB

Viscosity = +2.96*A+2.44*B-411.76*C+2.28*AB +462.90*AC+454.25*BC-291.23*A^2BC - 216.71 AB^2C -2377.51*ABC^2

FFA = +0.0597*A+0.0272*B+0.7114*C+0.1216*AB -0.7101*AC-0.1138*BC-2.40*ABC

PV = +1.96 A-1.13B-1859.23C+8.28 AB+2064.52AC+2026.88 BC-1358.46 A^2BC-901.39 AB^2C+11120.38 ABC^2

3.2. Physical characteristics of chocolate

The physical characteristics of produced chocolate compared with a control sample are shown below (Table 3).

Table 3: Physical characteristics of chocolate

Parameters	Chocolate from Cocoa liquor, Allanblackia and African elemi	Chocolate (Control sample)
Moisture (%)	1.18ᵃ±0.22	1.12ᵃ ±0.01
Melting pt ℃	35.5ᵇ ±0.09	36.55ᵃ ±0.03
Hardness (Nmm^2)	15.9ᵇ ±0.04	16.16ᵃ ±0.05
Viscosity (Cst)	3.54ᵃ ±0.05	2.95ᵇ ±0.03
FFA (%)	0.08ᵃ ±0.06	0.06ᵃ ±0.08
PV (meqKOH)	1.28ᵃ ±0.11	1.05ᵇ ±0.03

Values are means ±standard deviation of duplicate determination. Means that do not share a letter are significantly different(P<0.05)

Moisture content and melting point varied between 1.18% and 1.12%, 35.5 and 36.55℃ in Chocolate from Cocoa liquor, Allanblackia and African elemi and control sample respectively. Hardness, Viscosity, FFA and PV varied between 15.9 and 16.16Nmm^2, 3.54 and 2.95cst, 0.08 and 0.06% and 1.28 and 1.05 meqKOH.

4.0. Discussions

Model fitting from RSM

The effects of cocoa liquor, *Allanblackia floribunda* oil, and African elemi oil on moisture content, melting point, hardness, viscosity, free fatty acid content and peroxide value of dark chocolate are shown in Table 2. The independent and dependent variables were fitted to model equation and examined for the goodness of fit. The analyses of variance were performed to determine the significance of the linear, quadratic and interaction effects of the independent variables on the dependent variables (Table 3). Coefficient of determination (R^2) is the proportion of variation in the response attributed to the model rather than to random error and was suggested that for good fit model, R^2 should be at least 80% (Obinna *et al.*, 2023). The results showed that the models for all the response variables were highly adequate because they have satisfactory levels of R^2 of more than 80% and that there is no significant lack of fit in all the response variables. The regression coefficients are shown in Table 2 and the equations for each of the response variables could be derived from the predicted values of each response variable. The difference between adjusted R^2 and predicted R^2 values of all responses is less than 2 similarly, Adequate Precision for all responses is greater than 4; a ratio greater than 4 is desirable (Stat-Ease, 2019). Coefficient of variability which determines how means are distributed is less than 10% expect for peroxide value.

5.0 Conclusion

The physicochemical, fatty acid composition and phytochemical constituent of the oils used in the formulation of chocolate showed comparative features found in other edible oils. The statistical mixture design was used to determine the mixture's optimal composition to be adjusted to the response factor. Five solutions were found, however, only one appropriate solution for the optimization process was selected using the software package. That is, one combinations of cocoa liquor, *A. floribunda* and African elemi oil was used to minimize moisture, put melting in range, maximize hardness, minimize viscosity, FFA and peroxide value of the chocolate, The Predicted optimum condition and desirability index of 0.705 was obtained with preferred desirability blend; of CB, ASO and AE were 0.611, 0.350 and 0.038% for chocolate of acceptable quality attributes, this condition gave optimum moisture, melting point, Hardness, viscosity, FFA and PV of 1.116%, 42℃, 16.18Nmm^{2,} 2.51Cst, 0.05|% and 0.839meq/kg respectively. Production quality assessment of chocolate from the optimum blend showed physiochemical properties, proximate composition and sensory attributes comparable 55%, 70% chocolate used as control samples. The study showed that the mixture combination can be used in production of acceptable dark chocolate comparable to commercial equvalents.

References

Deus VL, Resende LM, Bispo ES, Franca AS, Gloria MBA. FTIR and PLS-regression in the evaluation of bioactive amines, total phenolic compounds and antioxidant potential of dark chocolates. Food Chemistry. 2021. 357:129754.

Kabari S. D. Umunna P. S. Gaimi S. Y (2018) Characterization of African Elemi fruit (C*anarium schweinfurtii*) pulp oil and its utilization in margarine formulation, *Journal of the Nigerian institute of Food Science.*page 126-127.

Nascimento MM, Santos HM, Coutinho JP, Lôbo IP, da Silva ALS Jr, Santos AG, et al. Optimization of chromatographic separation and classification of artisanal and fine chocolate based on its bioactive compound content through multivariate statistical techniques. *Microchemical Journal*. 2020. 152:104342.

Obinna-Echem P C, Okwechime U. J, Mepba H.D, (2023). Optimization of the properties of vegetable tallow (*Allanblackia floribunda*) and soybean oil blends for bakery fat formulation using response surface methodology, *Research Journal of Food Science and Quality Control* (RJFSQC)

Ostrowska-Ligęza E, Marzec A, Górska A, Wirkowska-Wojdyła M, Bryś J, Rejch A, et al. A comparative study of thermal and textural properties of milk, white and dark chocolates. Thermochimica Acta. 2019. 671:60-69.

Stat-Ease (2019). Design Expert V12. Stat-Ease, Inc., Minneapolis, MN

Suri, T., & Basu, S. (2022). Heat resistant chocolate development for subtropica and tropical climates: A review. *Food Science & Nutrition*, *62*(20), 5603-5622. PMid:33635177.

Urbańska B, Kowalska J. Comparison of the total polyphenol content and antioxidant activity of chocolate obtained from roasted and unroasted cocoa beans from different regions of the world. Antioxidants. 2019. 8:283.

Graphs

Effects of interaction of mixture components on *Allanblackia floribunda* oil and African elemi fruit pulp oil blend in dark chocolate formulation

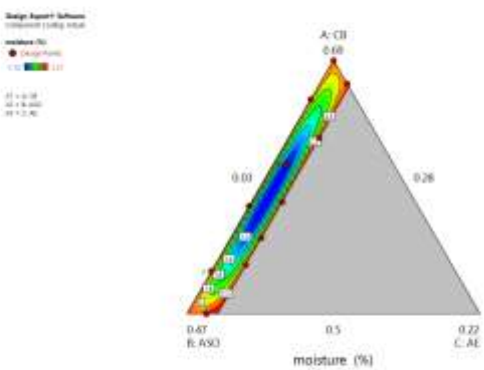


Fig.1 Effects on moisture content

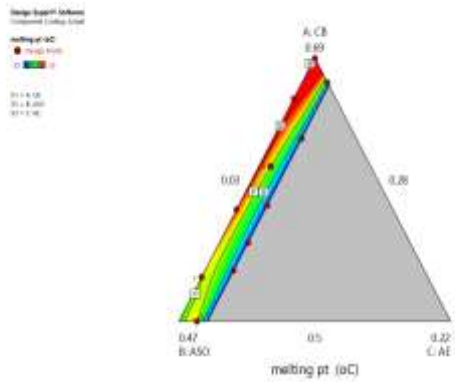


Fig.2 Effects on melting point

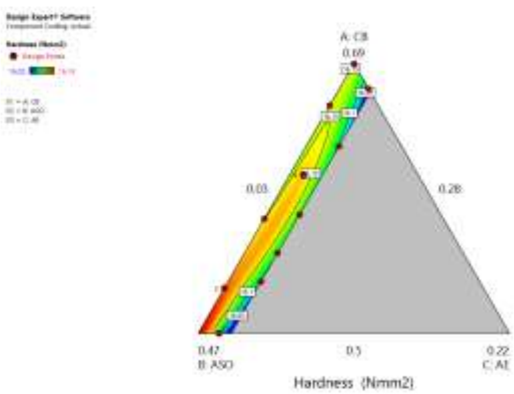


Fig.3 Effects on hardness

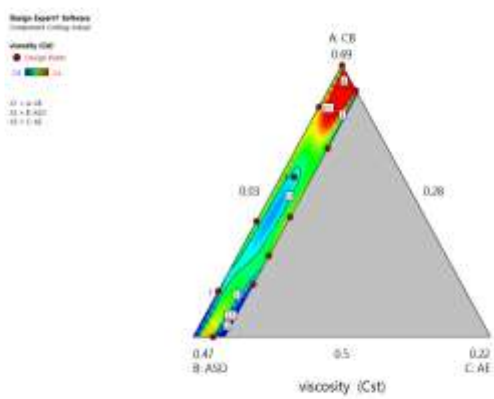
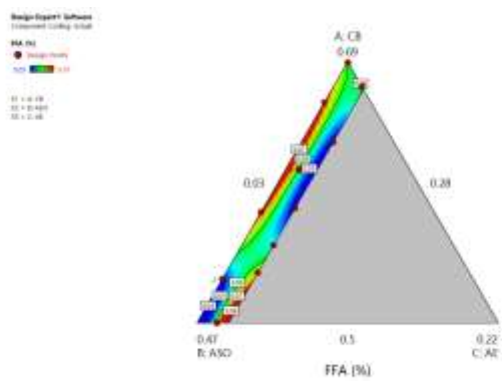


Fig.4 Effects on viscosity



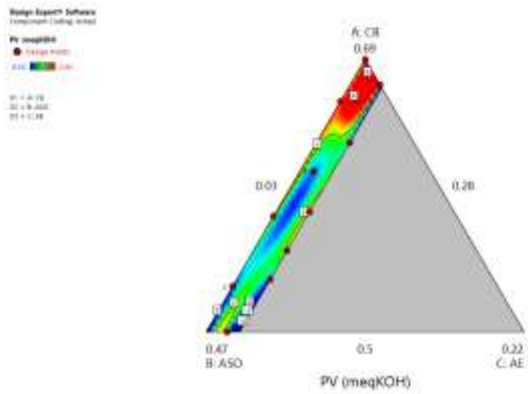


Fig.5 Effects on FFA content

Fig.6 Effects on Peroxide value