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Modelling The Physicochemical Characteristics Of Worts And Beers Made From Bitter Sorghum With Extracts Of *Balanites aegyptiaca*

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Key words

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Modelling,
Sorghum beer,
Wort.

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Abstract

The use of hops as a bittering agent in the brewing industry represents a major challenge for several African countries due to their dependence on imports and the resulting economic burden. Developing locally available alternatives is therefore of significant technological and economic interest. This study aimed to evaluate the brewing potential of *Balanites aegyptiaca* fruit extracts as a substitute bittering agent in sorghum beer production. Bitter compounds were obtained using two extraction methods, infusion and maceration, and incorporated during wort boiling. A mixture experimental design generated with Design-Expert® software was applied to optimize the proportions of infused and macerated extracts as well as the wort cooking time. Vitamin C, flavonoids, and total polyphenols were selected as response variables and modelled using Response Surface Methodology (RSM), resulting in multivariate polynomial models with high predictive performance. The infused extract showed titratable acidity, vitamin C, flavonoid, and polyphenol contents of 0.360 ± 0.029 g/L, 23.45 ± 0.42 mg/100 g, 0.0405 ± 0.0007 mg EQ/mL, and 0.158 ± 0.013 mg EAG/mL, respectively, while the macerated extract exhibited values of 0.586 ± 0.045 g/L, 15.58 ± 0.42 mg/100 g, 0.1309 ± 0.0007 mg EQ/mL, and 0.1309 ± 0.0007 mg EAG/mL. In bitter worts, vitamin C ranged from 0.93 to 2.56 mg/100 g, flavonoids from 0.068 to 0.142 mg EQ/mL, and polyphenols from 0.101 to 0.265 mg EAG/mL, while higher concentrations were observed in beers. Multi-response optimization identified optimal conditions consisting of 100% infused extract, 36% macerated extract, and a cooking time of approximately 52 minutes, confirming the suitability of *Balanites aegyptiaca* as a local alternative to hops in sorghum beer production.

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1. Introduction

Sorghum is one of the major cereals cultivated in Cameroon, particularly in the northern regions. Its use in the production of a traditional fermented beverage known as *bili-bili* by local populations is of particular interest. This beverage is considered a form of craft beer and is characterised by the absence of conventional bittering agents (Touwang et al., 2018). Sorghum is also widely used in the production of industrial beers (Palmer et al., 2001).

The production of sorghum beer (*bili-bili*) plays a significant role in the financial independence of women brewers. It has been reported that a woman processing between 100 and 150 cups of sorghum twice per week can generate an average monthly income of approximately 120,000 CFA francs (Zangue et al., 2013). In Cameroon, this activity is notably promoted by YIIH INDUSTRY SARL, while in Burkina Faso, *La Maison du DOLO* plays a similar role. These small- and medium-sized enterprises specialise in sorghum beer production and contribute to the promotion of this traditional beverage across the African continent.

Despite this potential, the development of the sector remains constrained. Production is still largely artisanal, even though preservation challenges have been partially addressed. In addition, the organoleptic properties of sorghum beer are often perceived as less appealing than those of industrial beers commonly consumed in African urban centres. To improve sensory quality, various tropical barks and plants are traditionally used as bittering and flavouring agents. Several studies have highlighted the potential of *Khaya senegalensis*, *Gongronema latifolium*, *Vernonia amygdalina*, *Nauclea diderrichii*, *Garcinia kola*, and other species to enhance bitterness and aroma in sorghum, cassava, and millet beers in Cameroon (KENNE, 2021; Zangue et al., 2013; Moneke, 2009; Okaro et al., 2010).

However, the use of these plants often results in excessive and persistent bitterness. This limitation justifies the exploration of alternative botanical sources with milder sensory profiles. The fruits of *Balanites aegyptiaca* exhibit a bittersweet taste, being green and hairy when unripe and becoming smooth and yellow to brownish upon ripening. The incorporation of *Balanites aegyptiaca* fruit extracts into sorghum beer therefore represents a promising approach for developing effective local substitutes for imported hops and for diversifying beer formulations.

2. Material and methods

2.1. Equipment

The following materials were used in this study. Djigari sorghum was obtained from the central market of Maroua II, with its cultivation mainly concentrated in the town of Mokolo. *Balanites aegyptiaca* fruits were harvested from the villages of Golonghini in the Far North Region of Cameroon and from the Mayo-Kani Department.

2.1. Methods

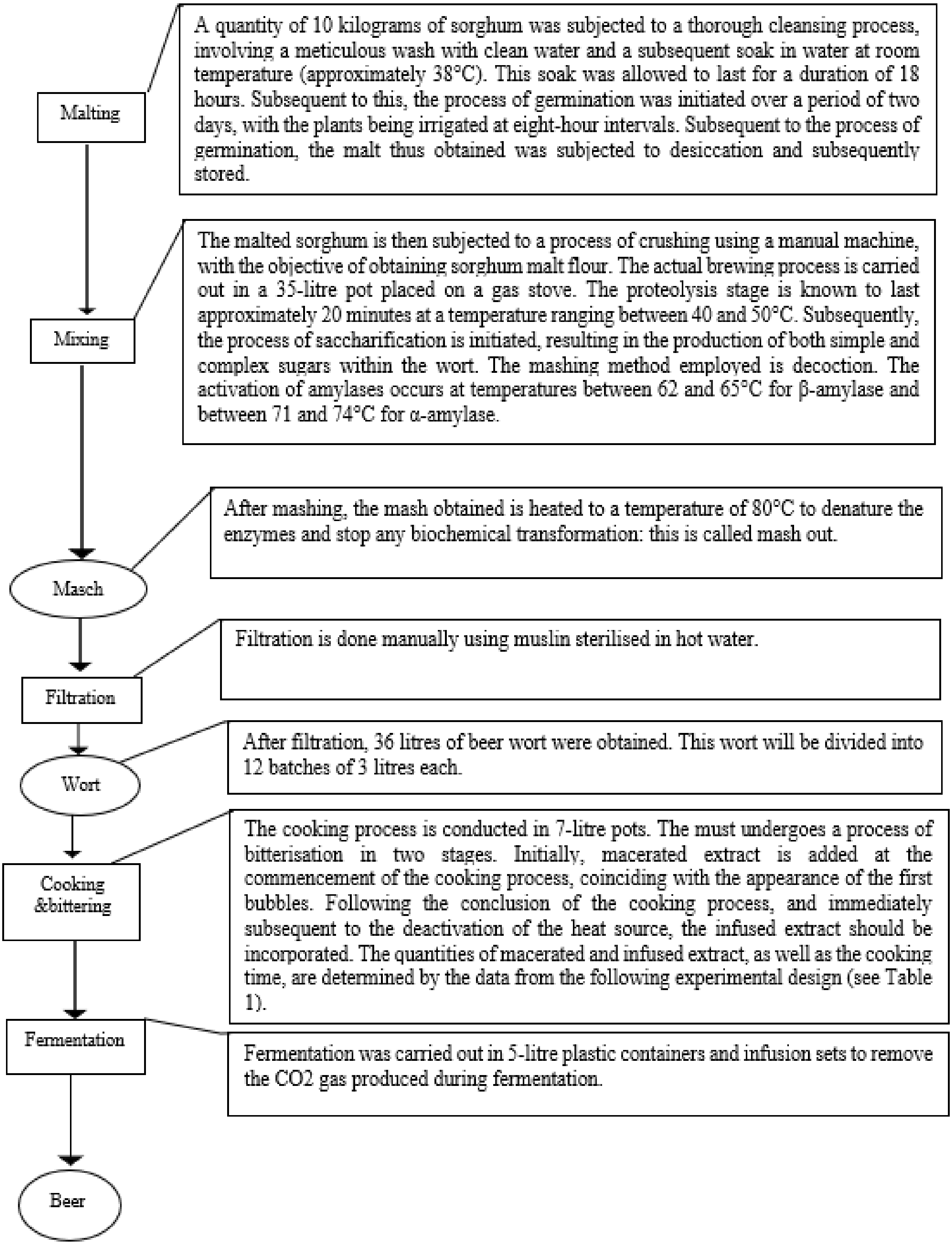
2.2.1 Extraction of bitter substances

Two extracts were obtained from the fruits of *Balanites aegyptiaca*: an infused extract and a macerated extract. Both extracts were produced using the same raw material, with the extraction steps differing only in the processing stage. For the infused extract, *Balanites aegyptiaca* fruits were immersed in distilled water at 80 °C and allowed to stand for 6 h. The resulting infusion was then separated from the solid material, filtered through muslin cloth, pasteurised, and stored under cool conditions.

Subsequently, the infused fruits were subjected to maceration in distilled water at 60 °C. The resulting macerate was pressed through muslin cloth to extract the fruit syrup, referred to as the macerated extract. This extract was then heated to 100 °C and stored in sterile containers under cool conditions.

2.2.2 Beer-making process

Figure 1 illustrates the different stages involved in beer production, including the bittering step using *Balanites aegyptiaca*.



A quantity of 10 kilograms of sorghum was subjected to a thorough cleansing process, involving a meticulous wash with clean water and a subsequent soak in water at room temperature (approximately 38°C). This soak was allowed to last for a duration of 18 hours. Subsequent to this, the process of germination was initiated over a period of two days, with the plants being irrigated at eight-hour intervals. Subsequent to the process of germination, the malt thus obtained was subjected to desiccation and subsequently stored.

The malted sorghum is then subjected to a process of crushing using a manual machine, with the objective of obtaining sorghum malt flour. The actual brewing process is carried out in a 35-litre pot placed on a gas stove. The proteolysis stage is known to last approximately 20 minutes at a temperature ranging between 40 and 50°C. Subsequently, the process of saccharification is initiated, resulting in the production of both simple and complex sugars within the wort. The mashing method employed is decoction. The activation of amylases occurs at temperatures between 62 and 65°C for β-amylase and between 71 and 74°C for α-amylase.

After mashing, the mash obtained is heated to a temperature of 80°C to denature the enzymes and stop any biochemical transformation: this is called mash out.

Filtration is done manually using muslin sterilised in hot water.

After filtration, 36 litres of beer wort were obtained. This wort will be divided into 12 batches of 3 litres each.

The cooking process is conducted in 7-litre pots. The must undergoes a process of bitterisation in two stages. Initially, macerated extract is added at the commencement of the cooking process, coinciding with the appearance of the first bubbles. Following the conclusion of the cooking process, and immediately subsequent to the deactivation of the heat source, the infused extract should be incorporated. The quantities of macerated and infused extract, as well as the cooking time, are determined by the data from the following experimental design (see Table 1).

Fermentation was carried out in 5-litre plastic containers and infusion sets to remove the CO_2 gas produced during fermentation.

Figure 1. Process for obtaining bitter sorghum beer with Balanites aegyptiaca.

In addition to the preceding figure, Table 1 presents the coded values of extract proportions and wort cooking times used for each experiment. The experimental matrix generated using Design-Expert® 11 software comprised a total of 12 runs involving the factors macerated extract (A), infused extract (B), and cooking time (C).

Table 1. Bittering mixture plan for wort

Manip Number	A	B	C
1	0,25	0,75	-1
2	0	1	-1
3	1	0	-1
4	0,75	025	-1
5	0,5	05	1
6	0	1	1
7	0,25	075	1
8	0,75	025	1
9	0,5	05	-1
10	1	0	1
11	1	0	-1
12	0	1	1

The coded values were converted into actual values using the equations presented below:

Equation 1 (Actual value)

$$U = U_0 + x\Delta U$$

where

$$U_0 = \frac{U_{\min} + U_{\max}}{2}$$

(**Equation 2:** Centre point value)

In these equations, U represents the actual value of the variable, U_0 corresponds to the value at the centre of the experimental domain, and x denotes the coded (reduced) value. ΔU represents the step size, while U_{min} and U_{max} correspond to the minimum and maximum values of the factor, respectively. This transformation allows the determination of real experimental values within the defined domain.

2.2.3 Raw material analysis

2.2.3.1 Determination of water content and dry matter

The term *dry matter*, also referred to as *total dry residue*, denotes the fraction of substances that do not volatilise under the drying conditions specified by the analytical method. Dry matter determination was performed on the raw materials according to the ASBC (2009) method. The initial mass of the fresh sample (M_0) was dried in an oven at 105 °C until a constant mass was obtained, followed by cooling in a desiccator for 24 h.
The total dry residue, expressed as dry matter (DM), was calculated as a percentage of fresh matter using Equation 3:

Equation 3 (Dry matter)

$$\%DM = \frac{M_2 - M_0}{M_1 - M_0} \times 100$$

where M_0 is the mass (g) of the empty capsule, and M_1 and M_2 are the masses (g) of the capsule containing the sample before and after drying, respectively.

The water content (H) was calculated using Equation 4:

Equation 4 (Water content)

$$\%H = 100 - \%DM$$

2.2.3.2 Germination capacity

The percentage of viable grains in a sample was determined using the ASBC (2009) method, with hydrogen peroxide employed as a germination stimulant. Three batches of 200 grains were immersed at ambient temperature (22–25 °C) in a 7.5 g/L hydrogen peroxide solution for 48 h. The exhausted solution was then replaced with a fresh solution, and the grains were soaked for an additional 48 h. After completion of the soaking period, the solution was decanted, and the germination rate was determined by counting the number of germinated grains.

Germination capacity (GC), expressed as a percentage, was calculated using Equation 5:

Equation 5 (Germination capacity)

$$GC = \frac{200 - N_{ng}}{2}$$

where GC is the germination capacity (%) and N_ng is the number of grains that did not germinate.

2.2.3.3 Mass of a thousand grains

The average grain mass is used as a quality parameter in the brewing process (MEBAK, 2020), as it provides information on the degree of grain filling. An increase in the mass of 1,000 grains is generally associated with a higher starch content and, consequently, greater extract potential. For this determination, three batches of 40 g of sorghum grains were weighed and cleaned to remove dust and foreign materials. The grains in each batch were then counted and weighed.

The mass of 1,000 grains was calculated using Equation 6:

Equation 6 (Mass of 1,000 grains)

$$P_m = \frac{1000 \times M \times (100 - H)}{100 \times N_g}$$

where P_m is the mass (g) of 1,000 dry grains, M is the mass (g) of the weighed batch after counting the grains, H is the water content of the grains (%), and $N_{(g)}$ is the number of grains counted in the batch.

2.2.3.4 Determination of germination energy (BRF method)

This method is based on determining the percentage of grains likely to germinate completely when the sample is malted under standard conditions (MEBAK, 2020). For this purpose, three filter papers were placed in each Petri dish, followed by the addition of 4 mL and 8 mL of distilled water, respectively. Three batches of 100 grains were then placed in the dishes, which were covered and kept in a light-protected environment.

The grains were incubated for 24, 48, and 72 h, respectively, after which the number of germinated grains was counted. Germination energy (GE) was calculated using Equation 7:

Equation 7 (Germination energy)

$$GE = 100 - N_{ng}$$

where GE represents germination energy (%) and N_ng is the number of grains that did not germinate.

2.2.4 Analysis of musts and beers

2.2.4.1 Determining the Brix degree

A drop of the sample was placed on the reading surface of the refractometer, and the Brix value was recorded. The Brix degree represents the percentage of soluble dry matter in the sample. This measurement is temperature-dependent, as variations in temperature can influence the refractive index. The refractive index reflects the degree to which light is bent as it passes through a medium.

2.2.4.2 Determination of vitamin C

The principle of this method is based on the redox reaction between vitamin C and iodine, in which a solution containing vitamin C at a known concentration is titrated with an iodine solution of known concentration. The reaction is described by Equation 8:

Equation 8 (Determination of vitamin C) $$\mathrm{C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2HI}$$

Procedure

A volume of 5 mL of the sample solution was transferred into an Erlenmeyer flask, followed by the addition of 45 mL of distilled water to obtain the required dilution. Subsequently, 1 mL of a 5% starch solution was added as an indicator, and the mixture was titrated with a 0.05 M iodine solution. The volume of iodine required to produce a stable deep blue colour was recorded. Each measurement was performed in triplicate, and the mean value was calculated.

A standard vitamin C solution of known concentration was titrated under the same conditions, and the corresponding volume of iodine required to reach the endpoint was recorded.

The vitamin C content of the sample was calculated using Equation 9:

Equation 9 (Vitamin C content $$Q = \frac{V_e \times f \times N \times m \times 100}{V_i}$$

where V_e is the volume (mL) of iodine solution used for the sample, f is the dilution factor, N is the normality of ascorbic acid, m is the mass (mg) of ascorbic acid, and V_i is the volume (mL) of iodine solution used for the vitamin C standard.

2.2.4.3 Determination of titratable acidity

Titratable acidity, also referred to as total or fixed acidity, is defined as the sum of free organic acids and hydrogen ions (H^+) present in a food or beverage.

Method

To prepare a 10% solution of *Balanites aegyptiaca* extract or syrup, 5 mL of the sample were diluted with 45 mL of distilled water. A volume of 0.5 mL of the diluted solution was then transferred into an Erlenmeyer flask, followed by the addition of six drops of phenolphthalein as an indicator.

The burette was filled with a sodium hydroxide (NaOH) solution of known concentration. The NaOH solution was added gradually to the Erlenmeyer flask under constant stirring until a persistent colour change of the indicator was observed. The volume of NaOH required to neutralise the acidity of the sample was recorded. Each determination was performed in triplicate, and the mean value was calculated.

Titratable acidity was calculated using Equation 10. The acidity (g/L) was determined by multiplying the volume of NaOH used (mL) by the molar concentration of NaOH (mol/L) and the molar mass of citric acid (g/mol), then dividing the result by the volume of diluted sample (mL) and multiplying by 1000.

Equation 10 (Titratable acidity)

$$TA\,(g/L) = \frac{V_{\text{NaOH}} \times C_{\text{NaOH}} \times M_{\text{citric}} \times 1000}{V_{\text{sample}}}$$

2.2.4.5 Determination of polyphenols

Determination of total polyphenols

Reagents. Folin–Ciocalteu reagent, sodium carbonate, and gallic acid were used in this analysis. The Folin–Ciocalteu reagent consists of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). During the oxidation of phenolic compounds, these complexes are reduced to a mixture of blue tungsten and molybdenum oxides. The intensity of the resulting colour, with maximum absorbance between 725 and 750 nm, is proportional to the total polyphenol content of the plant extracts.

Procedure. A volume of 0.02 mL of sample was mixed with 1.2 mL of distilled water, 0.2 mL of Folin–Ciocalteu reagent diluted to 1/10, and 0.4 mL of a 20% sodium carbonate solution. The mixture was incubated in the dark at room temperature (27 ± 3 °C) for 30 min.

Absorbance was measured using a UV–visible spectrophotometer at 760 nm. The blank consisted of 0.2 mL of Folin–Ciocalteu reagent diluted to 1/10 and 0.4 mL of a 75 g/L sodium carbonate solution. Gallic acid was used as the reference standard to construct the calibration curve, and total polyphenol content was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract). All measurements were performed in duplicate.

The total polyphenol content of the samples and fractions was determined from the calibration curve, described by the regression equation: $Y = 0.113\,X + 0.0514$, ($R^2 = 0.9695$) where gallic acid was used as the standard.

2.2.4.6 Determination of flavonoids

Reagents. Sodium nitrite, aluminium trichloride, sodium carbonate, and quercetin were used in this assay.

Principle. Flavonoids contain a free hydroxyl (–OH) group at position 5, which is able to form a coloured complex with aluminium chloride in the presence of a carbonyl (C=O) group. Flavonoids are known to form yellowish complexes through metal chelation, particularly with iron and aluminium ions. This property is attributed to the electronegativity of oxygen, which readily donates electrons. In this reaction, the phenolic molecule acts as an electron donor, forming a stable complex with the aluminium ion through coordination with oxygen atoms.

Procedure. Aliquots of 0.15 mL of 5% (m/v) sodium nitrite solution and 0.15 mL of 10% (m/v) aluminium trichloride solution were added to 5 mL of extract solution or a 1/100 dilution (m/V). After incubation in the dark for 30 min, absorbance was measured at 510 nm using a UV–visible spectrophotometer. The blank consisted of 0.15 mL of 5% (m/v) sodium nitrite solution and 0.15 mL of 10% (m/v) aluminium trichloride solution.

Quercetin was used as the reference standard for constructing the calibration curve, and total flavonoid content was expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract). All analyses were performed in triplicate.

The flavonoid content of the hydroalcoholic extracts and fractions was determined using the calibration curve described by the regression equation: $Y= 1.3605X - 0.0086$, ($R^2 = 0.9461$), where quercetin was used as the standard

3. Results and discussion

3.1. Characteristics of *Djigari sorghum*

The acceptance criteria and brewing potential used to assess the physicochemical profile of Djigari sorghum are presented in Table 2. Analysis of the results indicates that this variety exhibits satisfactory malting potential. The mass of 1,000 grains (29.07 ± 1.35 g) falls within the range reported in the literature (Steve, 2012). In addition, germination capacity and germination energy values of 95.75% and 96.37%, respectively, exceed the minimum threshold of 90% recommended by Touwang, Nso, and Ndjouenikeu (2018), thereby ensuring uniform grain germination and suitability for high-quality malt production.

The polyphenol content of the sample was 0.79 mg/mL, which is higher than the value of 0.64 mg/mL reported by Touwang (2018). Sorghum grains are widely available and cost-effective sources of various dietary and bioactive compounds, including proteins, minerals, dietary fibre, bioactive peptides, and polyphenols. These components contribute to the antioxidant and antidiabetic properties of sorghum (Rashwan A. K., 2021; Ofosu F. K., 2021).

Table 2. Physicochemical profile of *Djigari sorghum*

Features	Results
Mass of 1000 grains (g)	29,07 ± 1,35
Germination rate (%)	96,37
Germination capacity (%)	95,75
Total polyphenols (mg/mL)	0,79 ± 0,05
Water content (%)	9,88 ± 2,66

3.2. Characteristics of *Balanites aegyptiaca* fruit

3.2.1. Result of the choice of raw material

The selection of raw material was based on a detailed analysis of the morphological characteristics of desert date fruits, as presented in Table 3. The values reported for each parameter represent the mean of measurements performed on a sample of 30 fruits. As shown in Table 3, desert date fruits exhibit an ovoid shape, with an average length of approximately 2.5 cm and a diameter of about 1.4 cm. The fruits are characterised by yellowish to light brown coloration and an average weight of 6.12 g.

The differences between these characteristics and those reported by Jean Schunck De Goldfiem (2014) may be attributed to variations in climatic and geographical conditions affecting desert date palm growth.

Table 3. Morphological characteristics of desert date fruit

Characteristic	Average value
Fruit weight (g)	6.12
Weight of pulp(g)	2,93
Core weight(g)	3,58
Fruit length (cm)	2,50
Fruit diameter (cm)	1,40

3.2.2 Physicochemical characteristics of infused extract and macerated extract of *Balanites aegyptiaca*

pH is a key factor influencing the organoleptic properties of food products. The pH values of the infused and macerated extracts were 4.81 ± 0.01 and 4.95 ± 0.01, respectively. These values are comparable to those reported for tropical hop substitutes such as *Nauclea diderrichii* and *Vernonia amygdalina*, whose pH ranges from 4.15 to 6.9 (see references 3 and 11). The macerated extract exhibited higher acidity as well as higher total polyphenol and flavonoid contents than the infused extract. However, the total phenolic contents of both infused and macerated extracts were lower than the 2.65 g EAG/100 g reported by Lompo (1998). This difference may be attributed to variations in climatic conditions or to differences in the ratio of water to *Balanites aegyptiaca* mass used during extraction.

In contrast, the infused extract showed a higher vitamin C content than the macerated extract. Given the thermolabile nature of vitamin C and its sensitivity to heat and oxygen (Zangue et al., 2013; Solange, 2008), prolonged heating or cooking times should be avoided to preserve its content.

Table 4. Results of physico-chemical parameters of infused extract and macerated extract of Balanites aegyptiaca

	pH	Brix	ATT(g/L)	Vit C (mg/100g product)	Polyphenols (mgEAG/mL)	Flavonoids (mg EQ/mL)
Infused extract	4,95±0,01	18,96±0,01	0,360±0,029	23,414±0,057	0,158±0,004	0,0405±0,0017
Macerate extract	4,81±0,01	17,01±0,01	0,586±0,045	15,650±0,287	0,346±0.021	0,1309±0,0137

3.3 Characterization of Vitamin C content in beer

3.3.1 Changes in Vitamin C content before and after fermentation

Vitamin C is a compound known for its significant antioxidant properties. Its presence in wort is important not only for its free radical scavenging activity but also for its contribution to beer flavour and stability. As shown in Figure 2, vitamin C contents were determined in various worts and beers bittered with *Balanites aegyptiaca* extracts. Vitamin C concentrations in beers ranged from 2.44 ± 0.06 to 14.23 ± 0.57 mg/100 g, which were higher than those measured in the corresponding worts. This observation indicates an increase in vitamin C content during the fermentation process.

In addition to the vitamin C initially present in the worts, L-ascorbic acid can be biosynthesised from D-glucose by yeast cells during fermentation. This metabolic pathway is considered to contribute significantly to the increase in vitamin C content observed in the final beers (Paoala & Tiziana, 2020). The use of *Balanites aegyptiaca* extracts appears to further stimulate vitamin C biosynthesis, with an average threefold increase observed after wort fermentation.

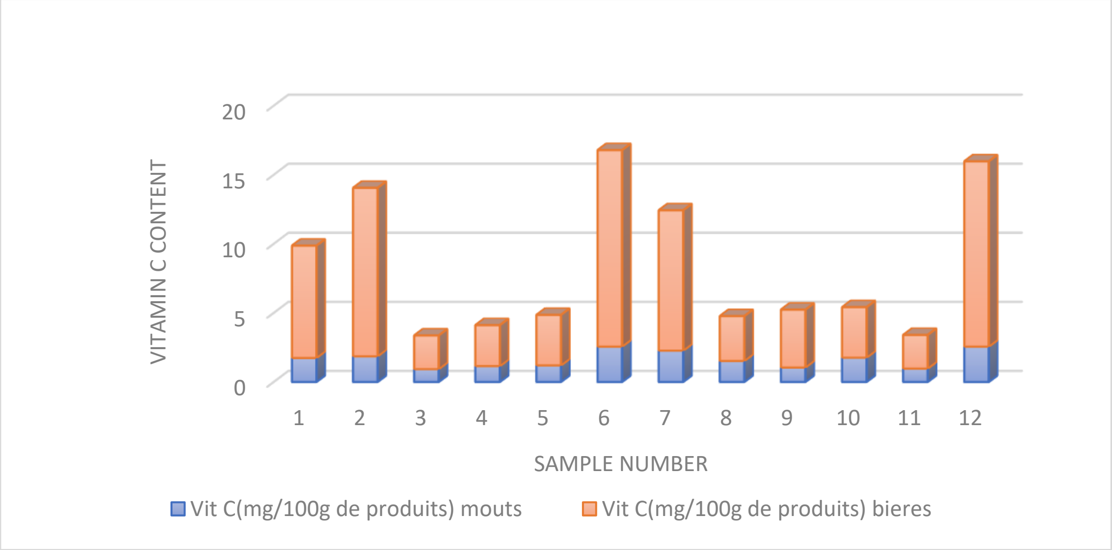


Figure 2. Vitamin C content in sorghum mashes and beers

3.3.2 Modelling vitamin C content in beer

The mathematical model used to monitor and predict vitamin C levels in beer as a function of formulation factors is expressed as follows: Vitamin C content=2.82A+13.49B−13.68AB

The model validation coefficients are presented in Table 5. The model is considered valid, as both the coefficient of determination (R^2) and the adjusted R^2 values exceed 0.8, indicating good predictive performance.

Table 5. Validation coefficient for the vitamin C monitoring model

R^2	0.9638
Adjusted R^2	0.9557
Predicted R^2	0.9370
Adeq Precision	22.0383

The p-values indicate the statistical significance of the model terms. Table 6 presents the model terms along with their corresponding p-values, showing that the overall model is statistically significant and that factors A, B, and their interaction (AB) significantly influence vitamin C content.

Table 6. Coefficient values for model factors

Source	p-value	
Model	< 0.0001	Significant
(1)Linear Mixture	< 0.0001	
AB	0.0006	
Residual		
Lack of Fit	0.1305	Not significant
Pure Error		
Cor Total		

3.3.2.1 Impact of the AB factor on vitamin C content

As illustrated in Figure 3, the effects of factors A and B, corresponding to the proportions of macerated extract and infused extract, respectively, on the vitamin C content of beer are shown. The negative coefficient of the AB interaction term in the model indicates that this interaction contributes to a reduction in vitamin C content. Analysis of the response surface reveals that vitamin C levels vary when the AB interaction is minimal and higher proportions of extract are added to the wort. This behavior can be attributed to the vitamin C content of the infused extract added at the end of the boiling process, as well as to the biosynthesis of vitamin C during fermentation.

The thermolabile nature of ascorbic acid and its sensitivity to heat and oxygen (Cruz, 2008; J. B. & Herbig, 2017) indicate that prolonged cooking times should be avoided to preserve vitamin C content. Historically, the industrial synthesis of L-ascorbic acid from D-glucose was developed by Tadeusz Reichstein in 1933 through the Reichstein–Grüssner process, which has been widely used to produce ascorbic acid supplements.

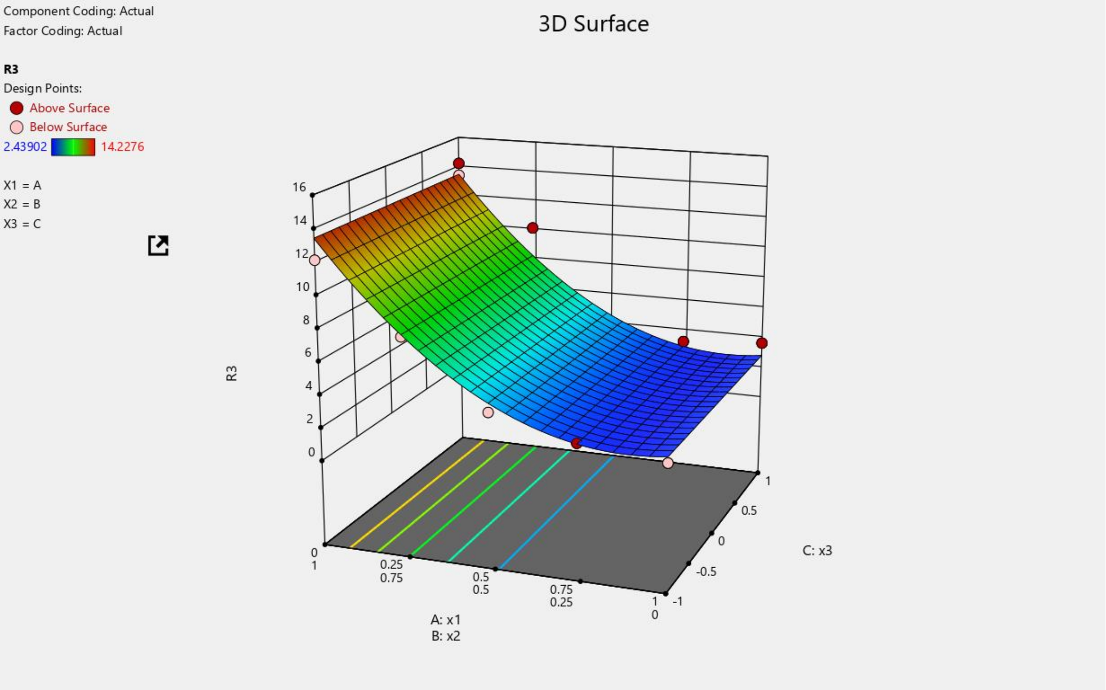


Figure 3. Influence of the AB interaction term on vitamin C concentration

2.4 Characterization of flavonoid content

2.4.1 Changes in flavonoid content in beer from wort fermentation

As illustrated in Figure 4, the effects of factors A and B, corresponding to the proportions of macerated extract and infused extract, respectively, on the vitamin C content of beer are shown. The negative coefficient of the AB interaction term in the model indicates that this interaction contributes to a reduction in vitamin C content. Analysis of the response surface reveals that vitamin C levels vary when the AB interaction is minimal and higher proportions of extract are added to the wort. This behavior can be attributed to the vitamin C content of the infused extract added at the end of the boiling process, as well as to the biosynthesis of vitamin C during fermentation.

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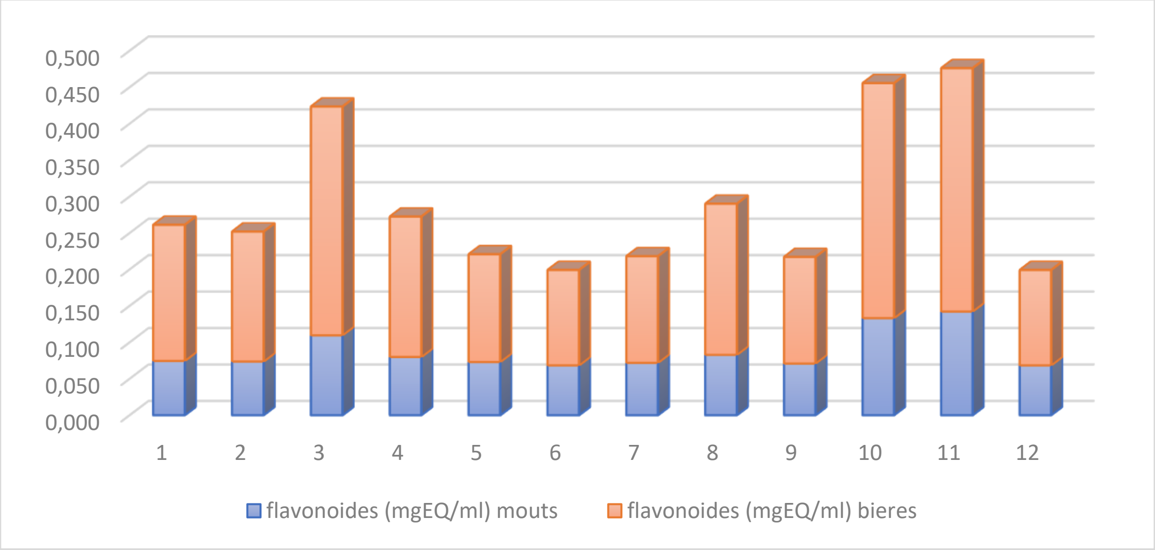

Figure 4. Flavonoid concentration in sorghum wort and beer bittered with *Balanites aegyptiaca*.

2.4.2 Flavonoid modelling

The mathematical model used to monitor and predict flavonoid levels in beer as a function of formulation factors is expressed as follows:

$$\text{Flavonoid concentration} = 0.3245A + 0.1478B - 0.3170AB - 0.2928AB(A-B)$$

The model validation coefficients are presented in Table 7. The model is considered valid, as the coefficient of determination (R^2) exceeds 0.8, indicating good predictive performance.

Table 7. Validation coefficient for the flavonoid tracking model.

R^2	0.9535
Adjusted R^2	0.9361
Predicted R^2	0.8910
Adeq Precision	15.8079

Table 8 shows the model terms followed by their p-values. It can therefore be seen that the model is significant, with term A and interaction AB the most significant terms in the model.

Table 8. Coefficient values for model factors

Source	p-value	
Model	< 0.0001	significant
(1)Linear Mixture	< 0.0001	
AB	0.0003	
AB(A-B)	0.0304	
Residual		
Lack of Fit	0.1870	not significant
Pure Error		
Cor Total		

2.4.2.1 Impact of AB on flavonoid concentration

As shown in Figure 5, the effect of the AB interaction on flavonoid content in beer is clearly observed. The negative coefficient of the AB term in the model indicates that this interaction contributes to a reduction in flavonoid concentration. Detailed analysis of the response surface shows that flavonoid levels vary when the AB interaction is minimal and higher proportions of infused extract (A) are added to the wort. Conversely, the absence of macerated extract leads to beers exhibiting optimal flavonoid content.

These flavonoid compounds are thought to contribute to the bitter taste and flavour of beer and are largely synthesised or released during the fermentation process. Flavonoids are known to degrade more rapidly under alkaline conditions and at elevated temperatures (Elise Emeraux, 2021; Ioannou, 2018), which supports their availability in sorghum wort and beer produced under controlled processing conditions. The fruit of *Balanites* *aegyptiaca* has been reported to contain several flavonoid compounds, including quercetin, isorhamnetin, isorhamnetin 3-*O*-glucoside, isorhamnetin 3-rutinoside, epicatechin *O*-glucoside, trigonelline, diosgenin, and yamogenin. The biological properties of plants are commonly attributed to their phenolic constituents, with flavonoids and tannins playing a particularly important role (Thomas Konan Kouamé, 2021; Hosakatte Niranjana, 2021).

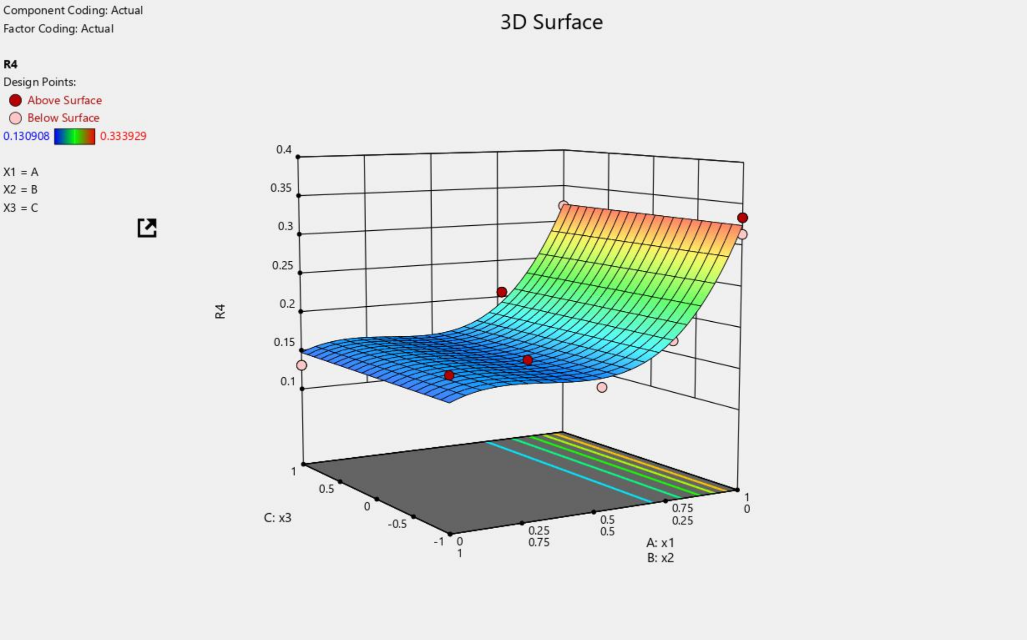

Figure 5. Influence of the AB interaction term on flavonoid concentration

2.5 Characterization of polyphenol content

2.5.1 Changes in polyphenol content in wort and in beer

As illustrated in Figure 6, the mean results of phenolic compound analysis in the formulated beers are presented. The values obtained ranged from 0.508 ± 0.001 to 0.723 ± 0.001 mg EAG/mL, which are comparable to the total polyphenol content reported for sorghum beer by Touwang, Nso, and Ndjouenikeu (2018), namely 0.64 ± 0.04 mg EAG/mL. Phenolic compounds have been shown to exhibit significant antibacterial activity, particularly against staphylococci (Laetitia, 2018). In beer, haze formation is mainly attributed to interactions between polyphenols, such as catechin, epicatechin, and their dimers, and proline-rich tannin-binding proteins (Rémi Bauduin, 2020).

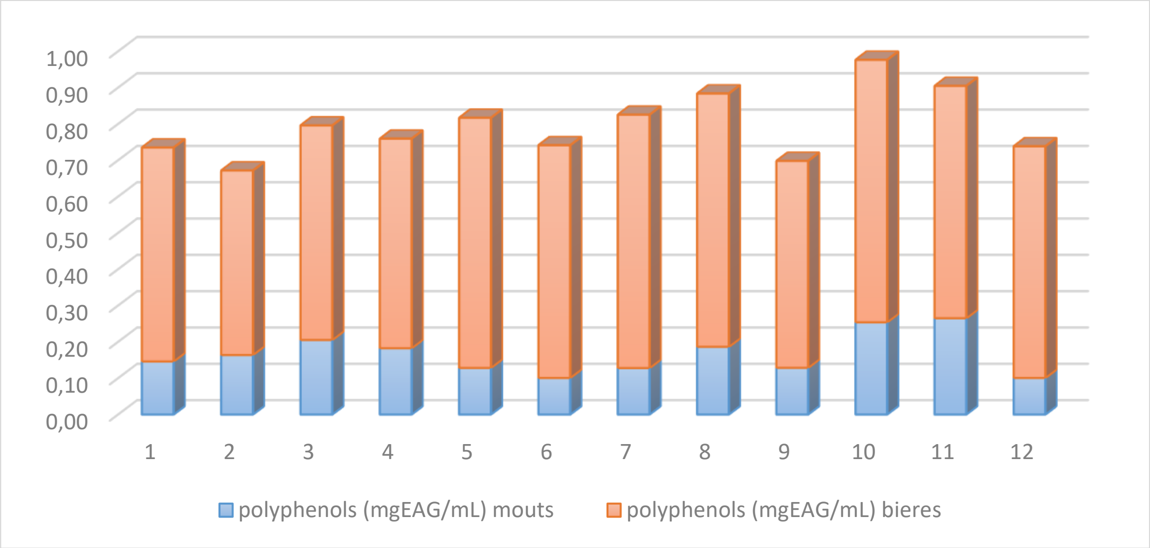

Figure 6. Polyphenol concentration in worts and beers made from bitter sorghum derived from *Balanites aegyptiaca*

2.5.2 Modelling polyphenols

The following equation represents the mathematical model used to monitor and predict polyphenol content in beer as a function of formulation factors:

Polyphenol concentration = $=0.6690A+0.5903B+0.0556AC+0.0593BC$

The model validation coefficients are presented in Table 9. The model is considered valid, as the coefficient of determination (R^2) exceeds 0.8, indicating satisfactory predictive performance.

Table 9. statistical values for polyphenol adjustments

R^2	0.9012
Adjusted R^2	0.8642
Predicted R^2	0.7238
Adeq Precision	14.0773

Table 10 presents the model terms along with their corresponding p-values. The results indicate that the model is statistically significant, with the AB interaction being the most significant term.

Table 10. Coefficient values of the model factors

Source	p-value	
Model	0.0002	significant
(1)Linear Mixture	0.0343	
AC	0.0014	
BC	0.0009	
Residual		
Lack of Fit	0.6051	not significant
Pure Error		
Cor Total		

2.5.2.1 Impact of AC and BC terms on polyphenol concentration

As illustrated in Figure 7, factors A, B, and C have a significant influence on the variation of polyphenol content in beer, particularly through the AC and BC interaction terms of the model. In this case, factors A and C evolve in the same direction, progressing from the blue region, which corresponds to the lowest polyphenol concentrations, toward the red region, which represents the highest polyphenol concentrations.

Phenolic acids are classified as non-flavonoid phenolic compounds and include both benzoic and cinnamic acid derivatives. The main phenolic acids identified in *Balanites aegyptiaca* include caffeic acid, ferulic acid, gentisic acid, *p*-coumaric acid, sinapic acid, syringic acid, and vanillic acid (Saker, 2000; Krusch, 2011; Hassan, 2017). These compounds help explain the combined effect of the infused extract and wort cooking time on polyphenol concentration in beer.

In contrast, the macerated extract shows a tendency toward negligible contribution under the studied conditions. Sorghum malt and infused extracts of *Balanites aegyptiaca* were found to contain sufficient levels of polyphenols to induce beer haze formation when long wort cooking times were applied.

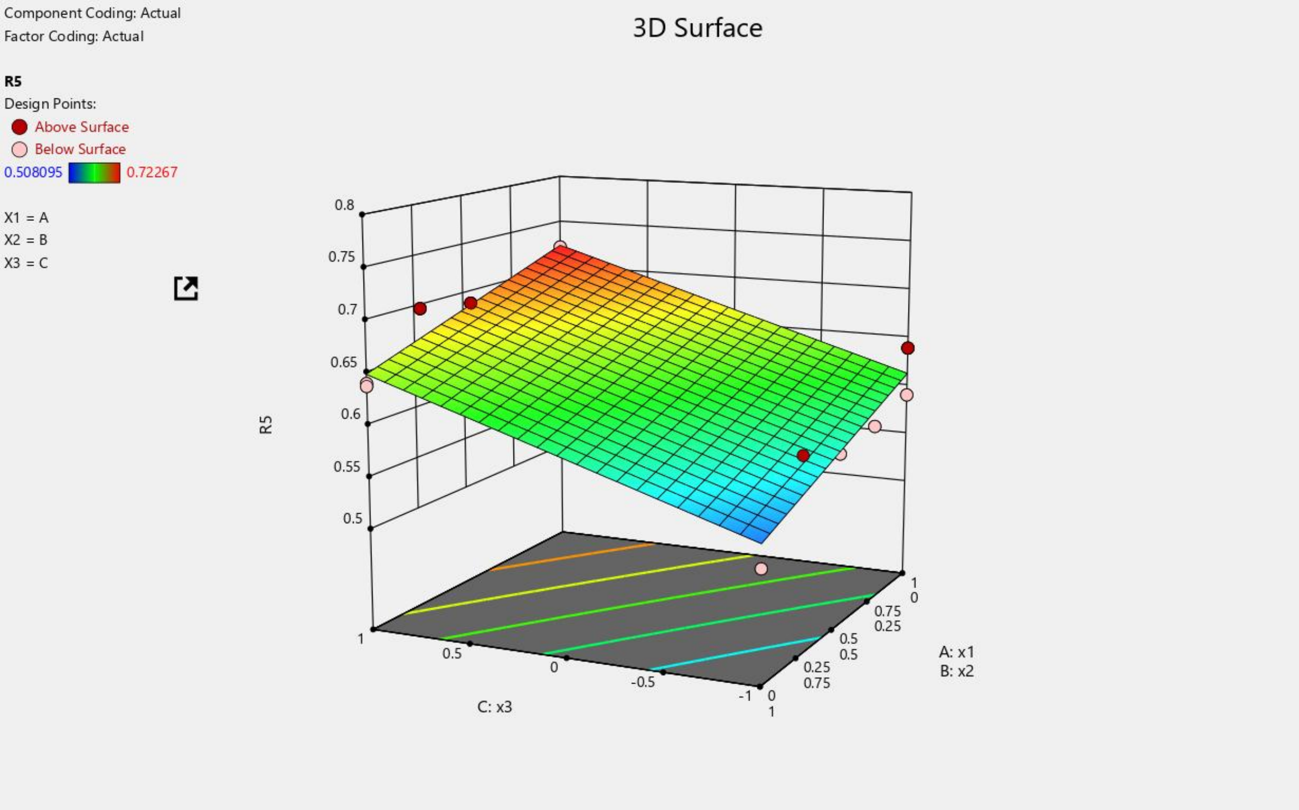

Figure 7. Impact of A and C on the variation in phenolic compounds.

4. Conclusion

The aim of the present study was to produce extracts from *Balanites aegyptiaca* for use as bittering agents in sorghum beer. The analysis of the physicochemical parameters of *Balanites aegyptiaca* fruits made it possible to identify the most effective extraction procedure and to determine an optimal formulation for imparting bitterness to the beer. The results obtained throughout the different stages of beer production demonstrate that sorghum beer can be effectively bittered using *Balanites aegyptiaca* extracts. Multi-response optimisation was performed based on the following criteria: minimisation of vitamin C content and maximisation of flavonoid and polyphenol contents. Using Design-Expert® software, an optimal formulation was identified, consisting of 100% infused extract, 36.1% macerated extract, and a wort cooking time of 51.8 min. Under these conditions, the predicted product characteristics were an acidity of 0.011 ± 0.001 g/L, a vitamin C content of 0.615 ± 0.012 mg/100 g, a flavonoid content of 0.260 ± 0.007 mg EQ/mL, and a polyphenol content of 0.701 ± 0.021 mg EAG/mL. Furthermore, the bitterness observed in *Balanites aegyptiaca* fruit pulp was confirmed to be partly associated with the presence of phenolic compounds, particularly polyphenols and flavonoids. Future studies should extend this work by incorporating sensory analyses, preferably using descriptive sensory evaluation methods.

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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