Optimization of an Edible Carnauba Wax Coating by means of a Screening Experimental Design of Experiments

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Abstract: This study aimed to identify and optimize the significant process parameters and formulation of a specific edible anionic Carnauba wax emulsion coating by means of design of experiments. A D-Optimal Combined screening experimental design was setup to identify the significant process parameter/s of a specific edible Carnauba wax coating. Three additional center points, two replicates and two additional points to estimate lack of fit were included in the design. The experimental runs were performed on a pilot plant that included a *6 liter* semi-batch reactor with a stirrer and high shear homogenizer. Formulation parameters were included in the screening experimental design to optimize the specific edible Carnauba wax coating formulation that consists of Carnauba wax, water and a surfactant (Potassium hydroxide, Ammonium hydroxide and Oleic acid in a fixed ratio). The parameters with fixed ranges determined by their capabilities and literature were; Carnauba wax [*10-15 wt%*], Water [*80-85 wt%*], Surfactant [*5-8 wt%*], Stirrer Speed [*450-1850 rpm*], High Shear Homogenizer (HSH) Speed [*3050-8050 rpm*], High Shear Time Interval [*0-55 min*], Cooling Rate [*-1,0,1*], Inverting Phase Addition Rate [*3-4 l/h*] and Temperature [*100-120 ^oC*]. Particle size and –distribution [*um*], Roughness [*Ra*], Gloss [*GU*], Dynamic Viscosity [*mPa.s*], pH and Density [*kg/m^3*]. These parameters were analyzed and recorded as responses. Due to the optimized formulation parameter not falling within the formulation ranges, a D-Optimal Mixture design was performed to optimize the formulation. The significant process factors with their optimized settings were identified as the Stirrer Speed [*800 rpm*], HSH Speed [*6800 rpm*], High Shear Time Interval [*10 min*] and Temperature [*120^oC*]. The optimized formulation was identified as; *75.3 wt%* Water, *15.8 wt%* Carnauba wax, *6.3 wt%* Oleic acid, *0.6 wt%* Potassium hydroxide and *2 wt%* Ammonium hydroxide.
By using the identified significant process parameters and optimized edible Carnauba wax coating formulation, a composite experimental design can be used to optimize the process parameters and achieve superior product characteristics. This can aid in the advancement of future applications of edible coatings in the food industry.

Keywords: Carnauba wax, post-harvest industry, screening experimental design, mixture experimental design, optimization

1 Introduction

The need to protect food products from physical, chemical and biological deterioration has always been a crucial objective for the food industry. Various techniques have been developed over the years to preserve the quality of food products, of which packaging is the ultimate one. When it comes to fresh fruit, synthetic packaging is not favoured since fruits, having living tissue, are considered active foods. The use of edible films and coatings have been suggested as an alternative packaging for these active foods, resulting in the development of new and/or modified edible coatings. However, improving the functionality and performance of these edible coatings has been one of the challenges of the post-harvest industry.

This development has been problematic due to the difference in requirements of certain fruits demanding the development of both natural and polyethylene coatings, in addition to having to comply with the ever changing United States (US) and European (EU) food regulations. One such edible coating used in the non-processed fruit industry is an anionic wax micro-emulsion consisting of a combination of wax, water, a fatty acid and a base. The base ionizes the fatty acid to form a soap, which stabilizes the wax droplets in the water to form an emulsion. The performance of any specific wax as a coating depends largely on the quality of the emulsion[1]. The characteristics of

emulsions are built into them during the manufacturing process and are not necessarily related to the properties and characteristics of the major ingredients. These characteristics include: appearance, viscosity, dispersibility, stability, wetting- and spreading capability and its particle size [2].

Hagenmaier and co-workers [1,3] studied three preparation methods for edible wax coatings for the fruit industry over a period of more than 10 years. Of the three preparation methods (the pressure method, semi-pressure method and the non-pressure method), the pressure method is most appropriate for Carnauba wax micro-emulsion formulations [3]. It is also a commonly used industrial procedure used for manufacturing micro-emulsions. Due to very limited literature available on edible Carnauba wax anionic micro-emulsion coatings, Hagenmaier [3] used a trial-and-error process to establish over 150 different formulations. That said there are no complete formulations, significant process parameters or manufacturing procedures for edible Carnauba wax coating published in the open literature.

Experiments on industrial scale is very expensive and therefore a scaled-down 6 litre reactor system (geometrically similar to an industrial plant) was designed and constructed that enabled a design of experiments approach. The aim of this study was to investigate a specific edible anionic Carnauba wax coating by performing experiments on pilot-plant scale. A *6 liter* semi-batch bench-scale reactor was used as part of a pilot plant to perform the experiments on (Figure 1). The significant process parameters were identified by means of a D-Optimal Combined screening experimental design. Statistical analyses were performed to optimize both the process- and formulation parameters. Due to the optimized formulation falling outside the ranges set during the screening experiment, the formulation was optimized by means of a D-Optmizal Mixture design. Various responses were measured and recorded for statistical analyses purposes. They include particle size and –distribution [*um*], gloss [*GU*] and roughness [*Ra*]. Because the study was done on a scaled version of an industrial plant it will be relatively easy to implement the findings of this study on the full-scale plant.

2 Materials and Methods

2.1 Materials

Carnauba wax is a vegetable wax produced by the leaves of the Brazilian palm tree. It has a pale yellow to light brown colour and consists mainly of long chained wax esters, -acids and –alcohols [4]. Carnauba wax has a melting point of *80-86^{o}C*. The Carnauba wax flakes that were used in this study were acquired from Croda© Chemicals South Africa (Pty).

Oleic acid ($C_{18}H_{34}O_{2}$) is a fatty acid that naturally occurs in various animal and vegetable fats and oils [5]. It is an odourless light yellow coloured oil. The Oleic acid used in this study was Priolene© 6940 acquired from Croda© Chemicals South Africa (Pty).

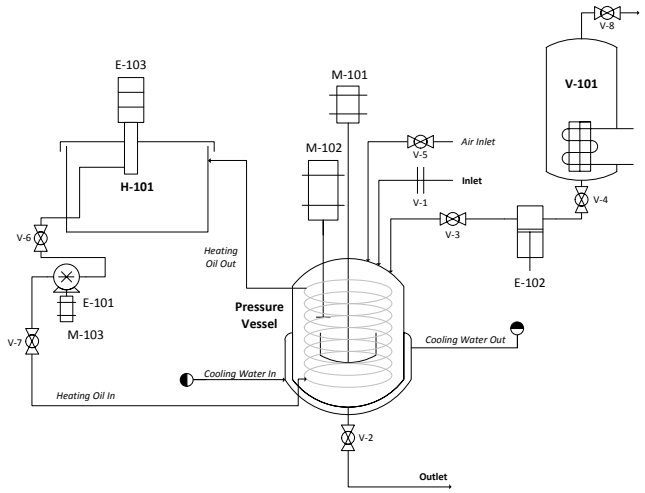

Figure 1: Process Flow Diagram showing the Pilot Plant used during this study

A 45% Potassium hydroxide solution was acquired from Protea Chemicals Cape (Pty). A 25% Ammonium hydroxide solution was also acquired form Protea Chemicals Cape (Pty).

2.2 Methods

2.2.1 Particle Size and -Distribution

The droplet size distributions of the Carnauba wax emulsions were determined by means of laser diffraction using a Saturn DigiSizer 5200 Particle Sizer (Micrometrics, UK) with a measuring range of *0.1-1000 µm*. Data from the laser diffraction and polarization intensity differential scattering (PIDS) were combined to calculate the particle size distribution with the Micrometrics© Saturn DigiSizer 5200 V1.10 software. Samples were diluted and measured in distilled water.

2.2.2 Roughness

The roughness (*Ra*) of the dried edible Carnauba wax layers was measured with a portable Time© Roughness tester TR110 (Time©) that was acquired from BAMR© South Africa. The TR110 has an accuracy of *±15%* and a repeatability better than *12%*. The TR110 was calibrated with the roughness test plate supplied with the instrument and set at an

evaluation length of *15 mm* (the longest length). An average of *10* readings was recorded for each dried coating.

2.2.3 Gloss

The gloss (*GU*) of the dried edible Carnauba wax layers was measured with a portable GT60 Gloss tester that was acquired from BAMR© South Africa. The GT60 gloss tester measures the gloss at an angle of 60^o It has an accuracy of *±1.5 GU* and a repeatability better than *±0.4 GU*. The GT60 was calibrated with the standard test plate supplied with the instrument. An average of *10* readings were recorded for each dried coating.

2.2.4 Dynamic Viscosity

The viscosity of the Carnauba wax emulsions manufactured throughout this study was measured with a MCR 501 Rheometer (Anton Paar©). It has a wide range of cone and plate tools available. Standard operating procedures were followed to perform viscosity measurements on the Carnauba wax emulsion samples.

2.2.5 pH

A digital pH-meter, which compensates for the effect of temperature, was used in accordance with an EC620131 (glass-body, open pore, for polymer gel applications) pH electrode (Eutech Instruments©) to measure the pH of the Carnauba wax emulsion samples. An average of three pH measurements was taken for each sample.

2.2.6 Density

The density of the Carnauba wax emulsion samples were measured with a calibrated density flask with a fixed volume of *24.873 ml*. A thermometer ensured that the readings were measured at a temperature of 20^oC.

2.3 Formulations

Due to there being both formulation- and process variables, a D-Optimal Mixture design was selected for the screening experiments to evaluate all the factors simultaneously. Guitterez et al. [6] (in their study on Nano-emulsions) evaluated the effects of both the composition variables and preparation variables by means of a mixture design. A fixed ratio of oleic acid:Ammonium hydroxide:Potassium hydroxide was assumed for the screening experimental design. This ratio was published by Hagenmaier [3] as *4.7:2.2:1*. The ranges of each component for the mixture part of the screening experimental design are presented in Table 1 (based on findings by Hagenmaier). To establish an optimal formulation a D-optimal Mixture design was set up which tested the ranges of the water, wax, oleic acid, ammonium hydroxide and potassium hydroxide to yield the optimal formulation. Three additional replicates, three additional points to estimate lack of fit and two additional centre points were included in the design. The candidate points that were selected include the vertices, axial check blends and an overall centroid. The ranges of each of the components were determined from literature and observations made during the experimental runs that were performed. The ranges are presented in Table 2.

Table 1 Formulation Ranges – Screening Experimental Design

Component	Minimum (wt%)	Maximum (wt%)
Water	80	85
Wax	10	15
Surfactant	5	8

Table 2 Formulation Ranges – Mixture Experimental Design

Component	Minimum (wt%)	Maximum (wt%)
Water	75	79.9
Wax	15	19.9
Oleic Acid	3	6.96
Ammonium Hydroxide	1.6	3
Potassium Hydroxide	0.5	1.3

2.4 Statistical analysis

Statistical analyses were performed using Design Expert® Version 7.1.5 (Stat-Ease, Inc. 2021 East Hennepin Ave., Suite 480, Minneapolis, MN 55413). A D-Optimal Combined design was selected for the screening experiments. A Point Exchange selection, with only the vertices and the overall centroid as design points, were selected. Three additional centre points, two replicates and two additional points to estimate lack of fit were included. To establish an optimal formulation a D-Optimal Mixture design was set up which tested the ranges of the water, wax, oleic acid, ammonium hydroxide and potassium hydroxide to yield the optimal formulation. Three additional replicates, three additional points to estimate lack of fit and two additional centre points were included in the design. The candidate points that were selected include the vertices, axial check blends and an overall centroid.

3 RESULTS

3.1 Screening Experimental Design: Particle Size-, Roughness- and Gloss Data

There is a significant difference between the particle sizes recorded by means of the volume of the particles (volume distribution), as it travels through the laser while it is analysed, and the particle size converted to the number- and area (surface area) distribution, by means of mathematical models. Volume distributions are very sensitive to the appearance of a few large particles due to their large volume. Conversely, number distributions are very sensitive to the appearance of fine particles (Saturn DigiSizer 5200 2000). There is also a significant difference between the median- and mean of a particle size data set. Median in statistics and probability theory is defined as the number separating the higher half of a data sample (population or probability distribution) from the lower half, thus it is the middle point of a number set (*D50*) (Stat-Ease 2010). The mean is defined as the sum of a collection of numbers (data sample, population or probability distribution) divided by the number of numbers in the collection (Stat-Ease 2010). With this in mind the particle size data was collected and analysed with Design Expert®.

The particle diameter sizes achieved with each screening experimental run for the volume-, area- and number distributions (showing both the median- and mean particle sizes) are presented in Figure 2. A secondary X-axis was included to present all three data sets (volume-, area- and number) more accurately. Initial analyses of the particle size and –distribution data indicated that there are a significant amount of outlier large particles in the samples. This is clearly visible in Figure 2 when comparing the mean and median calculated particle data sets.

Roughness- and gloss measurements were recorded for each dried sample obtained from the screening experiments. The roughness- and gloss results are presented in Figure 3. Both the roughness- and gloss data were sorted from high-to-low and low-to-high, respectively. In addition the volume mean particle size data was included to present a comprehensive representation of the data. The roughness- and gloss data was entered into the screening experimental design and analyzed with Design Expert®. Dynamic viscosity, density and pH measurements were recorded for confirmation purposes only. All samples obtained throughout this study fell within the ranges published in literature and material information sheets supplied by edible coatings manufacturers.

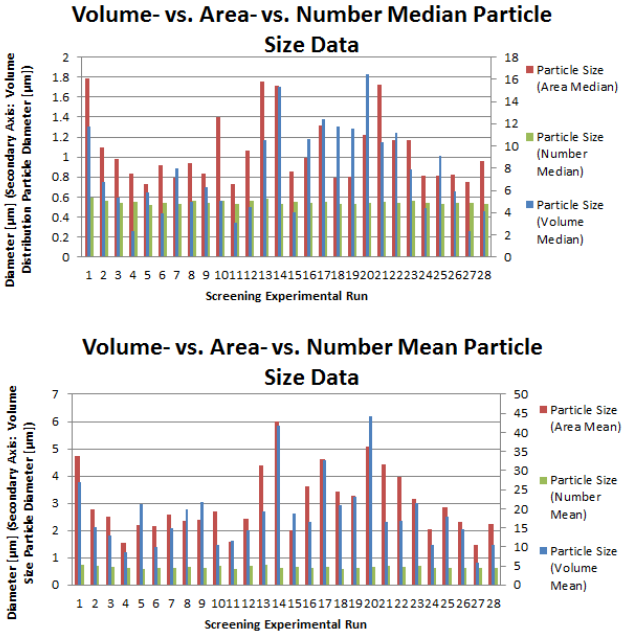

Figure 2 Particle Size Data (volume-, area- and number particle size data) for all Screening experiments

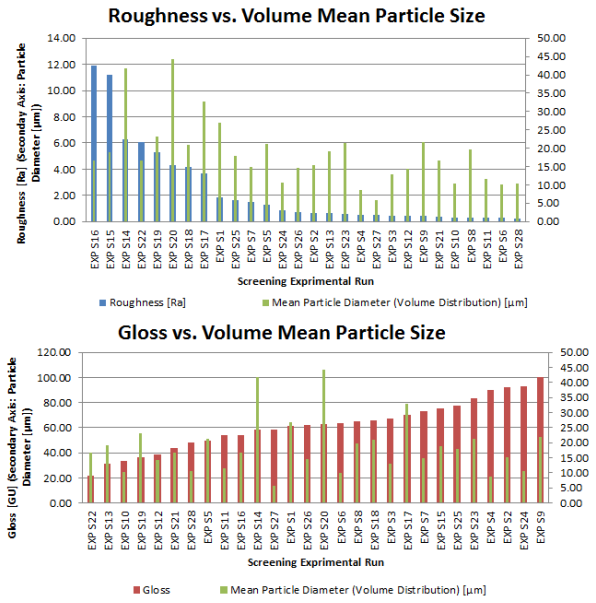

Figure 3 Roughness- and Gloss Data compared to the Volume Mean Particle Size Data

With the roughness model the significant process parameters were identified as the Temperature [^oC] and the Stirrer Speed [*rpm*]. From the results obtained during the particle size data analysis, it was concluded that the model derived from the volume mean particle size data set was the most appropriate model to use. This is supported by the fact that large particles are taken into account, thus it is a more accurate representation of the

design space. The trends obtained with the volume mean particle size model were all supported by literature. The significant process parameters of the particle size model were identified as the High Shear Time Interval [*min*] and the Cooling Rate [*-1, 0, 1*].Finally, the significant process parameters of the gloss model were identified as the HSH Speed [*rpm*] and the High Shear Time Interval [*min*].

3.2 Mixture Experimental Design: Particle Size-, Roughness- and Gloss Data

Once the data from the screening experiments were analysed with Design Expert©, it was concluded that the optimum formulation did not fall within the ranges of the components (%water, %wax and %surfactant).This was as a result of the oleic acid, ammonium hydroxide and potassium hydroxide being fixed at a certain ratio obtained from literature, which was not necessarily the optimal ratio for the specific experimental setup (bench scale pilot plant) that was used during this study. A D-Optimal mixture design was set up to determine the optimal formula. The process parameters were fixed at the optimum settings obtained during the screening experimental phase.

Once all the particle size data were entered into the mixture experimental design in Design Expert© it was found that the linear models were not statistically significant ($p>0.05$) for the volume-, area- and number particle size data sets. This applied to both the mean and median particle size data sets. As a result, reduced quadratic models were fitted to all six particle size data sets. The results indicated that the reduced quadratic model of the Area Median particle size data set was the only model that was statistically significant ($p<0.05$). In addition both the roughness and gloss data resulted in statistically insignificant models. The area median particle size model and actual particle size values, at a water content of *75 wt%*, are presented in Figure 4.

3.3 Optimization

The screening experimental design was optimized in according to three different response outputs. They are a target response output, a minimizing response output and a step response output. The screening experimental data (particle size, roughness and gloss data models) were optimized to minimize the particle size and roughness, while increasing the gloss (identified as favorable characteristics of edible wax coatings according to literature [6], [7], [8], [9], [10]. The final optimized process parameters settings are presented in Table 3.

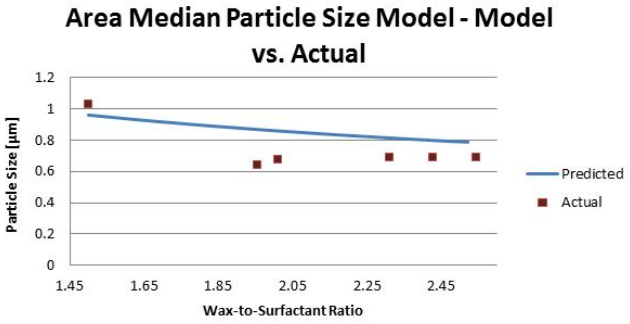

Figure 4 Area Median Particle Size Model compared to Actual Values obtained

Table 3 Optimized Process Parameter Settings

Process Parameter	Setting
Stirrer Speed [rpm]	800
HSH Speed [rpm]	6800
HS Time Interval [min]	10
Cooling Rate [-1/0/1]	1
Inverting Phase AR [l/h]	3
Temperature [°C]	120

Due to area median particle size being the only model that was statistically significant ($p<0.05$), the mixture design space was optimized by minimizing the particle size only. The same three response outputs used for the screening experimental design space were used to optimize the mixture experimental design space. The optimized formulation was identified as; *75.3 wt%* Water, *15.8 wt%* Carnauba wax, *6.3 wt%* Oleic acid, *0.6 wt%* Potassium hydroxide and *2 wt%* Ammonium hydroxide. A confirmation run was performed and the results are presented in Figure 5.

4 DISCUSSION

4.1 Screening Experimental Design: Particle Size-, Roughness and Gloss Data

Initial analyses of the particle size and –distribution data revealed that there are a significant amount of outlier large particles in the samples, as seen in Figure 2. As previously mentioned, the volume distributions are very sensitive to the appearance of a few large particles due to their large volume. Conversely, number distributions are very sensitive to the appearance of fine particles.

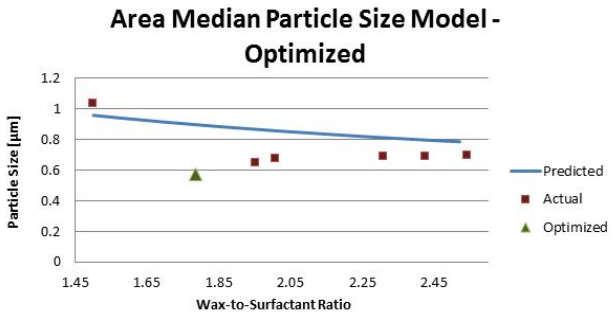

Figure 5 Optimized Formulation Particle Size Results

When comparing the mean- to the median-data sets in Figure 2, it is noted that the larger particles present in the samples are more significant with the mean- calculations than with the median-calculations. This confirms that the volume mean particle data set takes into account the larger particles and is a more accurate and true representation of the average particle size and –distribution of the emulsions obtained during the screening experiments. In addition, the trends obtained during the screening experimental phase were supported by literature. From the statistical modelling it was concluded that at a favourable low wax-to-surfactant ratio, the particle size decreases with an increase in the cooling rate. This trend is in agreement with Li et al. [11] who established in their study on the formation of paraffin wax emulsions, that increasing the emulsification temperature and cooling rate improves emulsion properties, i.e. it results in a smaller particle size [11]. In addition, the particle size decreases with a decrease in the high shear time interval. McClements [12] stated in his study on nano-emulsions that the particle size can be reduced by increasing the intensity or duration of homogenization. Adler-Nissen et al. [13] agreed with McClements conclusion in that there must be enough time given for a stable interface to form around the drops during the emulsification processes. The significant process parameters for the particle size model were identified as the High Shear Time Interval [*min*] and the Cooling Rate [*-1, 0, 1*].

The roughness- and gloss data were analyzed with Design Expert®. Both the roughness- and gloss data model is statistical significant ($p<0.05$). The roughness model indicated that the roughness decreases with an increase in the emulsification temperature. This is expected since an increase in the emulsification temperature results in a decrease in the particle size of the emulsion [11], [14]. It was also noted that at a favourable low wax-to-surfactant ratio, an increase in the stirring speed results in a decrease in the roughness. This finding is indirectly supported by McClements [12] who concluded that an increase in the intensity or duration of the energy input (stirring speed or high shear homogenizing speed) of an emulsification system results in a decrease in particle size, which indirectly will result in a decrease in the roughness. The significant process parameters of the roughness model were identified as the Temperature [^oC] and the Stirrer Speed [*rpm*]. The gloss model showed that at a low wax-to-surfactant ratio, the gloss increases with an increase in the high shear homogenizing speed. This is expected since an increase in the energy input (stirring speed or high shear homogenizing speed) results in a decrease in the particle size, which in return lowers the turbidity of the emulsion and will result in a clearer and more glossier (reflective) dried coating [4], [12], [15], [16]. In addition it was also noted that an increase in the high shear time interval decreases the gloss of the dried coatings. This could possibly be due to the wax being burnt as a result of being exposed to heating for a longer period of time. The burnt wax forms a more turbid emulsion and a less glossy dried coating. The significant process parameters of the gloss model were identified as the HSH Speed [*rpm*] and the High Shear Time Interval [*min*].

4.2 Mixture Experimental Design: Particle Size-, Roughness- and Gloss Data

The area median particle size model was the only statistically significant ($p>0.05$) model in the mixture experimental design. One of the trends that were identified was a clear increase in the particle size with a decrease in the oleic acid content. Thus a decrease in the surfactant content results in an increase in the particle size as supported by literature [4], [12],[16],[17]. When examining Figure 4 it is noted that the model is a fairly good representation of the design space. It should be kept in mind that the water content was kept at *75 wt%*. The area median particle size model was used for optimization purposes.

4.3 Optimization

The screening experimental design space was optimized according to three different response outputs. The final optimized process parameter settings are presented in ***Table 3***. A maximum desirability of *0.93* was achieved. All three response outputs yielded similar results with the exception being the cooling rate. The variation in the cooling rate could be due to it not being a significant process

factor. For this reason the cooling rate was not seen as a significant process parameter and was kept at *1* for the formulation experimental phase. The optimized process parameters were fixed on the values presented in **Table 3** for the mixture experiments.

The same three response outputs used for the optimization for the screening experimental design space were used for the mixture experimental design space. Due to area median particle size being the only model that was statistically significant ($p<0.05$), the mixture design space was optimized by minimizing the particle size only. The final optimized formulation, which was identified as *75.3 wt%* Water, *15.8 wt%* Carnauba wax, *6.3 wt%* Oleic acid, *0.6 wt%* Potassium hydroxide and *2 wt%* Ammonium hydroxide, was confirmed with an additional experiment. When examining Figure 5 it is clear that the confirmation run yielded the smallest overall particle size [*0.576 um*]. The roughness and gloss were measured and found to be *0.11 Ra* (*STD DEV = 0.021*) and *109.8 GU* (*STD DEV = 0.789*), respectively. Both the roughness and gloss values are overall optimums (the roughness a minimum and the gloss a maximum) for the screening- and mixture experimental phases. An overall optimum has thus been achieved.

5 CONCLUSIONS

The significant process parameters of a specific edible Carnauba wax coating were identified by performing screening experiments. Once the significant process parameters were identified, the screening experimental design space was optimized to yield a favorable final product (minimizing the particle size and roughness while maximizing the gloss). The significant process parameters with their optimized settings are; the Stirrer Speed [*800 rpm*], the High Shear Homogenizing Speed [*6800 rpm*], the High Shear Time Interval [*10 min*] and the Temperature [120^oC]. A D-Optimal Mixture design was performed to optimize the edible Carnauba wax coating formulation. The optimized formulation was identified as; *75.3 wt%* Water, *15.8 wt%* Carnauba wax, *6.3 wt%* Oleic acid, *0.6 wt%* Potassium hydroxide and *2 wt%* Ammonium hydroxide. A confirmation run confirmed the formulation by resulting in the smallest overall particle size achieved [*0.576 um*]. The roughness and gloss were also measured and found to be *0.11 Ra* (*STD DEV = 0.021*) and *109.8 GU* (*STD DEV = 0.789*), respectively (a minimum for the roughness and maximum for the gloss). A Composite experimental design can be used to optimize the process parameters by using the identified significant process parameters and optimized formulation. These findings can aid in the advancement of future applications of edible coatings in the food industry.

Acknowledgements

The National Research Foundation (NRF) of South Africa and Stellenbosch University are thanked for their financial support. The inputs of Dr JP Theron and Dr D De Wet-Roos are also acknowledged. Any opinion, findings and conclusions or recommendations expressed in this material are those of the author(s) and, therefore, the NRF and Stellenbosch University do not accept any liability in regard thereto.

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