Emulsion Quality Evaluation Using Automated Image Analysis

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***Abstract*— An emulsion represents a mixture of minute droplets of one liquid in another, which are normally non-mixable and non-blendable. The evaluation of emulsion quality is typically based on human assessment of microscopic images. This can lead to subjectivity in the interpretation as to whether the product meets requirements or not. An alternative approach is to automate the analysis of micrographs using image processing software that measures the emulsion quality based on the droplet characteristics.**

In this study, an automated image analysis of optical micrographs has been applied to detect the individual droplets and agglomerates and their characteristics in oil-in-water emulsions. A statistical examination of the characteristics obtained through image analysis is performed to evaluate the requirements of a desirable sample. The characteristics investigated in this study are droplet size and dispersion. Droplet size is measured in terms of area and Feret diameter and droplet dispersion is studied based on agglomeration.

A set of samples with desirable and undesirable properties were used to perform this investigative study. Absence of larger agglomerates of size greater than 150 µm^2, occasional presence of agglomerates (of size between 80 and 150 µm^2) and a droplet size distribution with more than 60% of the droplets having a size less than 3 µm^2 are identified as the desirable emulsion properties. The study proved that automated image analysis has the potential to assess the quality of emulsions in a more sophisticated way compared to human analysis. Therefore, the method can be recommended as a tool for in-situ process monitoring and real time quality control in emulsion preparation.

Keywords— emulsion; droplet characteristics; automated image analysis; quality control

I. Introduction

Emulsions are a mixture of two insoluble liquids such as oil and water which results in the formation of two different phases, a dispersed phase and a continuous phase. Meijer [1] first suggested emulsion quality control through human analysis of droplet size variation in emulsion samples using light microscopy as a better method compared to long term storage tests. The time consumption and subjective nature were identified as the two major downsides of human interpretation of droplet size [2, 3]. The usage of image processing software in the analysis of emulsion droplet size was found to be promising in overcoming the above stated limitations of human evaluation. Emulsion stability studies have been performed using light microscopy in conjunction with image processing and statistical analysis [4-6]. The previous studies have identified image processing of optical micrographs as an efficient and cost effective method for the analysis of emulsions in various aspects such as emulsion stability study, characterisation etc. Different image processing software such as ImageJ, Matlab, SASCHA software, Optimas etc have been used for the image processing of sample micrographs in order to achieve droplet size measurements in emulsions [4-7]. Droplet size distribution studies have been performed using image analysis techniques for the characterisation of double emulsions [8, 9]. Schuster [8] had suggested the analysis of maximum Feret diameter of the droplets (in addition to the area) in order to minimise the optical frame error. The maximum Feret diameter is the longest distance between any two points along the selected droplet boundary and it is applicable to non-circular shapes. Image processing has also been used to assess food quality based on colour, size, shape and texture and various image acquirement techniques, pre-processing procedures, segmentation and object measurement methods were explained [10, 11]. Scherze [9] has performed an automated image analysis of optical micrographs of multiple emulsions to control the yield of the inner continuous phase. The droplets of the external and internal phases of the multiple emulsion were detected from the micrographs with the help of a macro programmed by Scherze [9]. In the previous research of image processing in emulsions using ImageJ, the processing sequence applied are mostly based on noise filtering, thresholding, converting to binary, seperating the overlapped droplets by watershed segmentation or erosion and finally analysing the droplet characteristics [4-6]. Droplet detection in extremely concentrated emulsions using existing image processing techniques was identified as a challenge [12]. Boxall [13] found that the impact of human bias between two interpreters in the mean droplet size measurement has an average difference of 5.1% and their study concludes that an automated measurement through image processing shoud rectify this. The potential of automated image processing and analysis for inline droplet size monitoring in various polyphase systems was studied [7, 13]. The objective of the study by Maass [7] was to overcome the errors caused by manual droplet counting, physical sampling and usage of in-efficient image processing techniques. The drawbacks of external physical sampling have been mentioned in the literature [14, 15]. It has been found that even minute changes in the sampling time can result in substantial droplet size measurement errors. Maass [7] has implemented a two phase algorithm comprising of edge detection and circle evaluation using Hough transformation in Matlab for effective image processing. Previous research also shows that there is a future possibility of using automated image processing techniques in the investigation of agglomerates in emulsions [8]. Research has also shown that there is significant potential in using image processing tools combined with statistical analysis methods in the study of emulsions. However, there have been no studies done previously in evaluating the quality of oil-in-water emulsions through automated image processing and analysis of both droplet and agglomerate characteristics. This study is investigating the quality of oil in water (O/W) emulsions using automated image processing.

II. METHODOLOGY

A. Product Analysed

Micrographs of two samples of a lotion product (referred to as the emulsion), one with desirable properties (referred to as D) and the other with undesirable properties (referred to as UD) based on human assessment, were used for the analysis. The following steps were used for the sample evaluations.

- A study of droplets was conducted. The oil droplets of both the samples were detected and the droplet characteristics were retrieved through image processing and analysis. Statistical analysis of droplet size was performed to identify any differences in their distributions.
- A study of agglomerates (accumulation of oil droplets) was performed on both samples. Agglomerates were detected using a series of image processing steps and their count and size distribution were investigated statistically to facilitate a comparative study between the samples.

B. Software Used

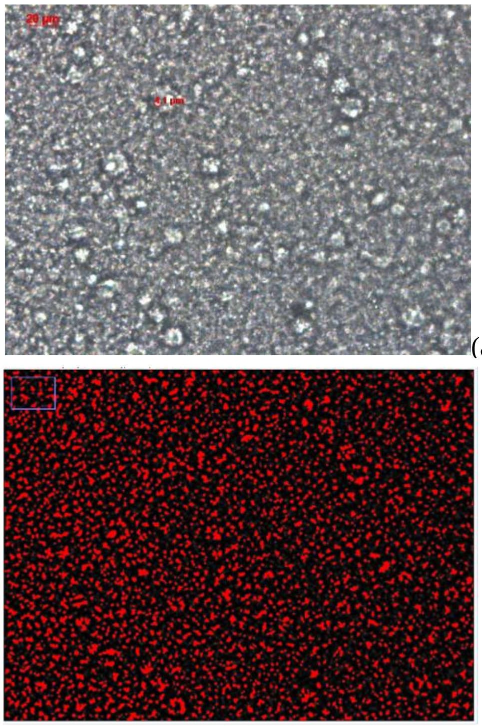
A scientific image processing software package called Fiji [16] was used for the processing of the micrographs to obtain the oil droplet characteristics and agglomerate features. Fiji is an open source distribution of ImageJ with several plugins built into an intelligible menu format [17, 18]. The measured droplet characteristics and agglomerate features were further statistically analysed using RStudio, Inc. version 0.99.903 [19], an integrated development environment for the programming language R (version 3.3.1) [20], used for statistical analysis and modelling.

C. Analysis of Droplet Characteristics

1) Image Processing: 400X magnification micrographs of samples comprising of desired (D) and undesired (UD) properties were used for the image processing and analysis of droplet characteristics. Around 2000 droplets were detected for each sample. The characteristics of the droplets were obtained through the following steps. A macro was programmed in Fiji to execute the steps dynamically in the given order.

- The micrographs were calibrated to the given scale (3.9 pixels/μm).
- Converted to 8-bit in order to enable further processing.
- Edge and Symmetry filter was applied to remove noise and to detect the droplets.
- The filtered image was thresholded though the red channel to highlight the droplets detected based on their pixel intensity.
- The image was then converted into binary and applied ‘watershed segmentation’ (separating droplets that touch/overlap each other).
- Using the ‘Analyse Particles’ option, droplet characteristics such as area, maximum Feret diameter, minimum Feret diameter, roundness (shape), Feret angle (orientation) and solidity were obtained for circular droplets. The circularity range specified was 0.90 to 1.00 (1.00 stands for a perfect circle). The given range of circularity filters out non-circular shapes.

An example of droplets detection from the original micrograph is shown in *Fig. 1*.



(a)

(b)

Fig. 1 (a) Example of original micrograph and (b) Corresponding image with droplets detected in red.

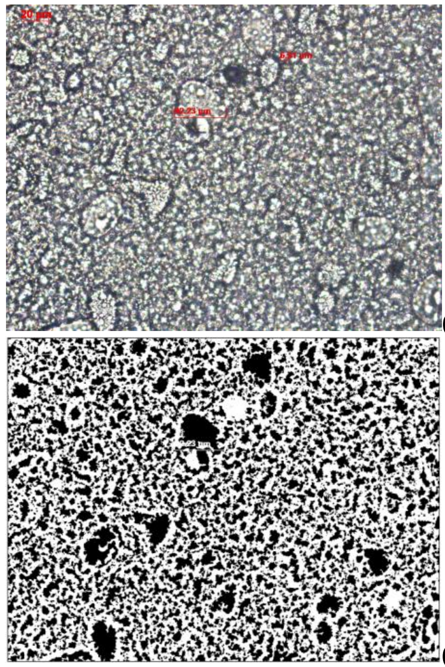
2) Statistical Analysis: A statistical investigation was performed on the droplet characteristics of the D and UD samples of the emulsion. The distribution of droplet size in terms of droplet area and Maximum Feret diameter were studied to distinguish between the samples. Normality of the droplet size data was also considered to identify the significance tests to be performed to find the difference between the samples and to evaluate the quality characteristics. The results of this analysis are shown in section III.

D. Analysis of Agglomerates

1) Image Processing: Agglomeration was visible to the human eye in the micrographs of the emulsion. Therefore, an automated image analysis of agglomerates was performed in Fiji. The presence of larger agglomerates of size > 80 μm^2 was analysed for both the samples comprising of desired (D) and undesired (UD) properties. The image processing procedure applied to detect the agglomerates in the samples and further, to retrieve their characteristics are explained in the following steps. A macro was developed in Fiji to execute the steps in the given order.

- Original micrographs were calibrated to 3.9 pixels/μm and converted into an 8-bit image in order to enable further processing.
- Applied thresholding to enhance the droplets based on their pixel intensity.
- Converted the image to binary with white background and droplets in black.
- Applied 'fill holes' process to convert the accumulated droplets into a single object (agglomerate).
- Applied 'watershed segmentation' to separate the identified agglomerates that touch or overlap.
- Obtained the characteristics of the agglomerates using the 'Analyse Particles' option.

An example of the agglomerates detection from the original micrograph is shown in *Fig. 2*.



(a)

(b)

Fig. 2 (a) Example of original micrograph and (b) Corresponding image with agglomerates shown in black.

2) Statistical Analysis: A statistical investigation of the agglomerate size was conducted and a comparative study was performed between the samples, D and UD. The size distribution of the agglomerates were analysed using box plots. The total count of the agglomerates present in the samples was also studied. The variation in the agglomerate size distribution and the dissimilarity in the count of the agglomerates of the samples were the two major factors considered to predict the quality requirements of the desired sample. The results of this analysis are shown in section III.

III. RESULTS AND DISCUSSION

The distinguishing features of the emulsion samples with desirable (D) and undesirable (UD) characteristics were evaluated based on the results obtained through the image processing and statistical analysis methods performed in this study. An analysis framework to determine the quality of a given sample was drawn from the methodology and the results obtained.

A. Droplet Characteristics

The droplet size distribution of the two samples, one with desirable properties (D) and the other with undesirable properties (UD), were studied in terms of area and maximum Feret diameter respectively. The mean and the median droplet size of the UD sample have slightly higher values when compared to the values of the D sample as shown in *Fig. 3* and *Fig. 4*. Other droplet characteristics, apart from area and Feret diameters, did not show any significant statistical difference between the samples.

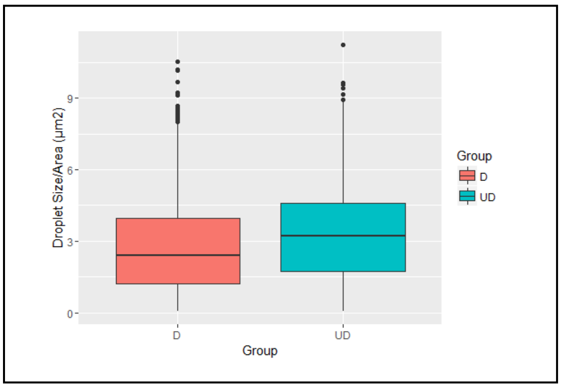


Fig. 3 Box plot of droplet area in µm^2 for the D sample (desirable) and the UD sample (undesirable).

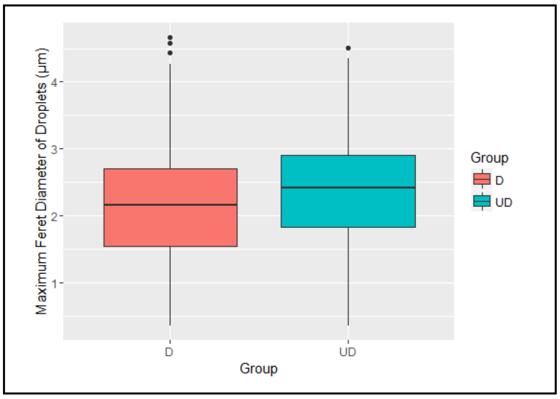


Fig. 4 Box plot of maximum Feret diameter in µm for the D sample (desirable) and the UD sample (undesirable).

The box plots in *Fig. 3* confirm that more than 60% of the droplets in the desirable sample, D have smaller size (< 3 µm^2) compared to the undesirable sample, UD. The results show that emulsions with a higher percentage of smaller droplets represent a desirable sample. Based on the analysis results of droplet size, the specifications for the average and median values of droplet area for a desirable sample was evaluated to be less than 3 µm^2.

The droplet size distribution in terms of area of the both the samples displayed non-normality. The statistical shift in the droplet size between the samples was investigated using a non-parametric significance test called Wilcoxon Rank Sum test/Wilcoxon test [21] and the results are shown in Table I.

The Wilcoxon test is a non-parametric equivalent of the independent t-test. The two samples are considered as two independent groups and the test assumes (null hypothesis) that there is no difference between the groups. The data is then ranked from the lowest to the highest (with the lowest value getting a rank of 1) irrespective of the group and the test statistic is calculated by finding the difference between the sum of the ranks from the mean rank. The results of Wilcoxon test gave a statistically significant p-value and resulted in a rejection of the null hypothesis. The effect size (a standardised measure) of the difference between the groups was calculated as 0.15, which is considered as a small effect [22]. The results of Wilcoxon test proves that the samples belong to two different populations.

TABLE I WILCOXON RANK SUM TEST

Wilcoxon rank sum test with continuity correction	
Data:	***Area by Group***
W = 1962600	p-value < 2.2e-16
Alternative hypothesis:	true location shift is not equal to 0

B. Agglomerate Characteristics

The size and the count of agglomerates were analysed for the samples and the results are presented in *Fig. 5* and *Fig. 6* respectively.

The size distribution of the agglomerates was studied using box plots. The mean, the median and the maximum value of the agglomerate size of the samples were calculated. The box plots presented in *Fig. 5* shows that more than 50% of the agglomerates in the D sample have size < 100 µm^2 and have no outliers. The maximum size of the agglomerates calculated for the D sample was 146.55 µm^2. This led to the conclusion that 100% of the agglomerates in the D sample (with desirable properties) have a size < 150 µm^2. The UD sample (with undesirable properties) shows a slightly higher median with agglomerates of size > 100 µm^2 in 50% of the sample and contains outliers of size ranging from 200 to 600 µm^2. The analysis of agglomerate count shows that the D sample has a lesser count of agglomerates of size greater than 80 µm^2 compared to the UD sample as shown in *Fig. 6*. The percentage of area occupied by the agglomerates in each of the sample micrographs were calculated. For the D sample (desirable), 1% of the total image area was occupied by the agglomerates while in the UD sample (undesirable), the area of the image occupied by the agglomerates was 5%.

It can be inferred from the analysis results that the presence of larger agglomerates in higher number represent undesirable emulsions and therefore, agglomerate analysis can be used as a tool to evaluate the quality of emulsions. The image analysis of agglomerates resulted in the development of the following characteristics for a desirable sample of the emulsion.

- Percentage of the total area of the micrograph occupied by the agglomerates (size > 80 μm²) should be ≤ 1%.
- No agglomerates of size > 150 μm² should be present.

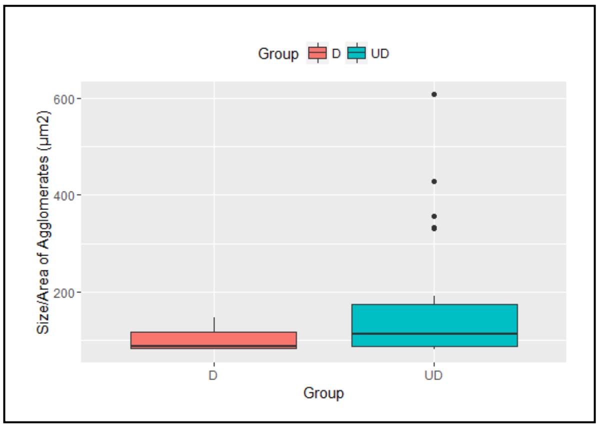


Fig. 5 Box plot of the agglomerate size distribution of the D sample (desirable) and the UD sample (undesirable).

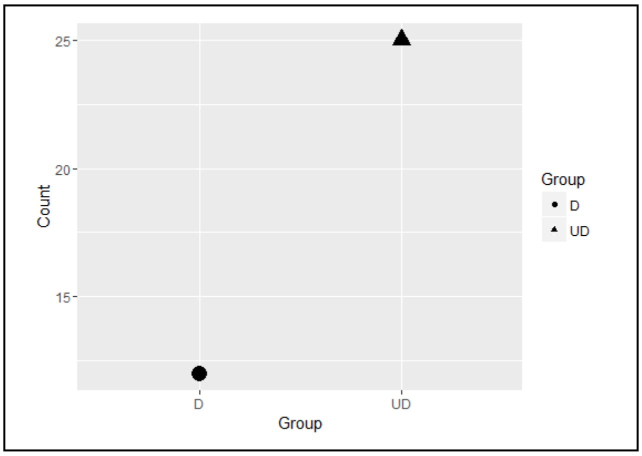


Fig. 6 Count of agglomerates in the D sample (desirable) and the UD sample (undesirable).

The results of the overall analysis of the lotion product in this study show that the following requirements should be met by the emulsion of desirable quality:

- A narrow droplet size distribution with more than 60% of the droplets having a size < 3 μm².
- Agglomerates of size > 80 μm² should not occupy more than 1% of the total area of the micrograph.
- No agglomerates of size > 150 μm².

The analysis results led to the design of a procedural framework to evaluate the emulsion quality, which is shown as a flowchart in *Fig. 7*. The automated image analysis procedure shown in *Fig. 7* can be implemented as a real time monitoring tool in emulsion processing. Based on the instantaneous feedback given by the analysis about the product quality, the processing time can be optimised and over processing can be avoided.

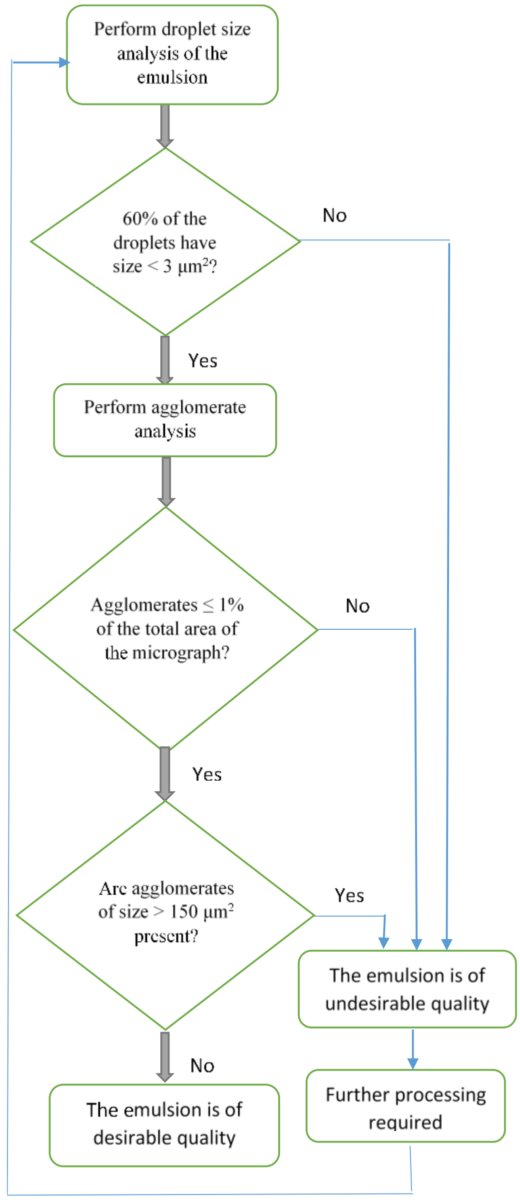


Fig. 7 Flowchart of the procedural framework developed.

The automated image analysis methodology applied in this study is performed after manual sample extraction. However, the procedural flow chart (Fig. 7) derived based on the results of the applied methodology can completely substitute humans in real time emulsion quality evaluation. Future work is planned to achieve this by integrating an endoscope attached digital camera coupled with suitable light sources and a laptop with the image processing software [7, 23]. The bias in microscopical analysis performed by different participants can be entirely rectified through automated image analysis [13].

The speed and the accuracy of the quality analysis can also be improved through automation.

Unlike previous studies, the current work analyses droplet size based on area and Feret diameter along with the study of agglomerates and other droplet characteristics, which demonstrates significant potential in emulsion quality assessment [7, 8]. The image processing algorithm used in previous research to substitute human analysis is entirely based on circular droplet detection [7]. The droplet detection technique applied in the current study is based on edge and symmetry detection, which is more robust in identifying droplets of any particular shape.

IV. Conclusion

This study has investigated the competence of automated image processing and analysis techniques, applied on optical micrographs of O/W emulsions, in the assessment of emulsion quality. The image processing technique applied in this study together with the statistical analysis methods present significant potential in determining the quality of emulsion samples. In the current study of the lotion product, the major distinguishing features between the analysed samples are identified as the frequent presence of larger agglomerates, the occurrence of agglomerate outliers and the droplet size distribution parameters. The emulsion sample with desirable properties is found to have no agglomerates of size > 150 μm^2 and has a narrow droplet size distribution with more than 60% of the droplets having a size < 3 μm^2. Hence for future work, automated image analysis of droplet size combined with the analysis of agglomerate size and count can be integrated as a tool for in-situ process monitoring to provide real time feedback on product quality. Other droplet characteristics such as shape, orientation and solidity can be studied as further distinguishing features of quality.

References

[1] N. Meijer, H. Abbes, and W. G. Hansen, "Particle size distribution and dispersion of oil-in-water emulsions: An application of light microscopy," *American Laboratory*, vol. 33, no. 8, pp. 28-+, Apr 2001.

[2] B. Junker, "Measurement of bubble and pellet size distributions: past and current image analysis technology," (in English), *Bioprocess and Biosystems Engineering*, Review vol. 29, no. 3, pp. 185-206, Aug 2006.

[3] J. E. Gwyn, E. J. Crosby, and W. R. Marshall, "BIAS IN PARTICLE-SIZE ANALYSES BY COUNT METHOD," *Industrial & Engineering Chemistry Fundamentals*, vol. 4, no. 2, pp. 204-&, 1965.

[4] A. Hosseini, S. Jafari, H. Mirzaei, A. Asghari, and S. Akhavan, "Application of image processing to assess emulsion stability and emulsification properties of Arabic gum," (in English), *Carbohydrate Polymers*, Article vol. 126, pp. 1-8, AUG 1 2015 2015.

[5] K. A. Silva, M. H. Rocha-Leão, and M. A. Z. Coelho, "Evaluation of aging mechanisms of olive oil–lemon juice emulsion through digital image analysis," *Journal of Food Engineering*, vol. 97, no. 3, pp. 335-340, 4// 2010.

[6] M. G. Freire, A. M. A. Dias, M. A. Z. Coelho, J. A. P. Coutinho, and I. M. Marrucho, "Aging mechanisms of perfluorocarbon emulsions using image analysis," *Journal of Colloid and Interface Science*, vol. 286, no. 1, pp. 224-232, Jun 2005.

[7] S. Maass, J. Rojahn, R. Haensch, and M. Kraume, "Automated drop detection using image analysis for online particle size monitoring in multiphase systems," (in English), *Computers & Chemical Engineering*, Article vol. 45, pp. 27-37, Oct 2012.

[8] S. Schuster *et al.*, "Analysis of W1/O/W2 double emulsions with CLSM: Statistical image processing for droplet size distribution," *Chemical Engineering Science*, vol. 81, pp. 84-90, 10/22/ 2012.

[9] I. Scherze, R. Knofel, and G. Muschiolik, "Automated image analysis as a control tool for multiple emulsions," *Food Hydrocolloids*, vol. 19, no. 3, pp. 617-624, May 2005.

[10] C. J. Du and D. W. Sun, "Recent developments in the applications of image processing techniques for food quality evaluation," *Trends in Food Science & Technology*, vol. 15, no. 5, pp. 230-249, May 2004.

[11] C. X. Zheng, D. W. Sun, and L. Y. Zheng, "Recent developments and applications of image features for food quality evaluation and inspection - a review," *Trends in Food Science & Technology*, vol. 17, no. 12, pp. 642-655, 2006.

[12] L. M. R. Brás, E. F. Gomes, M. M. M. Ribeiro, and M. M. L. Guimarães, "Drop distribution determination in a liquid-liquid dispersion by image processing " *International Journal of Chemical Engineering*, 2009, Art. no. 746439.

[13] J. A. Boxall, C. A. Koh, E. D. Sloan, A. K. Sum, and D. T. Wu, "Measurement and Calibration of Droplet Size Distributions in Water-in-Oil Emulsions by Particle Video Microscope and a Focused Beam Reflectance Method," (in English), *Industrial & Engineering Chemistry Research*, Article vol. 49, no. 3, pp. 1412-1418, Feb 2010.

[14] N. Niknafs, F. Spyropoulos, and I. T. Norton, "Development of a new reflectance technique to investigate the mechanism of emulsification," (in English), *Journal of Food Engineering*, Article vol. 104, no. 4, pp. 603-611, Jun 2011.

[15] C. Martinez-Bazan, J. L. Montanes, and J. C. Lasheras, "On the breakup of an air bubble injected into a fully developed turbulent flow. Part 1. Breakup frequency," (in English), *Journal of Fluid Mechanics*, Article vol. 401, pp. 157-182, Dec 1999.

[16] (22 February 2017). *https://fiji.sc/*.

[17] J. Schindelin, "Fiji Is Just ImageJ (batteries included)," in *ImageJ User and Developer Conference* 2008.

[18] J. Schindelin, C. T. Rueden, M. C. Hiner, and K. W. Eliceiri, "The ImageJ ecosystem: An open platform for biomedical image analysis," *Molecular Reproduction and Development,* vol. 82, no. 7-8, pp. 518-529, 2015.

[19] (22 February 2017). *https://www.rstudio.com/*.

[20] (22 February 2017). *https://www.r-project.org/*.

[21] F. Wilcoxon, "Individual Comparisons by Ranking Methods," *Biometrics Bulletin,* vol. 1, no. 6, pp. 80 - 83, 1945.

[22] A. Field, J. Miles, and Z. Field, *Discovering Statistics Using R*. SAGE Publications Ltd, 2012.

[23] A. Khalil, F. Puel, Y. Chevalier, J. M. Galvan, A. Rivoire, and J. P. Klein, "Study of droplet size distribution during an emulsification process using in situ video probe coupled with an automatic image analysis," Chemical Engineering Journal, vol. 165, no. 3, pp. 946-957, Dec 2010.