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Process optimization of gluten free cookies using cassava flour

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Abstract
Cassava is an important crop and is a source of food security for many countries. The unusual climatic variations witnessed in recent years, along with the prospect of global warming, highlight further advantages of this hardy, drought-resistant crop. Being Gluten free, it also addresses the problem of Gluten intolerance. Response Surface Methodology (RSM) was used to optimize the product, 20 sets of experiments were performed taking into account three factors viz., Cassava Flour, Soyabean Flour and Butter. Responses were examined for sensory attributes as well as physical characteristics. The data was analysed using Minitab17 software. Responses obtained after each and every trial were analysed to visualize the interactive effect of various parameters on sensory attributes and physical properties of gluten free cookies. After optimizing, Proximate analysis of the product and was carried out.

Keywords: cassava, response surface methodology, gluten

1. Introduction
The market for gluten-free products has grown tremendously in the last decade around the globe and is expected to increase due to the increasing number of celiac and gluten-intolerant consumers. There are different medical conditions caused by intolerance to gluten one of which is Celiac disease. Celiac disease is an autoimmune disorder in which the immune system responds abnormally to gluten. It is caused by a reaction to gliadin, a prolamin (gluten protein) found in wheat, and similar proteins found in the crops of the tribe *Triticeae*. One of the replacements of wheat flour and other common grains containing gluten is cassava flour. Cassava (*Manihot esculenta* Crantz) the "food of the poor" has become a multipurpose crop that responds to the priorities of developing countries, to trends in the global economy, and to the challenge of climate change (FAO, 2013) [8]. It is a nutty flavoured, starch-tuber in the spurge family (Euphorbiaceae) of plants. Advantages of cassava as a crop include flexibility in planting and harvesting time, drought tolerance and ability of cassava to grow and produce in low nutrient soils, it also provides more dietary energy per hectare and working hours than any other staple crop (Fregene *et al.* 2000) [9]. In India, it occupies 0.24 m ha with a production of 5.1 million tonnes. It is cultivated predominantly in the southern states of which Kerala and Tamil Nadu are responsible for 61% and 29% of area and 55% and 41% of production respectively (C.T.C.R.I., 2015). The major biochemical constituent in cassava is starch, which constitutes up to 65-70% of the dry matter, therefore Cassava is known to be a good and cheap source of carbohydrates. Earlier reports have shown that cassava flour blends have some functional properties due to which it can be used in bakery products. Consumption of bakery products has been increasing as a result of urbanization, and Food industries are exploiting this development by manufacturing nutritious bakery foods (Akubor, 2003) [3, 4]. Cookies, which are a major portion of bakery products are feasible fibre carriers because of their longer shelf-life. They have become one of the most desirable snacks for both young and elderly people due to their low manufacturing cost, more convenience and ability to serve as a vehicle for important nutrients (Amin *et al.* 2016) [5]. The project undertaken is to manufacture gluten free Cassava Cookies. However, on its own cassava flour provides little or no protein; it needs to be enriched by the addition of some protein source (CTA, 1995) [7]. Soyabean flour (*Glycine max*) which is an excellent source of protein was incorporated in the manufacturing process of the gluten free cookies in the undertaken project. The protein content of Soyabean flour is 37.81g/100g (USDA, 2016) [13] and contains all essential the amino acids required for proper growth and maintenance of body. Incorporating soyabean flour in the manufacturing process will not only improve the nutritional quality it may also improve the flavour and texture of the cookies.

2. Materials and Methods
2.1 Raw Material
Cassava Flour was procured from Indian Council of Agricultural Research-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, Kerala, India (Treated for cyanogenic glucosides). Soyabean Flour was purchased from the Local Market of Varanasi, India. Date Palm Jaggery was purchased from the Local Market of Kolkata, India. Other materials such as Butter, Eggs, Salt, Baking Powder, and Vanilla Essence were purchased from the Local Market of Varanasi, India.

2.2 Sample Preparation
The procedure for manufacturing gluten free cookies is given in the Flow Diagram:

Weigh All the Ingredients
↓
Beat butter for one minute
↓
Add Jaggery and beat till light and fluffy
↓
Add the eggs and Vanilla Essence and beat well for 30 seconds
↓
Add salt and Baking powder and mix well
↓
Sieve and mix Cassava Flour and Soyabean Flour
↓
Knead well to make a non-sticky dough
↓
Roll the dough on a pastry board in uniform thickness
↓
Cut the dough using a Cookie Cutter
↓
Line cookies on an oven try by placing them on a butter paper
↓
Bake at 180°C for 20 minutes
↓
Remove tray and cool for 30 minutes
↓
Packaging

2.3 Statistical analysis

The experimental data obtained was analysed with the help of Minitab 17 Software. Response Surface Methodology (RSM) was used to derive the optimum formulation conditions using a five parameter five level central composite rotatable design (CCRD) which generated 20 experimental runs. The independent variables affecting the quality of the end product were the different levels of Cassava Flour, Soyabean Flour and Butter. A total of 20 runs were conducted. Cassava Flour varied from 35-45g, Soyabean Flour from 5-15g and Butter from 20-30g. Factors such as Hardness, Fracturability, Colour and Appearance, Flavour and Overall Acceptability were used as quality attributes of the Cassava Cookies. After that each individual experiment and its responses were analysed to assess the effect of independent parameters on them. The rest of the ingredients used in manufacturing of cookies were used in quantities as follows Sugar – 25%, Egg – 30%, Vanilla Essence– 3%, Baking Powder – 1%, Salt – 0.5%

2.4 Chemical analysis of Gluten free Cassava Cookies

All chemical analyses were carried out in triplicate. Moisture, fat, ash, crude fibre, protein and carbohydrates of the product were analysed by the method given by (AOAC, 2002) [6].

2.5 Sensory Evaluation

The sample of each trial was evaluated for sensory attributes viz., Colour, Appearance and Overall Acceptability by an experienced panel comprising of five judges selected from the Centre of Food Science and Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi with the help of 9 point Hedonic Scale.

2.6 Analysis of physical characteristics of the Cassava Cookies

Hardness and Fracturability – The Cookie Hardness and Fracturability was measured by Texture Analyser (TA.XT Plus texture profile analyser, Stable Micro Systems, UK). The peak force (g) and the mean distance at break (mm) were recorded.

3. Results and Discussion

3.1 Optimization

RSM was applied to the experimental data with the help of Minitab 17 Software and the parameters taken into consideration were Cassava Flour, Soyabean Flour and Butter and second order polynomial models were developed. All the models exhibited statistical significance. It can be concluded that all the models were statistically valid for predicting the response. Table 1 shows the central composite rotatable design (CCRD) for the optimization of Gluten free Cassava Cookies.

3.1.1 Effect of ingredients on Hardness of Gluten free Cookies

The data fitted the following Quadratic Model.

Hardness = -22.9+ 1.030 Cassava F 0.317 Soya Bean F
+ 1.127 Butter - 0.01218 Cassava F*Cassava F
+ 0.02382 Soya Bean F*Soya Bean F - 0.02618 Butter*Butter
- 0.00200 Cassava F*Soya Bean F- 0.00100 Cassava F*Butter
- 0.00100 Soya Bean F*Butter

Figure (1) - a), b), c) shows the response surface plot for hardness as influenced by the level of Cassava Flour, Soyabean Flour and Butter. The coefficient of determination (R^2) was 93.65. The Linear and Square interactions of ingredients was significant ($P<0.05$). This model can be used to navigate the design space. The Analysis of Variance is given in Table 5.

By studying all the three graphs together we can see that Soyabean Flour is the major ingredient which is responsible for increasing the cookie hardness. Similar studies were carried out by (Lee *et al.*, 1991) who reported that more strength was needed to break cookies incorporated with legumes flour. This might have resulted from incorporation of protein rich flour which needs more water to obtain good cookie dough, and the cookies prepared from high-absorption dough tend to be extremely hard. The level of butter also affected the hardness, as the level of butter increased the hardness decreased, which is in agreement with the fact that one of the reasons for using fats in cookies is to soften and tenderize the texture besides adding to the flavour and assisting leavening.

3.1.2 Effect of ingredients on Fracturability of Gluten free Cookies

Figure (1) - d, e), f) shows the response surface plot for fracturability as influenced by the level of Cassava Flour, Soyabean Flour and Butter. The coefficient of determination (R^2) 92.44%. The Linear and Square interactions of ingredients was significant ($P<0.05$). This model can be used to navigate the design space.

By studying the results we observe that with increase in Soyabean flour the fracturability increased to a great extent, this could be as a result of oil in the Soyabean flour. Similar studies were carried out by (Agiriga, 2008) [1] who found that

Fragility of Cookie bars increased with increase in groundnut flour. However, fragility reduced when corn starch and cassava flour were added. This increase in rigidity is due to increase in carbohydrate starch granules, which is responsible for gel and structure formation in baked goods (Williams, 1974).

3.1.3 Effect of ingredients on Colour and Appearance of Gluten free Cookies

The coefficient of determination (R^2) was 96.40%. The Linear, Square and 2-Way interactions of ingredients was significant ($P<0.05$). Figure (1) - g), h), i) shows the response surface plot for Colour and Appearance as influenced by the level of Cassava Flour, Soyabean Flour and Butter. By analysing the results obtained it can be seen that with increase in Soyabean flour there is a decrease in the Colour and Appearance, a darker burnt colour was obtained as the soyabean flour was increased. Millard browning occurs during baking in the presence of amino acids and reducing sugars, it can be said that more Millard reactions occurred in cookies containing higher amount of soyabean flour as it contains higher amount of amino acids. With increase in Butter there was an increase in the Colour and Appearance. This observation is in agreement with the observations made by (Akoh *et al.* 1994) [2] who observed that fat enhances sensory characteristics such as Colour, Flavour, Palatability and Creaminess.

3.1.4 Effect of ingredients on Flavour of Gluten free Cookies

Figure (1) - j), k), l) shows the response surface plot for Flavour as influenced by the level of Cassava Flour, Soyabean Flour and Butter. The coefficient of determination (R^2) was 92.10%. The Linear, Square and 2-Way interactions of ingredients was significant ($P<0.05$). The Analysis of Variance is given in Table 6. Results of sensory analysis of flavour showed that increase in Cassava Flour reduced the Flavour of the cookies. An increase in Cassava Flour results in lower lipoprotein matrix formation which in turn reduces flavour, moistness and colour as it is unable to trap the fat which is butter in this case. The less the fat trapped the lower is the flavour. This is in accordance with the studies of (Akoh *et al.* 1994) [2] who found that cookies made with wheat flour had a better flavour compared to cookies made of composite flour due to the trapping of fat and cocoa powder by the lipoprotein matrix present in wheat flour. Increase in butter increased the flavour; this can be explained due to the fact that butter adds to the richness, palatability and creaminess of baked products such as cookies which is well liked by consumers.

3.1.5 Effect of ingredients on Overall Acceptability of Gluten free Cookies

Figure (1) - m), n), o) shows the response surface plot for Overall Acceptability as influenced by the level of Cassava Flour, Soyabean Flour and Butter. The coefficient of determination (R^2) was 93.83%. The Linear, Square and 2-Way interactions of ingredients was significant ($P<0.05$). The observations in the present study were in close agreement with the findings of (Akubor *et al.* 2003) [3, 4], who studied the effect of soyabean flour on the functional properties and the potential of soybean and cassava four blends in cookie production. They observed no significant differences in texture, flavour, taste and overall acceptability of both soy enriched and un-enriched four blend cookies. The increase in Butter increased the Overall Acceptability as increase in butter results in a better flavour, texture, aroma and palatability. (Akoh *et al.* 1994) [2] in their study also found that fat enhances sensory attributes such as flavour, palatability, texture and creaminess.

3.2 Optimization of the Product

On the basis of constraints suggested as given in Table 2, Minitab17 software selected solution as given in Table 3 with desirability 96.31%.

3.3 Proximate Analysis of Gluten free Cookies

The proximate composition of the optimized product is given in Table 4

From the proximate composition of the cookies we can see that the carbohydrate content is very high in the cookies which can be attributed to the extremely high content of carbohydrates in cassava. Protein which is very less in Cassava has been compensated by adding Soyabean flour to the cookies. The protein content was found to be 8.0g/100g. This result is in agreement with (Kumar *et al.* 2010) who reported that defatted soy flour incorporation increased the nutritional status of biscuits due to high protein content.

Table 1: The experimental data for response surface analysis of the effect different amounts of ingredients on the quality of Cassava Cookies.

Run	CF	SF	Butter	Hardness ($Kg/cm^{2)}$	Fracturability (mm)	Colour and Appearance	Flavour	Overall Acceptability
01	35	15	30	6.5	1.90	08	8.3	8.15
02	35	10	25	7.4	2.23	7.7	7.5	7.60
03	40	10	25	7.6	2.54	7.6	7.3	7.50
04	40	10	25	7.6	2.54	7.7	7.3	7.50
05	40	10	25	7.6	2.55	7.7	7.3	7.50
06	40	10	30	6.2	1.67	8.2	8.5	8.35
07	40	10	20	8.6	2.89	7.3	6.7	7.00
08	40	10	25	7.6	2.55	7.7	7.3	7.5
09	40	5	25	8.6	3.10	08	7.1	7.55
10	45	5	20	8.3	2.84	7.9	6.9	7.40
11	40	15	25	8.7	2.92	7.5	07	7.30
12	45	15	30	6.5	1.97	8.0	8.5	8.25
13	40	10	25	7.6	2.55	7.7	7.3	7.50

14	45	15	20	8.7	003	7.3	07	7.15
15	35	5	20	08	2.63	7.9	7.1	7.50
16	45	5	30	5.8	1.22	8.3	7.2	7.75
17	40	10	25	7.6	2.55	7.7	7.3	7.50
18	35	15	20	09	3.22	7.3	07	7.15
19	35	5	30	06	1.54	8.3	8.3	8.30
20	45	10	25	8.1	2.69	7.8	7.3	7.55

Table 2: Shows the constraints for the optimized solution

Response	Goal	Lower	Target	Upper
Overall Acceptability	Maximum	7.0	8.35	0
Flavour	Maximum	6.7	8.50	0
Colour and Appearance	Maximum	7.3	8.30	0
Fracturability	Minimum	0	1.22	3.22
Hardness	Minimum	0	5.80	9.00

Table 3: Optimized Product as chosen by Minitab 17 software

Factors			Responses				
Cassava Flour	Soyabean Flour	Butter	Overall Acceptability	Flavour	Colour And Appearance	Fracturability	Hardness
35	7.72727	30	8.33015	8.49015	8.19542	1.33109	5.62666

Table 4: Proximate composition of the Optimized Product

No.	Constituents	Amount (g/100g)
1	Protein	8.0
2	Fat	14.39
3	Ash	2.78
4	Moisture	3.5
5	Carbohydrates	69.83
6	Fibre	1.5

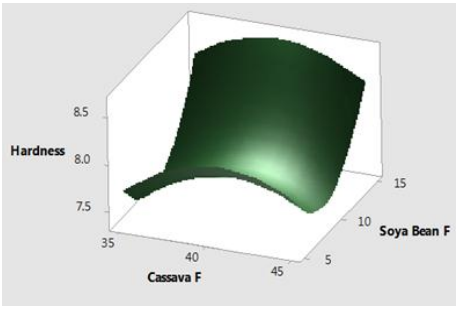
Table 5: Analysis of Variance (ANOVA) for the Effect of ingredients on Hardness of Gluten free Cookies

Source	DF	ADJ SS	ADJ MS	F-Value	P-Value
Model	9	16.3882	1.8209	16.38	0.000
Linear	3	14.2100	4.7367	42.60	0.000
Cassava Flour	1	0.0250	0.0250	0.22	0.646
Soyabean Flour	1	0.7290	0.7290	6.56	0.028
Butter	1	13.4560	13.4560	121.03	0.000
Square	3	2.1482	0.7161	6.44	0.011
Cassava Flour*Cassava Flour	1	0.2551	0.2551	2.29	0.161
Soyabean Flour*Soyabean Flour	1	0.9751	0.9751	8.77	0.014
Butter*Butter	1	1.1782	1.1782	10.60	0.009
2-Way Interaction	3	0.0300	0.0100	0.09	0.964
Cassava Flour*Soyabean Flour	1	0.0200	0.0200	0.18	0.680
Cassava Flour*Butter	1	0.0050	0.0050	0.04	0.836
Soyabean Flour*Butter	1	0.0050	0.0050	0.04	0.836
Error	10	1.1118	0.1112		
Lack-of-Fit	5	1.1118	0.2224	*	**
Pure Error	5	0.0000	0.0000		
Total	19	17.5000			

Table 6: Analysis of Variance for the Effect of ingredients on Flavour of Gluten free Cookies

Source	DF	ADJ SS	ADJ MS	F -Value	P-Value
Model	9	5.11866	0.56874	12.95	0.000
Linear	3	4.03400	1.34467	30.61	0.000
Cassava Flour	1	0.16900	0.16900	3.85	0.078
Soyabean Flour	1	0.14400	0.14400	3.28	0.100
Butter	1	3.72100	3.72100	84.70	0.000
Square	3	0.53091	0.17697	4.03	0.041
Cassava Flour*Cassava Flour	1	0.04778	0.04778	1.09	0.322

Soyabean Flour *Soyabean Flour	1	0.13091	0.13091	2.98	0.115
Butter *Butter	1	0.30278	0.30278	6.89	0.025
2-Way Interaction	3	0.55375	0.18458	4.20	0.036
Cassava Flour*Soyabean Flour	1	0.28125	0.28125	6.40	0.030
Cassava Flour *Butter	1	0.06125	0.06125	1.39	0.265
Soyabean Flour *Butter	1	0.21125	0.21125	4.81	0.053
Error	10	0.43934	0.04393		
Lack-of-Fit	5	0.43934	0.08787	*	*
Pure Error	5	0.00000	0.00000		
Total	19	5.55800			



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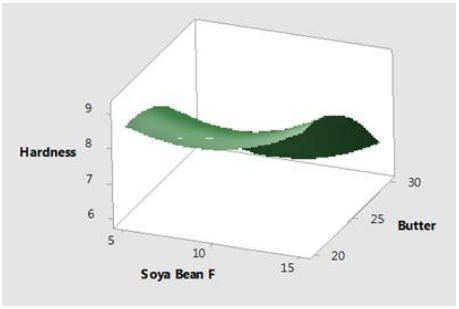

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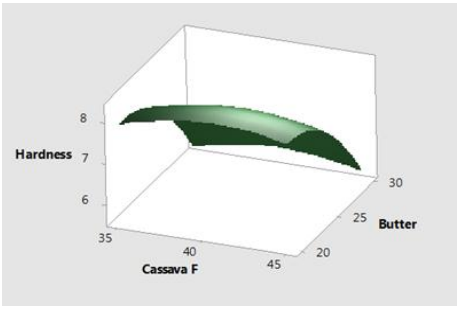

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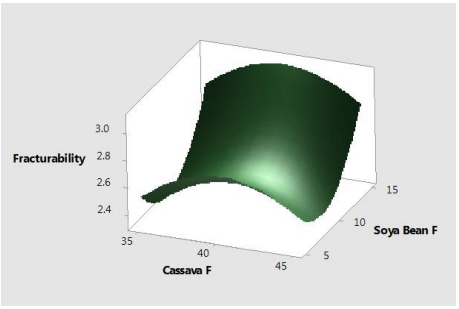

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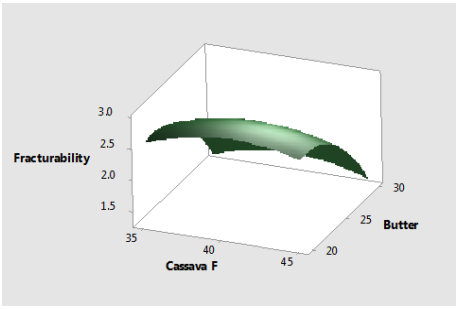

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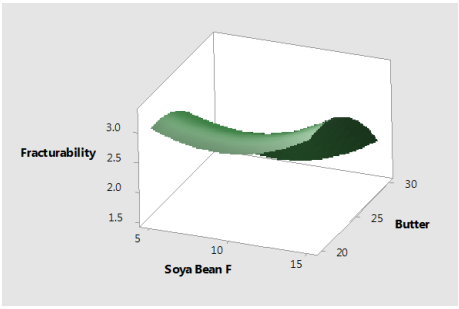

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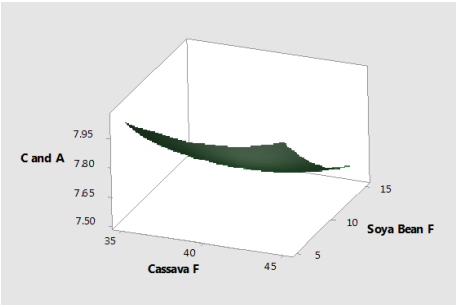

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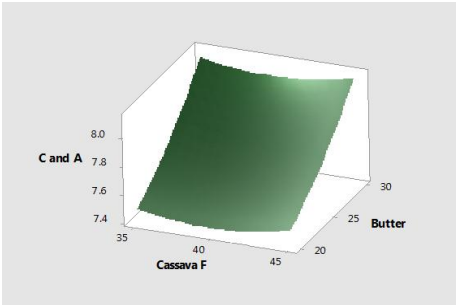

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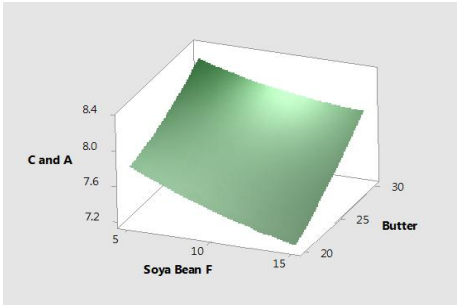

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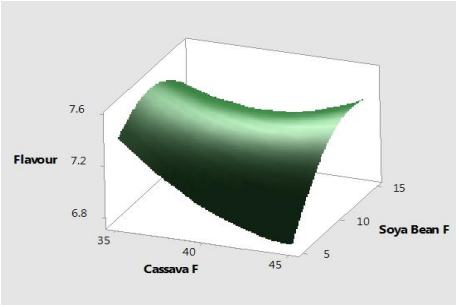

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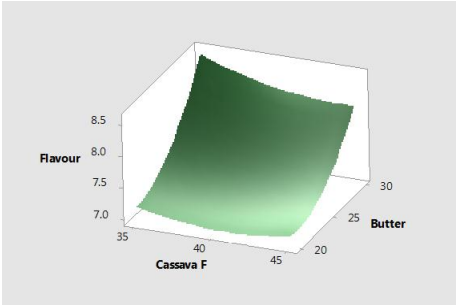

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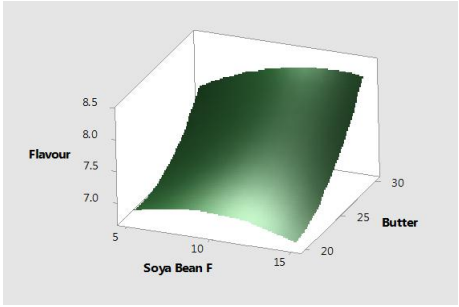

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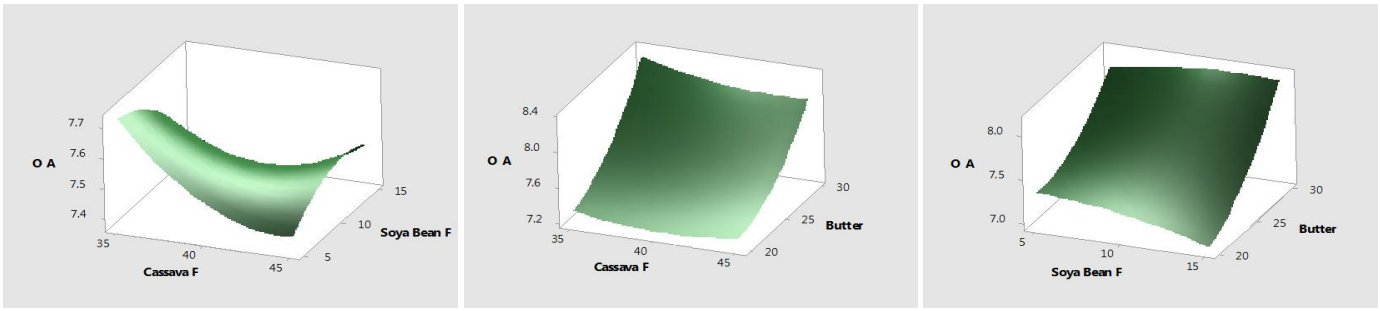

m) n) o)

Fig 1: Response Surface Plots

4. Conclusions

The optimized product chosen by the software was 35g of Cassava Flour, 7.72727g of Soyabean Flour and 30g of Butter that gave values of responses of sensory characteristics: Colour and Appearance – 8.19542, Flavour – 8.49015 and Overall Acceptability – 8.33015 and of textural properties: Hardness – 5.62666 kg/cm^2 and Fracturability – 1.33109 mm with an excellent desirability of 0.9631. So from the study it can be concluded that Cassava flour is an excellent replacement of wheat flour that can be used in many types of bakery products one of them being cookies, making it beneficial for people having gluten sensitivity. On the basis of proximate analysis of prepared cookies, it could be concluded that soyabean flour incorporation increased the nutritional status of the cookies by increasing the protein content which is quite less in Cassava flour. This study would also support industrial utilization and the consumption of under-utilized crops such as Cassava.

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