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Response Surface Methodology for Studying the Effects of Sugar Concentration and Acid Balancing on the Physico-Chemical Properties of Watermelon (*Citrullus Vugaris*) Jam

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Abstract Response surface methodology and central composite rotatable design for K=2 was employed to analyze the combined effect of sugar concentration and acid balancing on the soluble solids, refractive index, water insoluble solids total acidity and pH of watermelon (*Citrullus vulgaris*) jam. Regression models were developed to predict the effects of the processing parameters on the studied indices. Significant interactions were observed between all the factors with high regression coefficients (61.78-98.34%). There was a significant ($p \leq 0.05$) influence of the quadratic factors of pH and sugar concentration as well as the linear factor of pH on the soluble solids content, pH and refractive index but no significant influence of total acidity and water insoluble solids on watermelon jams. However, the linear and quadratic factors of pH showed no significant ($p \leq 0.05$) influence on the refractive index of the watermelon jams. Sugar concentration influenced most of the quality indices whereas pH had only marginal influence on the studied parameters.

Keywords: *response surface methodology, refractive index, regression models, soluble solids, water melon jam*

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1. Introduction

Watermelons (*Citrollus vulgaris*) belongs to family Cucurbitaceae. They are cultivated worldwide and since time immemorial, it is widespread in all tropical and subtropical regions of the world and is mostly grown for fresh consumption of the juicy and sweet flesh of mature fruit. Lycopene, Vitamins A, B6, C, carotenoids, and antioxidants are some nutrients found in watermelon [1]. In Ghana, the crop is cultivated mostly in the Bono region, Greater Accra region, Accra plains to be precise and in some parts of Takoradi and Kumasi in the Western and Ashanti regions, respectively [2]. Due to the high perishability of fruits especially watermelons, it is imperative to preserve them for consumption in the off-season and also to reduce its postharvest loss [3]. Postharvest factors basically involve production practices that have tremendous explicit effect on the microbial quality of fruits [4]. There is a possibility that management practices can affect product quality since stressed produce or mechanical injuries permit microbial contamination that inadvertently affects the final product make from such fruits.

Nutritionally they are low in calories but rather a high value of moisture approximately 92%. Sugar composition of watermelon is about 8%; it also contains a bioactive compound known as a carotenoid [5]. The type of carotenoid found in watermelons (*Citrollus vulgaris*) is called lycopene [6,7]. Lycopene, a phytochemical, is an important intermediate in the biosynthesis of many carotenoids, including β-carotene, responsible for yellow, orange, or red pigmentation [3]. Lycopene has been identified as a major antioxidant that helps to prevent some degenerative diseases such as some cancers and cardiovascular diseases [8].

The manufacture of jams serves as a means of preserving fruit, corresponding to the period when the fruit are harvested. During jam preparation consistency cannot be overemphasized. The consistency of jams depends on the presence of pectin; however, jam manufacture is largely based on the correct application of the pectin- sugar- acid gel formulations [9]. The right proportions pectin, sugar and acids are essential and have a resultant bearing on the definite equilibrium of the jam [10]. In jam making, the fruit mixture is concentrated by heat to form a uniform gel. The gel formation is very critical to the development of a high standard jam. The strength of the gel is very important as to prevent syneresis during storage [11]. The strength of the gel depends on the concentration of the gelling agent, the level of acidulant used and the concentration of the sweetening agent. According to Hui [7], the range for the concentration of pectin (gelling agent) is 0.5- 1.5% and an optimum of 1%, acidity ranging from 2.7 – 3.6 with an optimum pH value of 3.0 whilst the concentration of the sugar (sweetening agent) ranges from 64 – 71% with an optimum value of 67.5% [12].

Processing of jams requires attention to the high quality and delicate nature of the fruit, ingredients and finished goods. The object is to produce consistent products, requiring blending of fruit, ingredients and other additives to achieve a fairly tight formulation. Pectin, acid and soluble solids levels, in addition to cooking times and temperatures, must be uniformly practiced to achieve consistent products. One of the most important characteristics of pectin from the standpoint of manufacture of fruit jellies is the methoxyl content. This characteristic controls some of the important properties of pectins in relation with gel formation [13,14]. The role of pectin in jam is to impart a texture to the jam or jelly that allows that allows transportation without changes, that gives a good flavour release and that minimizes synersis [15]. Other added advantages include shorter cooking time, larger yield and allowing of product to retain its natural fruit flavour. Watermelon contains little pectin, so pectin has to be added to ensure the jam will have a good set.

In jam technology, no gel will form even in the presence of sufficient pectin and sugar until the pH is reduced below some critical value [9]. The addition of acid will suppress the dissociation of pectinic acids in solution and this reduces the positive charges and increases the tendency of the molecules to associate. Sugar is one of the most stable raw materials used in jam production and its presence exerts a dehydrating influence in a jelly. The higher the concentration of sugar, the less water there is for the structure to support [7]. Sugar disturbs the balance between the pectin solutions decreasing its stability. When the concentration of sugar is sufficiently high and acid is also present, the hydrogen ions complete the destabilization and a jelly forms as a result of partial precipitation [7].

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving and optimising processes in which a response of interest is influenced by several variables and the objective is to optimise the response. Baş and Boyacı [16] noted that RSM has important applications in the design, development and formulation of new products, as well as in the improvement of existing product designs. It defines the effect of the independent variables, alone or in combination, on the processes. In addition to analysing the effects of the independent variables, this experimental methodology generates a mathematical model which describes the chemical, biochemical or physical processes involved [17,18,19,20,21].

The objective of this work was to study the effects of sugar concentration and acid balancing on the physico-chemical properties of watermelon (*Citrullus Vugaris*) jams using response surface methodology.

2. Materials and Methods

2.1. Materials

Fresh watermelon fruits, sugar and lemon were purchased from the Lapaz market in Accra Ghana and transported to the food processing laboratory where they were stored in the cold room under cool hygienic conditions. High methoxy pectin from apple peel was obtained from the department. The ingredients used in the processing of the watermelon jams were: watermelon fruit; sugar (sucrose); lemon and pectin (from apple peel; H_2O<10 %, ash~6 %, degree of esterification: 70-75 %, Hydroscopic).

2.1.1. Processing of Watermelon Jams

Fully ripe watermelon was used for making the jams. The whole fruit was washed in clean water and all bad parts of the fruit discarded. The rind was removed from the melon and the flesh was cut into small pieces and seeds removed. The pieces of fruit were placed in a Hobart cutter and blended until smooth and consistent fresh fruit pulp was obtained. A pH meter was used to determine the pH of the fruit pulp and lemon juice was added until the required pH was attained. The required quantity of pulp stated was transferred into the jacketed pan. The fruit was boiled for about 40 minutes with consistent stirring to reduce water content. The stated amount of sugar was dissolved in 250 ml of water and boiled for 2 minutes before adding to the cooked fruit pulp. About 25 % of the stated amount of sugar was blended with the required amount of pectin and added to the boiling mixture. It was allowed to boil for 30 minutes.

2.1.2. Testing for Gel Formation

The refrigerator test was used to test for thickness and gel set. A small amount of boiling jam was poured on a cold plate and placed in the freezing compartment of the refrigerator for about a minute. The mixture gels if done, it is then removed from heat and quickly skimmed off from using a metal spoon.

2.1.3. Filling and Storage

The processed jams were filled into sterilized glass bottles and allowed to set by placing in a cool dark place for 12 hours. The jams were then stored in a cold room prior to analysis.

2.1.4. Preparation of Jam Samples for Analysis

About 50 grams of the watermelon jam was removed for analysis from the container and blended for 2 minutes in a warring blender. A portion of the sample was scooped from the container. The blended portion was stirred with a spoon before taking each sample for weighing. The processed jams were analyzed for their physico-chemical properties (water-insoluble solids, total acidity, pH, refractive index and total soluble solids) chemical composition (moisture, ash and total carbohydrate content), textural properties and colour.

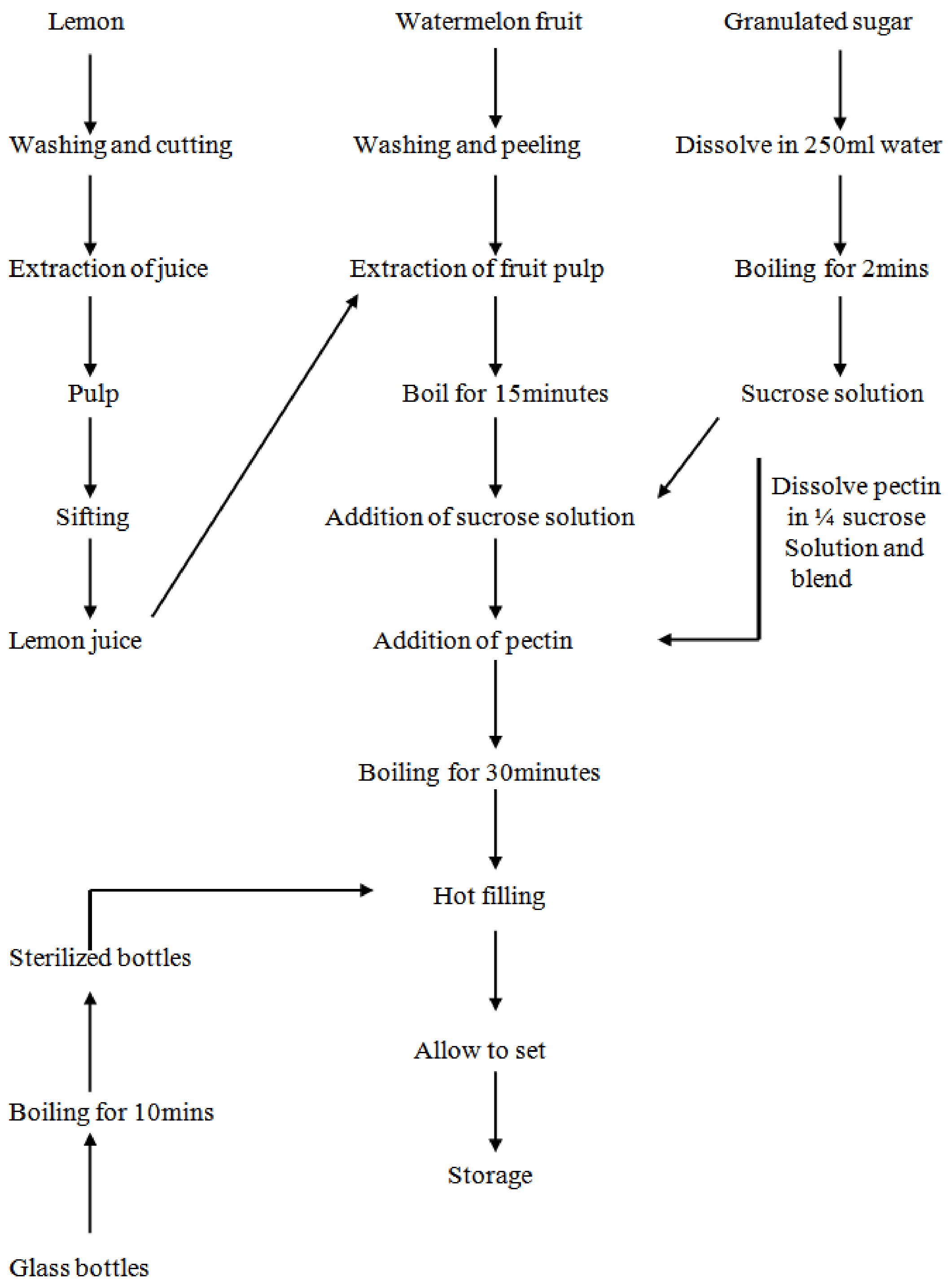

Figure 1. Process flow chart for watermelon jam

2.1.5. Experimental Design for the Processing of Watermelon Jams

Fourteen different batches of watermelon jams were processed based on the experimental design shown on Table 2 below.

2.1.6. Experimental Design for Response Surface Methodology

A Central Composite Rotatable Design of the experiment was set up using the stat graphics software with experimental study variable number K = 2, for independent variables including, acid and sugar concentrations. The process variables to be used in the Central Composite Rotatable Design (CCRD) For K = 2 were processed using the software. This indicates the dependent variable limits and their limits and values. The dependent variables studied included the following, total soluble solids, water insoluble solids, pH, moisture, total Acidity, ash, total carbohydrate and colour.

Table 1. Process variables and their levels used in the Central Composite Rotatable Design for K=2

Variable	Code	Level (Y)				
		-1.414	-1	0	1	1.414
Sugar Conc	X1	0	15	50	70	100
Acid Conc	X2	3	3.07	3.25	3.42	3.5

Fourteen sample combinations were generated from the software in experimental design using the design matrix and variable combinations in experimental runs as follows.

Table 2. Design matrix and variable combinations in experimental runs

Sample number	Codes		Coded variables	
	X1	X2	Sugar concentration (%)	pH
1	-1	-1	15	3.42
2	-1	1	15	3.42
3	1	-1	70	3.07
4	1	1	70	3.42
5	0	0	50	3.25
6	0	0	50	3.25
7	0	0	50	3.25
8	1.414	0	10	3.25
9	-1.414	0	0	3.25
10	0	1.414	50	3.50
11	0	-1.414	50	3.0
12	0	0	50	3.25
13	0	0	50	3.25
14	0	0	50	3.25

2.2. Analytical Methods

2.2.1. Refractive Index and Total Soluble Solids

The refractive index and total soluble solids contents of the watermelon jam samples were determined using the procedure outlined in the Indian Institute of Food Processing Technology [22]. Representative samples of a well mixed portion of jam or jelly – free from seed and fiber was taken and placed on absolutely dry refractometer prisms and read directly at 20°C.

2.2.2. Water – Insoluble Solids

The water insoluble solids content of the watermelon jam was determined using the procedure outlined in Ajenifujah-Solebo and Aina [15]. About 25 g duplicate samples of the blended portion were weighed to the nearest 0.01 g. Each duplicate was transferred into a 400 ml beaker with hot water and diluted with water to about 200 ml. It was allowed to boil gently for 15 to 20 minutes. One of the duplicate samples was transferred to a 250 ml volumetric flask, cooled and made up to volume at 20° (filtrate from this was later used for determining acidity. Mark filtrate "A"). Each sample from the volumetric flask and that from the beaker were filtered separately through No.4 whatman paper (15.0 cm) that was previously washed with hot water, oven dried for 2 hours at 100°C to 105°C, cooled in a dessicator and weighed in a covered weighing dish (7 cm diameter). The samples were each washed with 800 ml of hot water, loosening the water-insoluble solids from the filter paper with each addition. The filter paper was transferred to the original weighing dish. It was dried overnight at 100°C to 105°C, cooled in a dessicator and weighed.

2.2.3. pH

The pH of the watermelon jam samples, were determined using the procedure outlined in Egan *et al.* [23]. The pH meter was standardized with a standardized pH 4.0 buffer solution and 50 g of well mixed portion was placed in a 100 ml beaker and read on the pH meter. When the first reading was completed, the electrodes were wiped with a small piece of cotton soaked in distilled water. The electrode was rinsed with water from a washed bottle and dried with a piece of filter paper prior to the next determination.

2.2.4. Total Acidity

The total acidity of the watermelon jam samples were determined using the procedure outlined in Ogbeide *et al.* [24]. The filtrate from the previous water-insoluble solids determination was used. Fifty (50) ml of the filtrate was pipetted into a 250 ml beaker. About 100 ml of water was added and titrated with 0.1 N NaOH to pH 8.1, using a pH meter. The amount of NaOH required was recorded. Total acidity is expressed as a percentage of the malic acid in the fruit.

3. Results and Discussion

3.1. Effect on the Soluble Solids Content of Watermelon Jam

The model obtained for soluble solids content of the watermelon jams was:

$$Z = 1184.21358 + 0.848488X_1 - 717.349463X_2$$
$$-0.006836X_1^2 + 111.144965X_2^2 + 0.10038X_1X_2,$$

with an R^2 of 96.77%

There was a significant ($p \leq 0.05$) influence of the quadratic factors of pH and sugar concentration as well as the linear factor of pH on the soluble solids content of watermelon jams. The model could explain about 96.77% of the variations in soluble solids meaning only 3.23% of the variation was due to other factors not included in the model.

Sugar is one of the most stable raw materials used in jam production and its content of soluble solids is about 100 percent [25]. According to Cruess [26], finished jelly may contain a sugar concentration varying from 40 to 70% including that present in the fruit extract. This means that both the fruit and sugar contribute to the total soluble solids content of the finished product. The response surface plots developed (Figure 2) showed linear plots for both sugar concentration and pH. The plots revealed that increasing sugar concentration resulted in an increase in the soluble solids content of watermelon jam. However above 60% sugar concentration, the soluble solids content of the watermelon jams did not change appreciably, but remained between 66 and 76% soluble solids content.

The Food and Drug Administration requires that all jellies and jams be made from a mixture composed of not less than 45 parts by weight of fruit and 55 parts by weight of sugar which has been concentrated by heat until the soluble solids content is not less than 65 percent as determined by a refractometer [27]. Since the soluble solids content of the watermelon jams did not increase above sugar concentration of 60 % and this sugar concentration resulted in the prescribed soluble solids content of jams, it is not necessary to use sugar concentration above 60%, as it would not increase soluble solids content or further improve the quality of the finished jam. Therefore, to attain the required soluble solids, 60% sugar concentration would be optimal for water melon jams processing.

The plots revealed that increasing pH had no significant effect on the soluble solids content of the watermelon jams. Nartey [28], reported that, using different lemon juice concentrations in the processing of pineapple jams did not lead to any significant change in the soluble solids content of the jams. This therefore indicates that pH values between 3.0 and 3.5 do not decrease or increase the soluble solids content of the finished jams and that any concentration in that range is ideal for water melon jams.

It can be deduced from this study that the optimal sugar concentration and pH required to achieve the prescribed soluble solids content of jams is as follows: 60 % sugar concentration and pH between 3.0 and 3.5.

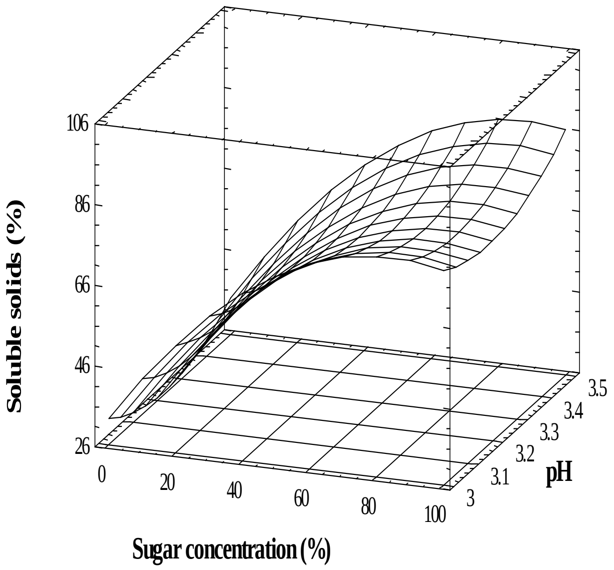

Figure 2. Effect of sugar concentration and pH on the soluble solids content of watermelon jams

3.2. Effect on the Refractive Index of Watermelons Jam

The model obtained for refractive index was:

$$Z = 1.986107 + 0.00254 X_1 - 0.368668 X_2$$
$$-0.000013 X_1^2 + 0.055776 X_2^2 - 0.000059 X_1 X_2,$$

with an R^2 of 98.34%.

There was a significant ($p \le 0.05$) influence of the quadratic factor of sugar concentration on the refractive index of watermelon jam. However, the linear and quadratic factors of pH showed no significant ($p \le 0.05$) influence on the refractive index of the watermelon jams. The model could explain 98.34% of the variation in refractive index; meaning only 1.66% of the variation was due to other factors not included in the model. The response surface plots (Figure 3) generated showed linear plots for both sugar concentration and pH. The plots revealed that increasing sugar concentration resulted in an increase in the refractive index of the watermelon jam. Increasing pH however had no significant effect on the refractive index of the jam.

The removal of water during boiling of jam produces a rise in the total soluble solids content of the mixture as indicated by the refractive index [29]. The rise in the soluble solids content of the jams with increasing sugar concentration as shown by the plots is therefore accountable for the increasing refractive index values observed. However, it can be observed from the plots that the refractive index did not increase significantly above 1.46 which corresponds to a sugar concentration of 60%. According to Pearson's, 1976 a refractive index of 1.46058 corresponds to a soluble solids level of 68 %. This implies that sugar concentration of 60 % is sufficient to generate a refractive index of 1.460 which corresponds to the prescribed soluble solids level of above 65 %.

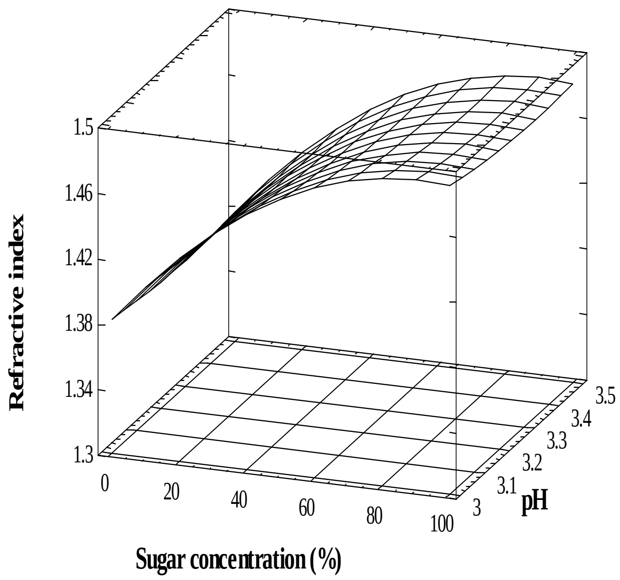

Figure 3. Effect of sugar concentration and pH on the refractive index of watermelon jam

3.3. Effect on the Water Insoluble Solids Content of Watermelon Jams

The model obtained for water insoluble solids of the watermelon jam was:

$$Z = -23.062146 + 0.068823 X_1 + 13.970255 X_2$$
$$-0.00009 X_1^2 - 1.967594 X_2^2 - 0.017536 X_1 X_2,$$

with an R^2 of 61.78% .

There was no significant ($p \le 0.05$) influence of the linear and quadratic factors of pH and sugar concentration on the water insoluble solids content of the watermelon jams. The model could explain 61.78% of the variations in water insoluble solids, meaning that the remaining 38.22% was due to other factors not included in the model.

The response surface plots generated (Figure 4) showed linear plots for both sugar concentration and pH. The water insoluble solids content of the watermelon jams, increased gradually with increasing sugar concentration from 0% to 100%, meaning increasing sugar concentration led to increase in the water insoluble solids content. There was a slight decrease in the water insoluble content of the jams with increasing pH. This suggests that sugar adds some level of water insoluble solids to jams during processing which adds to the adjunct content of the jam.

The insoluble solids content of jams is mostly derived from the fruit used and makes constitute the fibre content of jams. The British standards for jam specify minimum fruit content for fruit jam. However no standard exists for watermelon jam.

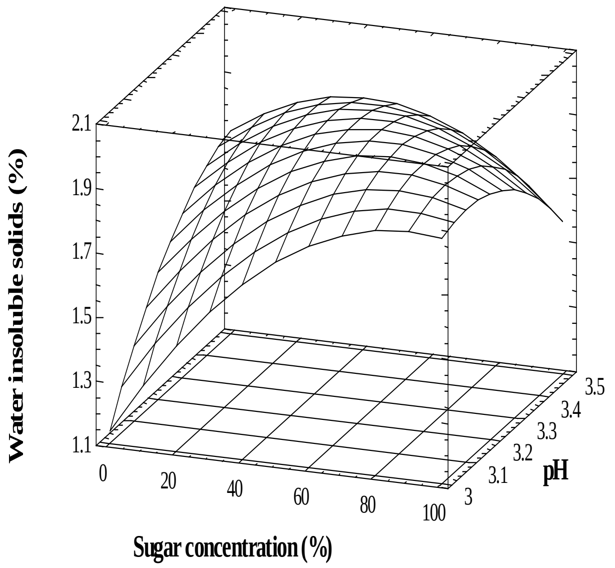

Figure 4. Effect of sugar concentration and pH on the water insoluble solids of watermelon jams

3.4. Effect on the Total Acidity of Watermelon Jam

The model obtained for total acidity was:

$$Z = 3.698529 + 0.002965X_1 - 1.476362X_2$$
$$+6.209394E - 6X_1^2 + 0.206539X_2^2 - 0.00338X_1X_2,$$

with an R^2 of 98.26%.

There was no significant ($p \leq 0.05$) influence of the linear and quadratic factors of pH and sugar concentration on the total acidity of the watermelon jam products. The model could explain 98.26% of the variation in total acidity, implying that only 1.74% of the variation was due to other factors not included in the model. The response surface plots (Figure 5) generated showed linear plots for both pH and sugar concentration. The total acidity decreased with increasing sugar concentration. Increasing pH on the other hand led to a slight decrease in the total acidity of the jam from 1.2 to 0.6.

It is necessary to keep the acid content of jam fairly constant by increasing it in some and neutralising it in others. The total acidity should not exceed 0.8 percent, but 0.5 percent can be taken as a general standard and 0.3 percent a minimum figure [24]. It can be observed from the plots that the total acidity of the watermelon jams attained the general standard of 0.5 percent at sugar concentration of 60 %. Above this level the total acidity reduced below the minimum figure of 0.3 percent, indicating that 60 % sugar concentration is sufficient to give total acidity that meets requirements. The total acidity remained within acceptable limits for all pH levels studied, indicating that pH level between 3.0 and 3.5 would produce jams that meet the standards for total acidity and hence jams of acceptable quality.

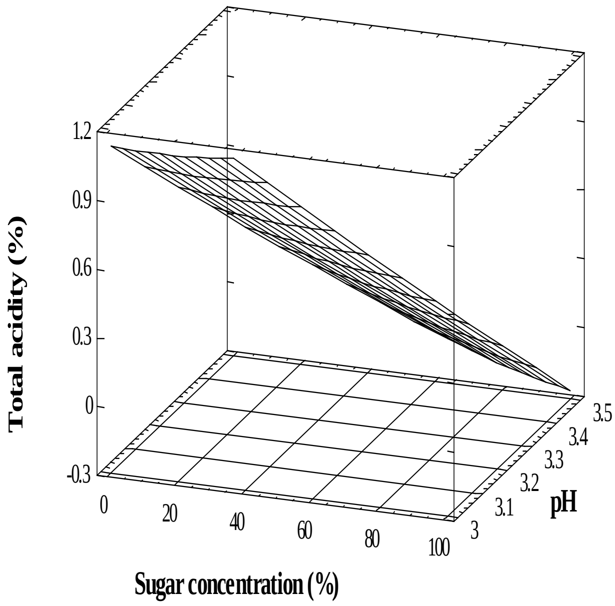

Figure 5. Effect of sugar concentration and pH on the total acidity of watermelon jam

3.5. Effect on the pH of Watermelon Jam

The model obtained for pH was:

$$Z = -23.285636 + 0.008358X_1 + 15.223774X_2$$
$$-0.000122X_1^2 - 2.193413X_2^2 + 0.001722X_1X_2,$$

with an R^2 of 95.90%.

There was a significant ($p<0.05$) influence of the quadratic factors of pH and sugar concentration and the linear factors of pH on the pH of the watermelon jam products. The model could explain 95.90% of the variations in pH implying that only 4.1% of the variation was due to other factors not included in the model. The response surface plots generated (Figure 6) showed a curvilinear plot for sugar concentration and a linear plot for pH.

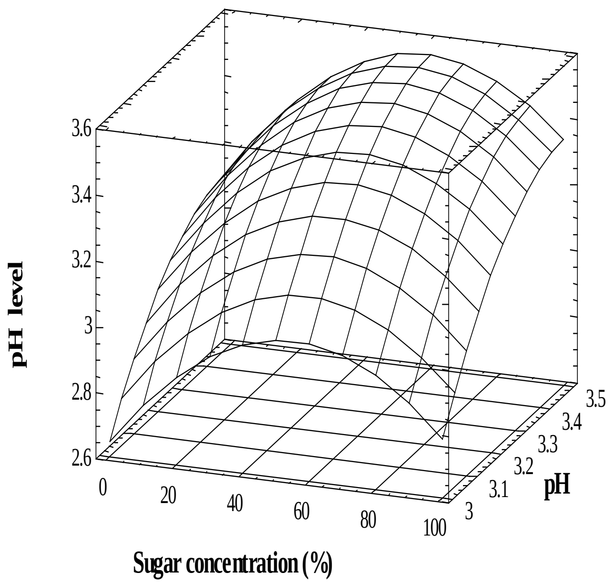

Figure 6. Effect of sugar concentration and pH on the pH of watermelon jam

There was an initial increase in pH level followed by a decline in pH level with increasing sugar concentration. The plots revealed that sugar concentration of 60% resulted in a pH of 3.0. It has been found that gel formation occurs only within a certain range of hydrogen ion concentration, the optimum acidity figure for jam and jellies being 3.0 [24]. For the pH of the product there was a gradual increase in the pH of the watermelon jam as the pH of the sample increased from 3.0 to 3.5. According to Duckworth [30], the optimum pH for gel formation is not fixed but varies depending on the type of fruit. However the range is between 3.0 and 3.7. It can therefore be deduced from the study that, the optimal ingredient balancing required to achieve optimum pH was sugar concentration of 60 %and pH between 3.0 and 3.5.

4. Conclusion

Central Composite Rotatable Design (CCRD) and Response Surface Methodology (RSM) were effectively used to estimate the effect of sugar concentration and pH balance on the physiochemical properties of watermelon jams. The results revealed that sugar concentration influenced most of the quality indices whereas pH significantly influenced only a few of the indices. Increasing sugar concentration had a significant effect on the physiochemical characteristics of the watermelon jams. From the study, it was observed that the optimal processing conditions required to achieve the optimum quality of watermelon jam were sugar concentration of 60 %, pH range of 3.0 to 3.5 with a pectin concentration of 0.5 %. These levels give the best quality watermelon jams with acceptable quality characteristics.

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