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Research Article

Study on deterioration of milk-food texture caused by heat treatment using flow cytometry and microscopy

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Abstract

Particle size and its distribution is one of the major textural attributes of liquid and semi-solid food systems. The objective of this study was, therefore, to investigate the transformation in the particles' properties of different constituents of food system as a function of varied degree of heat treatment using Flow Cytometry and Microscopy (FlowCAM) technique. Three different temperatures (22, 60, and 91°C) for 4 different holding times (30, 60, 90, and 120 s) were chosen to determine the changes in particle size and its volume distribution in three food systems (2% milk, 36% whipped cream, and cream based sauce). The particles of milk were found to shift to bigger size distribution with severity of treatment and agglomeration was clearly observed in images so obtained. Similar results were obtained with both cream and cream based sauce. However, experimental data of cream sauce indicated that the particles were more stabilized due to the use of emulsifiers and higher viscosity. The experiment with viscous sauce (viscosity: 400 mPa.s) as compared to milk (1.8 mPa.s) and whipped cream (8.7 mPa.s) also revealed that abundance of particles protected the disruption of particles which helped in preserving their native properties. We found that increasing viscosity and heating to minimum required temperature can preserve the native textural properties of fat particles.

Flow Cytometry and Microscopy (FlowCAM) has been exclusively used for oceanographic research. However, it can be considered an emerging technique in food applications giving both particle size, volume distribution along with images and count. Use of FlowCAM helps to predict and thereby control the changes in the particle properties (especially for fat particles) for food systems such as milk, ice cream, cheese, and cream based sauces. It can also be used to examine other food system particulates like protein and starch particles so as to control the texture and mouth-feel of food products.

Keywords: FlowCAM, cream, particle, texture, mouth-feel, colloid, sensory, India.

Introduction

Textural properties of food are important aspect in mouth-feel, especially for semisolid or fluid food. The particle size of dairy and other food emulsions play an important role in defining both structural and sensory characteristics. Consumer acceptance of dairy based foods is highly based on their mouthfeel and its uniformity. Therefore, dairy and food industries spend a substantial part of their revenue on maintaining the texture of ice cream, cream, cream based sauces, and so on. Noticeable textural difference can be observed in cream if the particle size changes from 0.1-2 μm to >3 μm. The smaller the size of the particles the more creamier would be the product i.e. cream with 0.1-2.0 μm particle size would be lot creamier than cream with 3 μm or larger particles [1]. Degree of smoothness depends on the sizes of particles suspended in dispersed phase of the product. For instance, in ice cream these particles would be cream or fat particles, ice crystals and casein micelles suspended in water dispersion. However, the particles may vary in their characteristics. Suitable image analysis can help in characterizing these particles in such food systems.

Particles possess broadly two types of physical properties leading to texture attributes important with respect to mouth-feel. One is the property of material such as its elemental structure or molecular composition and second is its geometrical properties such as its size, shape and surface structure. To characterize a particle, some or all of these properties can be explored [2]. Common technologies used in particle analysis or characterization are various types of mass-spectrophotometry, X-ray crystallography, electron diffraction, laser diffraction, infrared microspectrophotometry, etc. The goal of these technologies is not only to count the number of particles but also calculate their average size, distribution and shape. However, particles are three-dimensional objects for which at least three parameters (length, breadth and height) are required in order to provide a complete description. Most sizing techniques, therefore, assume that the material being measured is spherical and report the particle size as the diameter of the "equivalent sphere" which would give the same response as the particle being measured.

With the advent of automated imaging systems, it is now possible to obtain statistically significant size distributions using image analysis. This opens up a range of applications which cannot be developed using more established techniques such as laser diffraction. In laser diffraction, particle size distributions are calculated by comparing a sample's scattering pattern with an appropriate optical model using a mathematical inversion process [3]. The particle sizing and characterization have been undertaken in dairy and food Industries using laser diffraction technique which provides particle size and volume distribution. However, Flow Cytometry and Microscopy (FlowCAM) is an emerging technique which is a combination of flow cytometry and miscroscopy giving both particle size, volume distribution, along with images and count. This technique was originally designed for oceanographic analysis but due to its versatility and user friendly functions, it has now been used in dairy and food industries to study particle properties of food systems. It is well known that food properties can be easily affected by thermal, chemical, physiological and biological factors. However, it would be interesting to observe how these factors affect individual particles within the food matrix. Bazmi and Relkin [4] recently found that the pasteurization holding time and aging time affected the structural and functional properties of whipped cream. They reported that increasing aging time reduced changes in the dynamic rheological and structural properties.

The hypothesis for this study was that particle size and its distribution would change by applying heat treatment and would affect overall quality of the food. To test this hypothesis, the objective of this investigation was to apply different degrees of heat treatment to milk, cream and cream based sauce (already pasteurized) for 4 different holding times (30, 60, 90, and 120 s) well within processing tolerance and to analyze their particle properties before and after heat treatment using FlowCAM technique.

Materials and Methods

Starting materials

The material used for the study was milk (2% fat), heavy whipped cream (36% fat) and Alfredo sauce. The composition of cream based sauce was comprised of 77% cream (11% fat); 2.3% anhydrous milk fat; 8.9% Parmesan cheese; 3.2% Romano cheese; 2.1% modified corn starch; 1.2% salt; 0.1% black pepper; and 0.3% emulsifier. All these materials were procured from the local grocery store. The samples were diluted in 1:100 proportion or until the suspension looked almost transparent. To calibrate and standardize the technique, it was recommended to run some known samples such as baker's yeast (20-50 μm particle size).

FlowCAM imaging

The procedure for operating FlowCAM (Fluid Imaging Technologies, Yarmouth, ME) was adopted fully from the user manual and as followed by Buskey and Hyatt [5]. The signal switch was turned on which automatically turned the computer on. Then the funnel holder was connected and the funnel was placed on to it followed by assembling the tubing from the funnel to the peristaltic pump. It is made sure that the pump and tubing are tightly connected and pump rollers are at right place. Then the funnel was filled with distilled water for calibration. The pump was then run with *Forward* and *Prime* setting until the tube showed no air-bubbles and water flowed thoroughly from funnel to the waste collector. The setup was configured as suggested by the user manual. The images were observed with water which looked empty since water had no particles. The samples were prepared as described earlier and run through the machine. Data were collected with the help of assembled micro-processor and saved. After all the experiments, the sample flow was cleaned from the system and the machine was closed as per the instructions in user manual.

FlowCAM is an instrument for continuous analysis and digital imaging of microscopic particles in a fluid stream. The Portable FlowCAM as shown in Figure 1 [12], is mounted in a rugged, water-resistant housing for safe transportation, rapid set-up and ease of use. It can operate on either 120/240 volt or 12 volt battery power. Ideal for water or industrial process monitoring, the FlowCAM detects, counts, measures and provides shape analysis of cells or particles in a fluid stream or a real-time basis. VisualSpreadsheet enables FlowCAM users to visually post-process particle data. The proprietary processing system of the FlowCAM captures a digital image of each cell or particle and presents the data in an easy-to-read VisualSpreadsheet or through our patented Interactive Scattergram feature. VisualSpreadsheet is as simple as using standard office software. It then automatically counts, images, and analyzes cells or particles from a discrete sample or continuous flow, providing significantly increased data collection. It eliminates slide preparation and provides a complete picture of your fluid sample in a fraction of the time needed for traditional microscopy.

Figure 1. **Portable fluid imaging FlowCAM technique.**

Heat treatment

Milk, cream and cream based sauce were heated by convection method (water bath) at 60°C and 91°C and analyzed at 4 different holding times, i.e. 30, 60, 90, and 120 s, with slow stirring to avoid charring. Convectional heating was used to provide a common heat treatment and uniform temperature distribution. A number of trials were carried out to obtain most representative trend in each case. The temperature range selected was based on the heat treatment range used in processing plants for making dairy-based sauces.

Data analysis

All data collected were analyzed for mean size of particles, minimum, maximum, standard deviation and coefficient of variance (CV%). Significance level of mean particle size among samples was tested using Fisher's Least Significance Difference (LSD) feature of SAS software at 95% level (SAS Institute Inc., Cary, NC). Particles were also imaged at every 5 seconds and the most representative image has been included in this paper for each sample at different temperature.

Results and Discussion

The experiment investigated the effect of severity of heat treatment on particle size in various samples. FlowCAM technique was very helpful in delivering results. However, the particle size below 1 µm were not detected successfully due to its detection limitations. There was no difference observed in particle size distribution at 4 different points (30, 60, 90, and 120 s) of analysis with respect to holding time in water bath (data not shown). Hence, only data obtained at 60 s are included here in this paper. The study showed that the mean particle size in 2% milk which was previously pasteurized and homogenized significantly increased from 1.35 µm to 4.36 µm on heating at 60°C but the size of particles remained unchanged on further heating up to 91°C as summarized in Table 1.

Table 1. **Particle properties of milk, cream and cream sauce at three different temperatures at 60s of holding time.**

Item	Mean diameter (μm)	Std. Dev.	%CV
2% Milk (22°C)	1.35[a]	0.23	63.17
2% Milk (60°C)	4.36[b]	1.45	61.86
2% Milk (91°C)	5.44[c]	1.67	65.81
Cream (22°C)	21.27[d]	4.32	36.72
Cream (60°C)	24.76[d]	3.78	40.35
Cream (91°C)	35.12[e]	5.41	37.68
Cream sauce (22°C)	27.89[de]	5.67	31.44
Cream sauce (60°C)	35.54[e]	6.31	33.45
Cream sauce (91°C)	41.23[e]	7.12	35.51

[a-e]Superscripts with common letter within same column are not significantly different ($\alpha = 0.05$)

The particle size distribution also followed almost normal distribution and ranged from 1 μm to 5 μm (Figure 2) at 22°C. There was clear shift in particle size distribution of milk when heated to 60°C and 91°C (Figure 3 & 4, resp.). The particle images captured by inbuilt camera in FlowCAM as illustrated in Figure 5 showed that upon heating particle size might show increment in size due to the agglomeration of particles smaller than 1 μm. However, the particles could still be seen as individually intacted. In 2% milk, protein is the predominant constituent in the milk system. Increment in particle size on heating can be explained as the heat induced protein denaturation and subsequently combining with other mineral constituents of the food system.

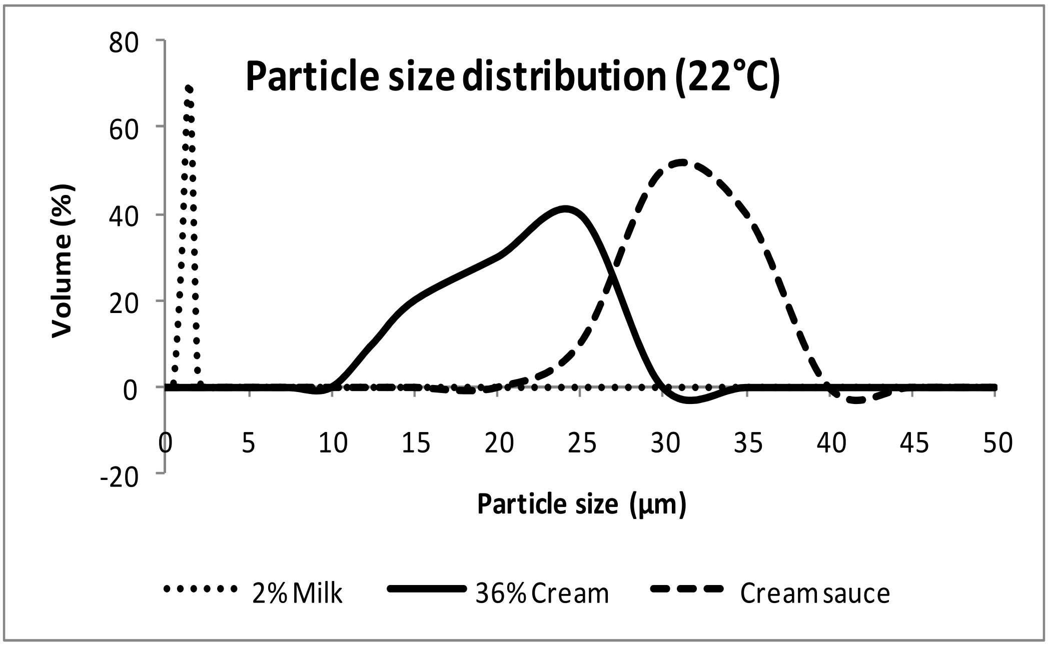


Figure 2. **Particle size distribution of milk, cream and cream sauce at 22°C**.
(holding time = 60 s)

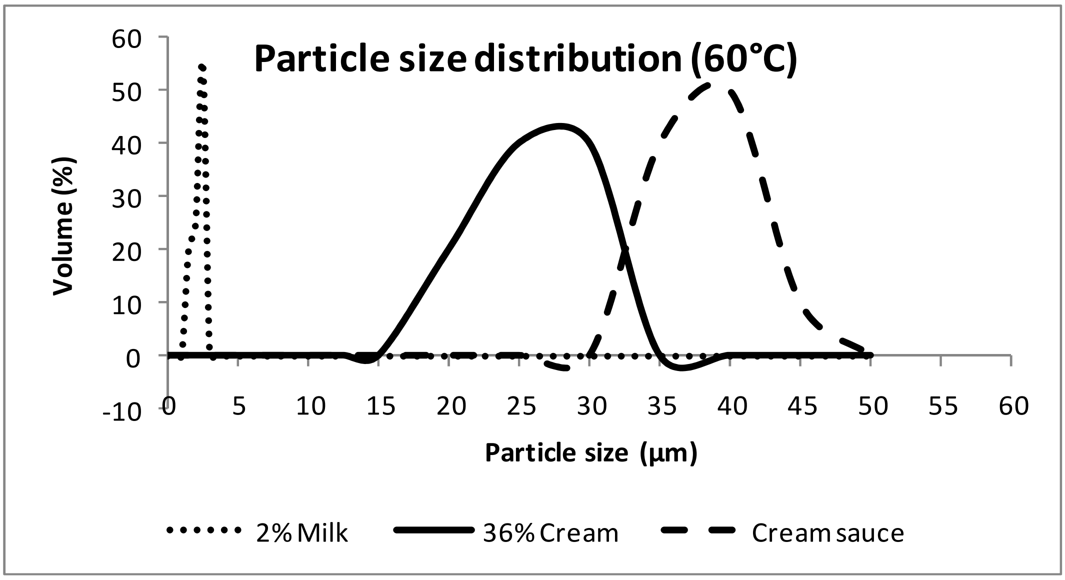


Figure 3. **Particle size distribution of milk, cream and cream sauce at 60°C.**
(holding time = 60 s)

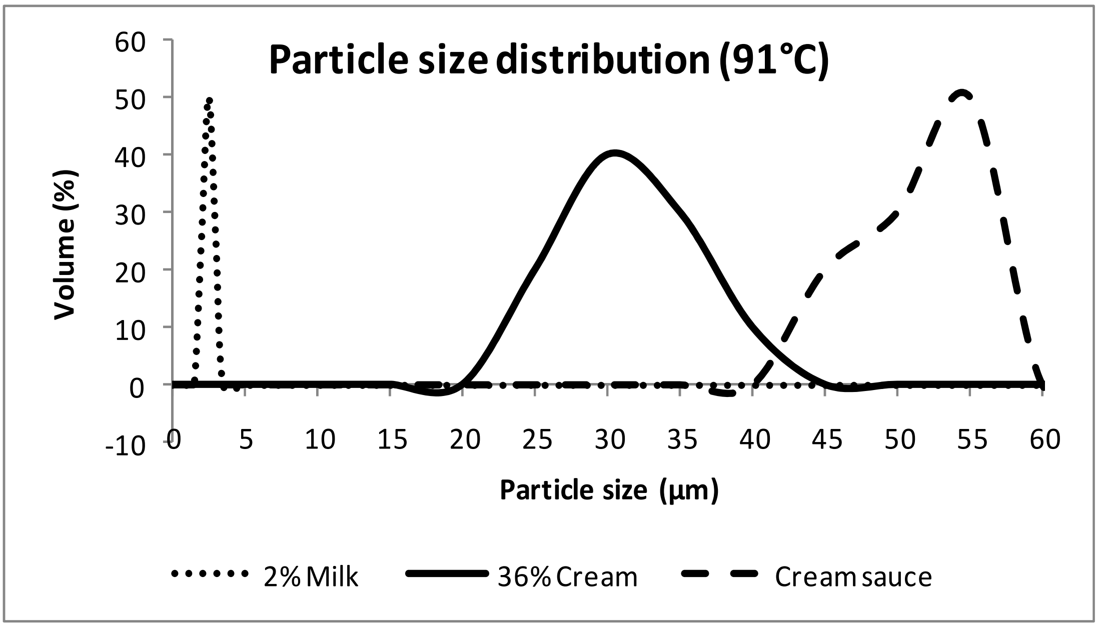
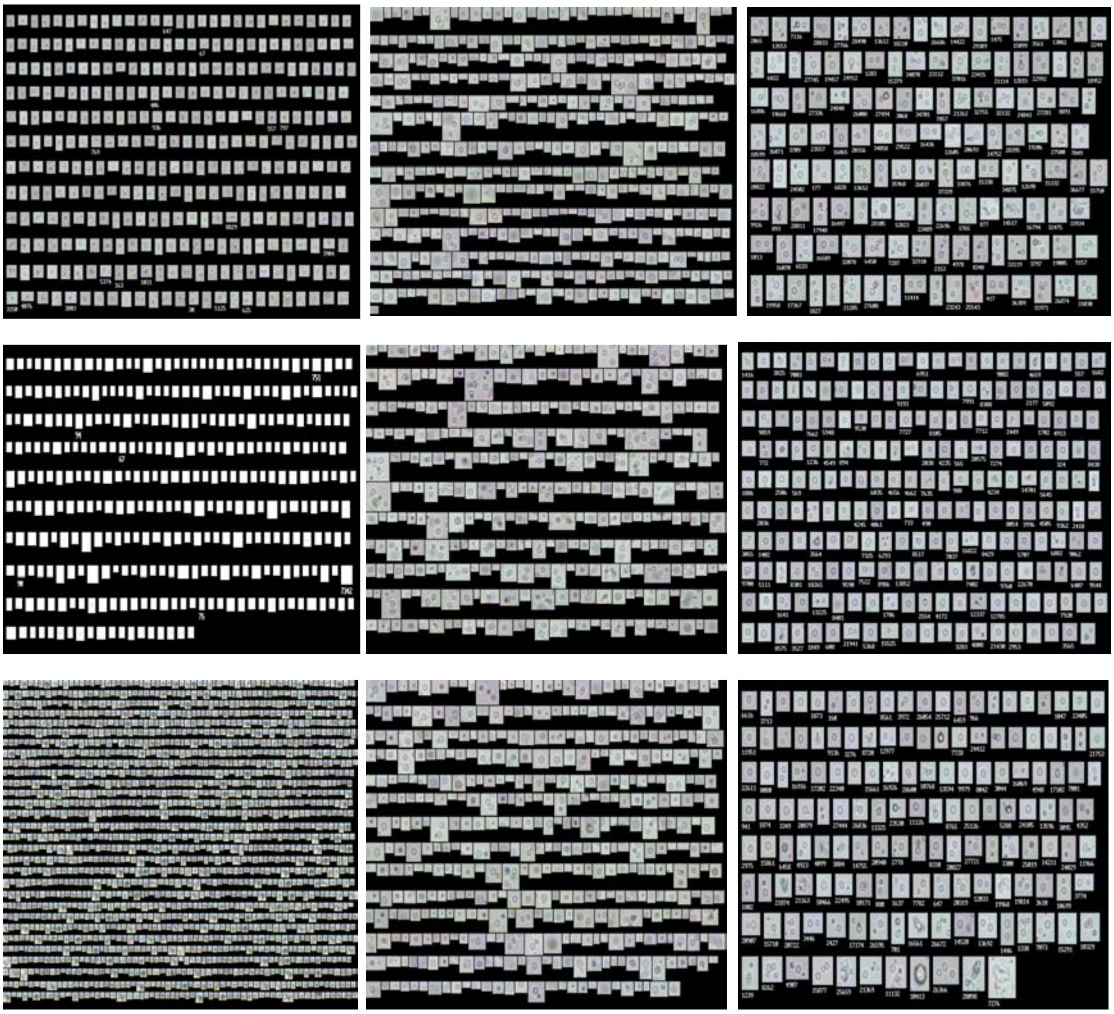


Figure 4. **Particle size distribution of milk, cream and cream sauce at 91°C.**
(holding time = 60 s)

Heat treatment of milk at temperatures of 70 - 90°C for 60 s causes denaturation of whey proteins, which may result in the exposure of reactive groups that are normally burried within the native conformation [6]. Of particular importance is the increased reactivity of the free thiol group of β-lactoglobulin, which can be involved in thiol–disulphide exchange reactions with other denatured whey proteins and with κ-casein at the casein micelle surface. As a further consequence, the denatured whey proteins may either form whey protein aggregates or may provoke a whey protein coating of the casein micelles, which in turn leads to an increased particle size [7]. The 2% milk used in this study was homogenized and hence the fat particles detected were considered below 2 µm. All particles above this value were protein particles or fat entrapped in casein micelles.



2% MILK 40% CREAM CREAM SAUCE

Figure 5. **Imaging of milk, cream and cream sauce at 22, 60 and 91°C (top to bottom).**

Particle size analysis of whipped cream (36% fat) was studied using FlowCAM at three different temperatures (22, 60, 91°C). These temperatures were applied on already pasteurized cream. As summarized in Table 1, particle size of whipped cream increased from 21.27 to 24.76 µm and on further heating it increased to 35.12 µm. The explanation for this enormous increase in particle size as compared to milk is firstly due to unhomogenized fat globules and secondly to the incorporation of air cells while whipping operation. Particle size analysis on whipped cream demonstrated a significant increase ($P<0.05$) in fat globule volume-surface diameter as a result of an increased severity of heat treatment which was in agreement with the results obtained by Smith and group [8]. The role of protein in stabilizing aircell-fat globule particles in whipped cream system is predominantly important in increasing size of the cream particles and has been well documented [9]. Whipped cream also showed a clear shift in particle size distribution when applied different temperature of heating as shown in Figures 2, 3 and 4 as similar to that of 2% milk. The image analysis for cream at different temperatures were also depicted in Figure 5 which evidently confirmed the air incorporation as well as particles approaching towards one another due to increase in kinetic energy due to the thermal energy [10].

The effect of severity of heat treatment was also examined on cream based sauce procured by local grocery store. The purpose of investigation was to determine the deterioration of sensory quality of sauce due to heat treatment so as to regulate texture. As discussed previously, size of particles in a food system plays a very vital role in contributing richness and mouth-feel. Results obtained from the study for cream based sauce is summarized in Table 1. It was observed that there was no significant effect of heat treatment on sauce particles ($p>0.05$). The sauce particles were mainly milk fat, protein, soy oil, starch, and emulsifier. The variable sauce particles can be confirmed by images obtained from FlowCAM (Figure 5). Although the particle size did not alter with different temperature, the distribution shifted towards slight bigger sized particles as depicted in Figure 2, 3, and 4 which is one of the important mouthfeel textural qualities. The stability of particles size was rewarded to the action of emulsifiers and the viscosity of the food system. The abundance of varied particles inhibited the change in particle properties and also the particles were entirely safeguarded by layer of emulsifiers and protein undoubtedly [11].

Conclusion

This study confirmed the hypothesis that heat treatment does affect the particle properties in some food systems such as milk and whipped cream. However, cream based sauce showed no major change on heating which was reflected by using emulsifiers and the abundance of particles obstructing the movement of particles and get disrupted or defaced. It is recommended to apply mild heat to protect the ingenious particle properties in a food system especially in case of milk and cream or their products to regulate the textural properties. Also, the textural quality of fat rich sauce can be regulated by the extent of heating in the range of 60-90^{o}C.

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