KAUNAS UNIVERSITY OF TECHNOLOGY
FACULTY OF CHEMICAL TECHNOLOGY

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THE MODIFICATION OF POLY-DISPERSE MODELS ON THE STABILITY OF EVAPORATED MILK

Master's Degree Final Project

Supervisor
prof. dr. Daiva Leskauskaite

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"THE INFLUENCE OF MODIFICATION OF POLY-DISPERSE MODELS ON THE STABILITY OF EVAPORATED MILK"

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TABLE OF CONTENTS

Janarthanan Jayalakshmi. THE INFLUENCE OF MODIFICATION OF POLY_DISPERSE MODELS ON THE STABILITY OF EVAPORATED MILK: *Master's* thesis in Food Science and Safety / supervisor Prof. Daiva Leskauskaite. The Faculty of Chemical Technology, Kaunas University of Technology.

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SUMMARY

This thesis provides a quantification of physical changes in the evaporated milk stored at 30°C temperature, with the aim of increasing the stability of the product during storage that would favor the hot places and for long distance transportation. Thus, many types of research work have been performed to stabilize evaporated milk using different food hydrocolloids. This research thesis focuses on the utilization of hydrocolloids such as k- carrageenan (marine polysaccharide), carboxymethylcellulose and an enzyme microbial transglutaminase to enhance the stability and increase the viscosity of evaporated milk stored at 30°C. The steady-shear rate rheological properties, L*a*b* values, pH, dry matter content, fat content and oil droplet particle size distribution of EM under supplementation of different concentrations of k- carrageenan (0.001 %, 0.002 %, 0.003 %, 0.004 %, 0.005 %), carboxymethylcellulose (0.001%, 0.00050% and 0.00025%) and inoculation with transglutaminase (120U g^{-1} of protein) at different pH from (6.3 to 6.7) were evaluated. There was a greater influence on the physio-chemical properties of the EM by the addition of k-Car and CMC and by the treatment of EM with TG. The changes in colour values of the samples during the storage manifested the occurrence of Maillard reaction. The proteolysis in the samples was evident and this can be attributed to the decrease in the pH of the samples. The dry matter and fat content was higher in the upper phase than the lower phase after the separation of two phases in the samples. The flow behavior had a good fit with an Ostwald model (R^2 > 0.97) and the EM samples exhibited pseudoplastic behavior and poly dispersal distribution of particle size. Due to cross-linking of milk protein, higher molecular weight macromolecules found at pH 6.6 and 6.7 in the skimmed milk. The addition of different concentration of CMC to evaporated milk had no positive impact on the stability of EM stored at 30°C. The samples with 0.005% of k-carrageenan had no cream separation throughout the storage period. The cross-linking of proteins by the inoculation of TG into evaporated milk did not prevent the seperation of fat and protein in the evaporated milk during the storage at 30°C.

Janarthanan Jayalakshmi. POLIDISPERSINIŲ MODELINIŲ SISTEMŲ MODIFIKAVIMO ĮTAKA SUTIRŠTINTO PIENO STABILUMUI. *Magistro* baigiamasis projektas / vadovė prof. dr. Daiva Leskauskaitė; Kauno technologijos universitetas, Cheminės technologijos fakultetas.

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SANTRAUKA

Šiose magistro tezėse aprašyti sutirštinto pieno fizikiniai pokyčiai laikymo 30 ^{o}C temperatūroje metu, siekiant padidinti produkto stabilumą laikant, o tai savo ruožtu sudarys palankias sąlygas jo gabenimui ilgais atstumais į šiltus kraštus. Iki šiol atlikta nemažai tyrimų , siekiant stabilizuoti sutirštintą pieną, panaudojant skirtingus biopolimerus. Šiose tezėse dėmesys sutelkiamas į polisacharidų – k-karagenino ir karboksimetilceliuliozės, bei fermento – transglutaminazės, panaudojimą sutirštinto pieno stabilumui pagerinti ir jo klampai padidinti, laikant 30 ^{o}C temperatūroje. Buvo vertinama, kaip keitėsi sutirštinto pieno reologinės charakteristikos, spalvos rodikliai - L*, a*, b*, pH, sausosios medžiagos ir riebalų kiekis, riebalų rutulėlių dydžio pasiskirstymas sutirštinto pieno indelio viršutiniame bei apatiniame sluoksniuose laikymo metu, kai produktas buvo pagamintas pridedant κ -karagenino (0,001 – 0,005 %) arba karboksimetilceliuliozės (0,00025 – 0,001 %) arba apdorojant pieno baltymus fermentu transglutaminaze (120 TV/1g baltymo), esant skirtingam pH (6.3 – 6.7). Nustatyti ženklūs sutirštinto pieno fizikinių cheminių savybių pokyčiai, pridėjus κ-karagenino arba karboksimetilceliuliozės, arba paveikus pieną transglutaminaze. Nustatyti spalvos pasikeitimai rodo, kad sutirštinto pieno laikymo metu jame vis dar vyksta Maillard reakcija, o nustatyti pH pasikeitimai rodo galimus proteolizės procesus. Sutirštintam pienu praradus stabilumą, jis išsisluoksniavo į dvi fazes; didesnis sausosios medžiagos ir riebalų kiekis nustatytas viršutinėje fazėje. Sutirštinto pieno tekėjimo savybėms aprašyti gerai tiko Oswald matematinis modelis ($R^2 > 0.97$), kuris parodo, kad sutirštintam pienui būdingas pseudoplastinių skysčių elgesys ir polidispersinis dalelių pasiskirstymas. Nustatyta, kad paveikus liesą pieną transglutaminaze dėl papildomų skersinių ryšių tarp pieno baltymų, esant pH 6.6 ir 6.7, susidarė didesni baltymų agregatai. Tyrimų rezultati parodė, kad sutirštinto pieno stabilumui laikymo 30 ^{o}C temperatūroje metu karboksimetilceliuliozės įdėjimas neturėjo teigiamos įtakos. Tačiau sutirštinto pieno mėginiai, pagaminti pridedant 0.005 % κ-karagenino ir taip sutirštinant nepertraukiamą vandens fazę produkte, sustabdė fazių atsiskyrimą produkte laikymo metu. Bandymas sustabdyti fazių atsiskyrimą sutirštinto pieno laikymo metu, sukuriant papildomus skersinu ryšius tarp pieno baltymų molekulių, buvo nesėkmingas.

ABBREVIATIONS

EM	Evaporated milk
k-Car	k-Carrageenan
CMC	Carboxymethylcellulose
MTG	Microbial Transglutaminase
L*a*b*	Ligtness, redness and yellowness axes
K1	EM with 0.001% of k-car from 1% stock solution of k-carrageenan
K2	EM with 0.002% of k-car from 1% stock solution of k-carrageenan
K3	EM with 0.003% of k-car from 1% stock solution of k-carrageenan
K4	EM with 0.004% of k-car from 1% stock solution of k-carrageenan
K5	EM with 0.005% of k-car from 1% stock solution of k-carrageenan
C1	EM with 0.001% of CMC from 1% stock solution of CMC
C2	EM with 0.00050 % of CMC from 1% stock solution of CMC
C3	EM with 0.00025% of CMC from 1% stock solution of CMC
TG1	Transglutaminase samples with 6.3 pH
TG2	Transglutaminase samples with 6.4 pH
TG3	Transglutaminase samples with 6.5 pH
TG4	Transglutaminase samples with 6.6 pH
TG5	Transglutaminase samples with 6.7 pH
DMC	Dry matter content
FC	Fat content

INTRODUCTION

In the most developing countries due to the population growth, there is an increasing demand for dairy products in the urban areas. This became an ultimate challenge to the dairy industries to stipulate safety, nutritious, easily digestible and long shelf life dairy product to the consumers at any time. Evaporated milk satisfies all these requirements.

Evaporated milk has a large market especially in the tropical countries, at sea and in the armed forces. Generally, it remains acceptable for 2 years when it is stored between 5 to 16^0C and the humidity should be low around 50%. As far as evaporated milk for export to the tropical countries concerned, it must meet the standards under storage at higher temperatures – up to 30^0C. The major quality problems of evaporated milk in warm climates are the following: heat coagulation, fat separation, age gelation, age thinning and color changes (Maillard reaction).

The aim of the study was to enhance the stability of evaporated milk stored at 30°C and validate it with experimental shelf life study. In this study, we hypothesize that the stability of evaporated milk can be improved by the increasing of the viscosity of continuous phase with the help of two different thickening agents: k-carrageenan and carboxymethylcellulose. Another approach which we used in our study was to modify the proteins phase in the system of evaporated milk by cross-linking proteins with the help of enzyme microbial transglutaminase.

For the verification of these hypotheses the following objectives of the thesis were formulated:

1. To choose the optimum concentrations of thickening agents - k-carrageenan and carboxymethylcellulose, to be added to the evaporated milk made under laboratory scale and stored at 30°C, for the increasing the stability of product during its storage.
2. To assess the influence of thickening agents - k-carrageenan and carboxymethylcellulose, on the stability of evaporated milk during storage at 30°C for 12 months by measuring chemical and physical characteristics of the product made in the industrial scale.
3. To investigate the impact of enzymatic cross-linking of proteins by microbial transglutaminase on the physical properties of skimmed milk.
4. To assess the influence of enzymatic cross-linking of proteins induced by transglutaminase on the stability of evaporated milk during storage at 30°C for 12 months by measuring chemical and physical characteristics of the product made in the industrial scale.

1. LITERATURE REVIEW

1.1. Characteristics of evaporated milk

During the evaporation, a lot of nutrients and calories are concentrated in the milk naturally, which makes it more calorie-laden, but at the same time, more nutritious than the fresh milk [1]. The standard defines ideal evaporated milk should contain at least 7.9% milk fat and 25.5% milk solids. EM is usually manufactured in one of two different standards which are reported in Table 1.1. The colour of evaporated milk is a bit darker and has a thicker texture than the normal fresh milk. There is a good variety of evaporated milk available in the market such as skim, low fat, whole milk, etc. While Vitamin D and C are present in every variety of evaporated milk, low fat and skim milk has Vitamin A additionally [2]. EM comes in a canned container and does not require refrigeration until the seal is broken. EM has a creamy texture and is very delicious to taste. Most of the people add water in the evaporated milk just to serve it as normal milk and lower down the calorie.

Table 1.1. UK and US standards for the composition of EM [3].

Compositions	**UK standard (%)**	**US standard (%)**
Milk fat	9	7.9
Non-fat milk solids	22	18
Total solids	31	25.9
Dry matter content	29	30.5

1.2. Reasons of instability in EM

There are many problems occurred in the evaporated milk during the storage above 21°C. Some major problems such as heat coagulation, fat separation, age gelation, age thinning and Maillard reaction were discussed. Their chemistry and the factors responsible for the instability were reviewed.

1.2.1. Heat coagulation

Heat stability of milk is defined in terms of the time required to induce coagulation at a given temperature say at 115^0C [4]. In the successful processing of milk and the manufacture of dairy products, the heat stability of milk is of tremendous importance. The destruction of spore resistant microorganisms can be done by the sterilization process. However, milk should not coagulate during heating which is caused chiefly by de-stabilization of milk proteins. Caseins

which are mentioned as milk proteins are stabilized by an electric charge and are in constant kinetic motion. Coagulation takes place when these casein molecules join to form aggregates. The optimum heat stability is also dependent on a ratio or balance of certain calcium and magnesium ions to phosphate and citrate ions. Heat coagulation could be accelerated by salt imbalance due to the deficiency of either group [5].

Coagulation is due to colloidal instability and not because of polymerization of the protein [6]. It generally occurs in two steps. The foremost is heat-induced disassociation of k-casein followed by denaturation of whey protein and its interactions with casein micelles caused during heating of milk at heat stability assay temperature. The formation of a specific disulfide-linked complex ensues between the casein micelles on the surface of k-casein and whey protein β-lactoglobulin [7]; [8] shown in the Fig. 1.1.

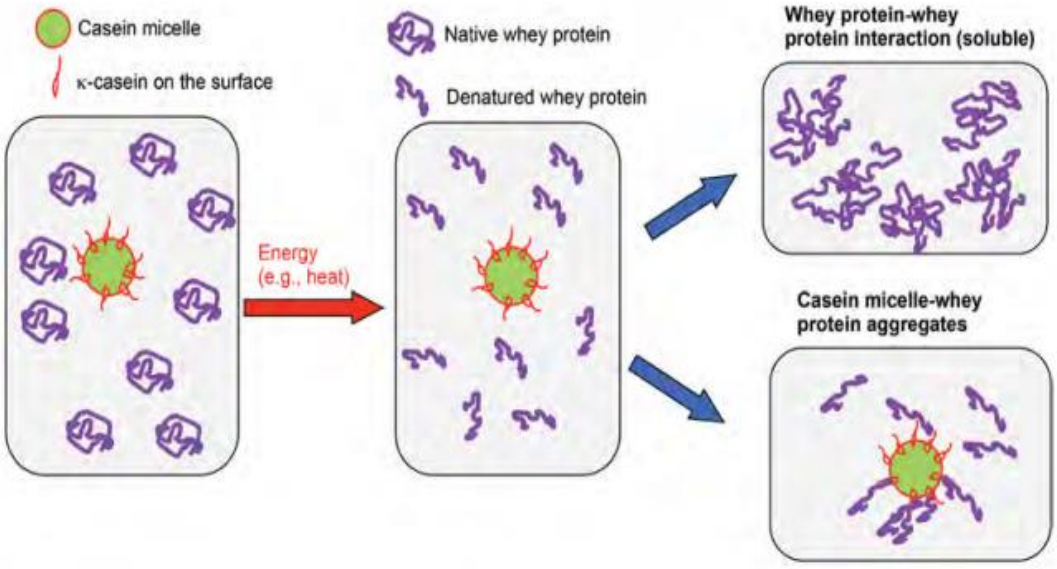


Figure 1.1. Casein-whey protein complex [9].

There are many factors that influence the heat stability of milk such as pH of milk, the concentration of total solids, the concentration of salts and ions, pre-heating or fore-warming of milk, homogenization, heat treatment after condensing or evaporation, the addition of chemical stabilizer and mineral-ion exchange treatment [2]. Among these, pH is the most important factor upon which milk has been classified into type A and type B. The milk salts are modified artificially which influences heat coagulation time (HCT) – pH profile. HCT is defined as the time that elapses between placing a sample of EM in an oil bath and the onset of coagulation. In type A milk, the stability increases to pH 6.7 and decreases at 6.9 and again increases at higher pH values. In type B milk, the stability increases with increase in pH of the milk except being lower in the region of the maximum and higher in the region of minimum when compared to type A milk. Whereas in

concentrated milk (20% total solids), the stability is maximum only in the pH range 6.4 to 6.6 which is shown in the Fig. 1.2.

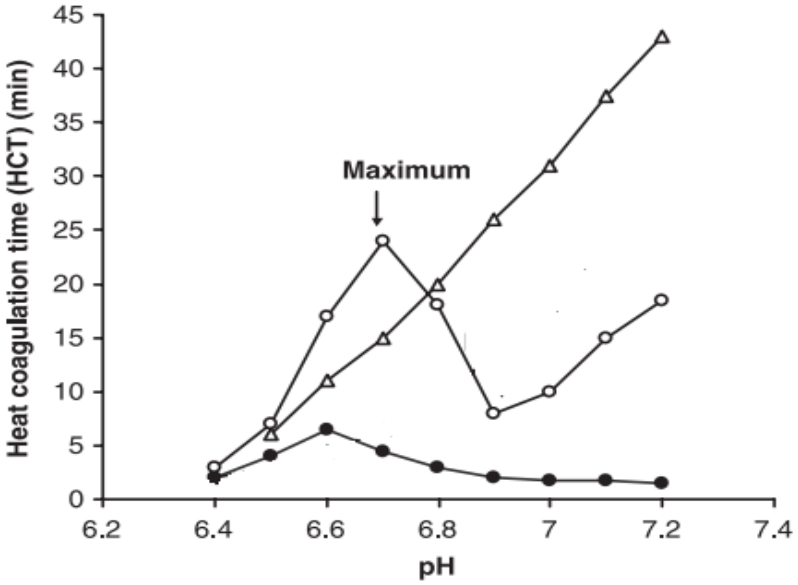


Figure 1.2. Heat coagulation time (HCT) vs pH profile 1. Type A milk (○) 2. Type B milk (Δ) 3. Concentrated milk (•) [10].

Heat stability of milk can be assessed by various methods. The generally used method to assess heat stability of milk involves heating the milk placed in a glass tube sealed in the temperature-controlled water bath at 140ºC and 120ºC for normal and concentrated milk respectively [10]. The other methods to determine heat stability of milk are ethanol test, whitening test, a protein sedimentation and a viscosity determination test. For more reliable results and prediction of the behavior of milk, the use of a pilot-scale or laboratory-scale sterilizer is recommended.

1.2.2. Fat separation

The separation of fat and serum into distinct layers is known as fat separation of milk which generally rises to the top forming leathery, viscous layer of about 0.64 to 1.27 cm that is not miscible with the remainder of the milk. However, smaller globules have a higher density than the plasma which settles at the bottom forming thick layers rather than cream. A can that has been stored undisturbed for several months is found to have higher fat content accumulated in the top and bottom of a can than in the middle.

The rate of rising of the individual fat particles is directly proportional to the square of the radius of the size of the fat particles. The velocity of the rise of individual fat globules is in good agreement with the Stroke's law in unheated skimmed which is given by the equation

$$V_s = a(\rho_p - \rho_f)\, d^2/18\eta_p$$

where V_s is the constant rising speed, a is the acceleration defining the field, ρ is the mass density, *d* is the fat globule diameter, η is the viscosity, subscripts p and f refer to plasma and fat respectively. The fat globules reduced in size by homogenization process and by coagulation; on the other hand, protein particles increased in size than the fat globules which are difficult to explain by stroke's law [11].

Creaming affects the flow properties of the milk. It mainly appears due to the inexorable reason since the density of the fat particles is lower than that of the concentrated milk plasma in which they are suspended. This might also occur due to the excessive addition of water during final standardization of EM. Discs of free fat referred to as moon spot appears as a 0.08 to 0.032 cm in diameter on the surface of EM rarely. The fat seems yellowish, crystal clear and as flattened spheres irregularly on the top [12].

A disruption of the ionic equilibria due to the adsorption of cations or anions is the outcome of destabilization [13]. Fat separation can be hindered by the subsequent ways such as reducing the size of fat globules, increasing the viscosity of the suspending phase and a modification of the density difference between the fat particles and the suspending medium [11]. Some of the attempts followed by industries seeking to diminish fat separation are effective homogenization, attainment of the maximum viscosity, cool storage and periodic inversion of cans during storage.

1.2.3. Age gelation

The formation of a gel or a capacious sediment or a creamy layer during the storage of sterilized EM is referred as age thickening or age gelation. The gel appears like a three-dimensional matrix which is instigated by the interactions between k-casein and β-lactoglobulin via the extreme heat treatment shown in the Fig. 1.3. The occurrence of gelation is early in evaporated milk stored at a higher temperature. There are four steps involved in the age gelation of evaporated milk. 1. the formation of aggregates by the aggregation of separate casein micelles, 2. the formation of larger metastable aggregates by further aggregation, 3. the formation of strands and 4. the formation of a continuous gel network.

One of the major cause for age gelation of the product is extracellular proteinases which are secreted by psychotropic bacteria comes from the poor quality of raw milk [14]. The other reason of the age gelation of EM seems to be an anonymous physicochemical change which occurs from dissociation of protein from the casein-whey protein complexes in the casein micelles,

although the raw milk is of good quality. On storage, gelation of a product is largely conveyed by an increase in non-protein nitrogen which is deceptively not brought about by enzymes.

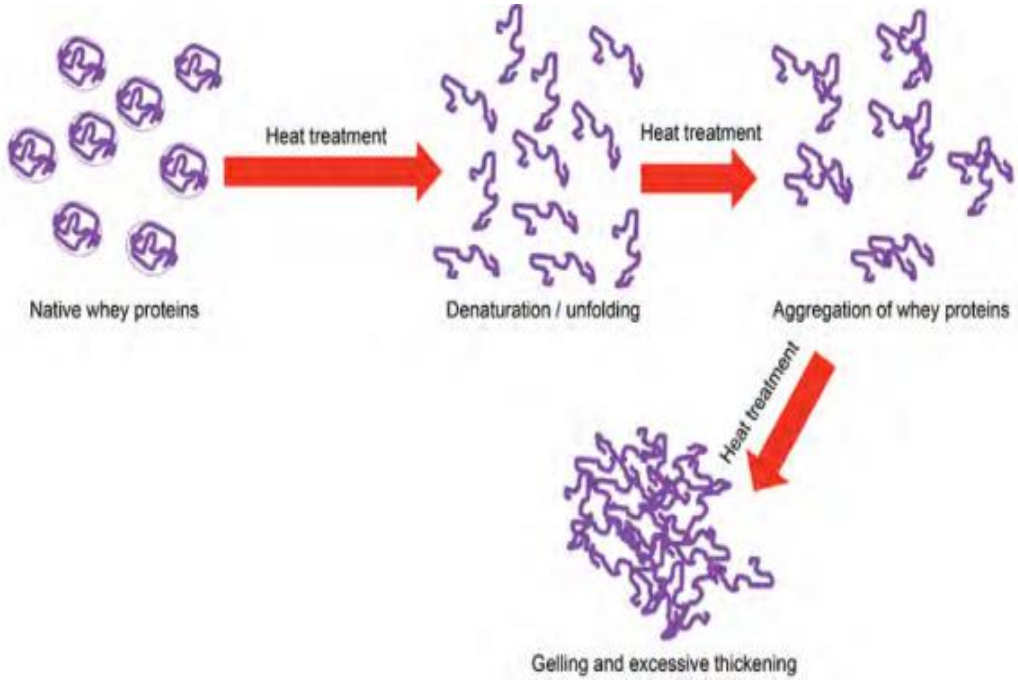


Figure 1.3. Gelling of proteins under heat treatment [9].

1.2.4. Age thinning

Age thinning arises due to insufficient concentration, improper homogenization or sterilization and it is indirectly related to the logarithm of storage temperature and time. Age thinning generally commences in the early phase of storage immediately after sterilization in which viscosity is found to be similar to that of normal milk [15]. The best way to avoid this defect is by storage at low temperature and processing in good condition.

1.2.5. Maillard reaction

There is a development of cooked flavor when EM is processed at a higher temperature for a longer period. Among the three process such as conventional, HTST and aseptic; HTST found to have a better flavor with less deterioration whereas convention was good in flavor initially but not later and aseptic was consistently the poorest in flavor. EM tends to be darker in colour in the direct relationship with the concentration of total solids, age and intensity of sterilization. This flavor and colour changes are mainly due to the Maillard reaction which might also reduce product's nutritional value.

Some of the factors that influence the Maillard reaction are composition of the raw material, processing and storage temperature-time combination, pH and water activity. The

reaction between a reducing sugar such as lactose and amino acid residues predominantly a Ɛ-amino group of lysine causes Maillard reaction explained by the Fig. 1.4. This results in the formation of brown coloured melanoidin by a variety of reactions such as cyclizations, dehydrations, realdolisation, isomerisation and condensation [16]. Besides, some intermediate compounds such as hydroxymethylfurfural and formic acid are formed that is attributed to the reduction pH during storage [17].

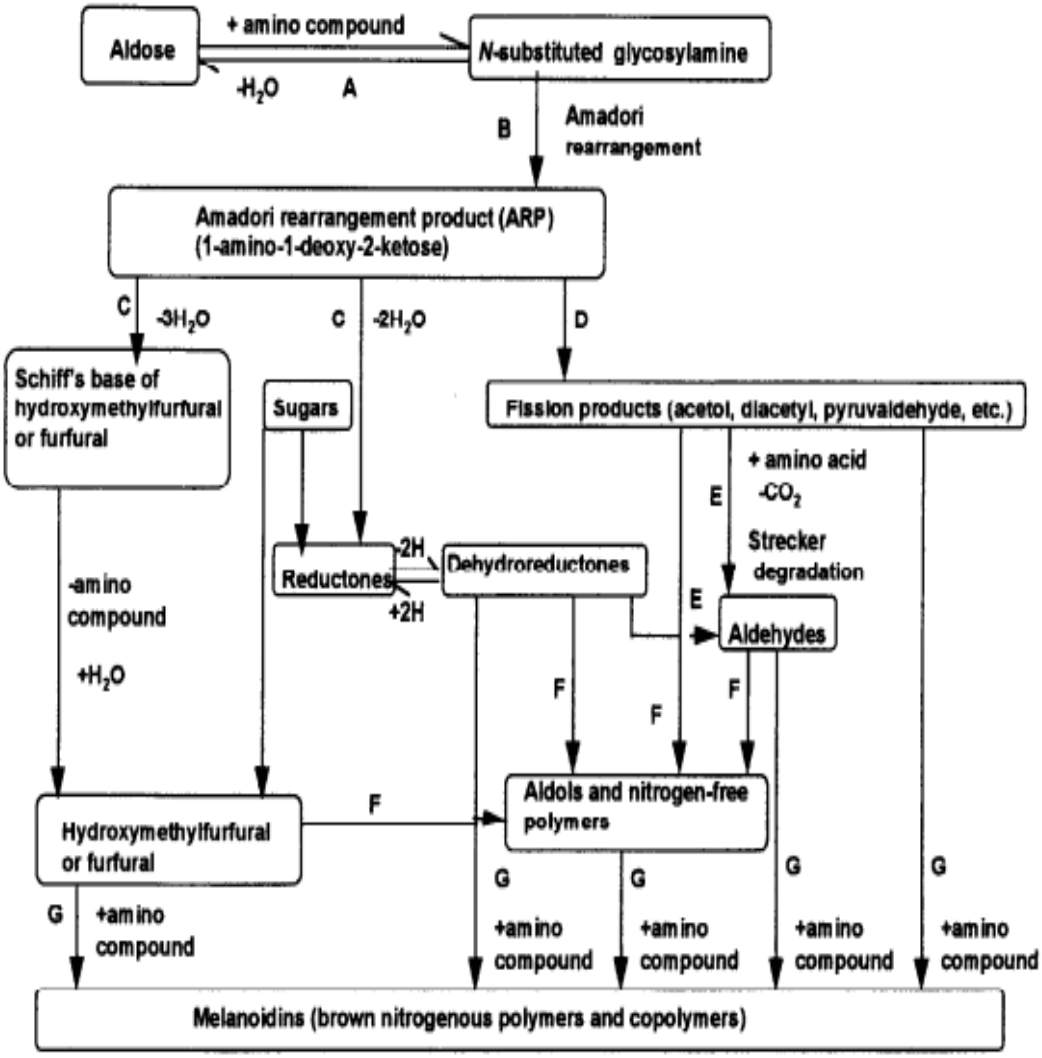


Figure 1.4. Hodge's schematic representation of the formation of melanoidin [16].

1.3. Steps to enhance the stability of EM

There are steps that can be done during the processing of EM to overcome the hindrance that is responsible for the instability of EM and to improve the shelf life of the product. EM does not undergo significant changes if it is well processed and stored at a lower temperature. There are many steps discussed below where the effect of the addition of hydrocolloids and inoculation of enzymes was the interest of this study.

1.3.1. Influences of preheating / fore-warming

During the manufacture of evaporated milk, preheating of EM can be done before sterilization in order to improve the heat stability of EM. Preheating is generally done through direct steam injection at 110 to 125^{0}C for 2 minutes which tend to higher heat stability of the evaporated milk [18]. Technically, serum proteins are denatured during preheating to avoid the gel formation which happens due to the high concentration of serum proteins in milk.

It also aids in killing microorganisms as well as bacterial spores and to inactivate enzymes especially lipases to prevent the development of rancidity. Besides, it increases the effectiveness of the evaporation by heating the milk to a higher temperature and to make the EM more resistant to the sterilization heat. The other changes involved due to pre-heating are a reduction in calcium ion activity, the interaction between casein micelles and whey proteins, reduction of sensitivity of casein to calcium ions etc. which increases the stability of evaporated milk [10].

1.3.2. Effect of pH and temperature

The pH of milk is greatly dependent on the heat stability and heat-induced complex interactions between whey protein and casein micelles of milk [19]; [20]. EM is much less heat stable than the normal milk. At pH (6.5), an increase in the size of the casein micelles occurs due to the association of the denatured whey proteins with the casein micelles whereas at pH (7.1), a small reduction in the size of the casein micelles happens due to the disassociation of k-casein from the low levels of denatured whey proteins associated with the casein micelles and at pH (6.5 to 7.1), an intermediate behavior is observed [21]. The casein composition of the disassociated protein is significantly dependent on the temperature-time relationship [22].

The heat stability of EM can be improved by reducing the calcium content of the milk before evaporation by ion exchange [23] or by the addition of stabilizing salts such as disodium hydrogen phosphate and trisodium citrate which reduce the calcium ion activity. The occurrence of disassociation of k-casein of concentrated milk influenced by pH and heating temperature of about 120°C shown in the Fig. 1.5. At pH from 6.5 to 6.6, extensive disassociation of k-casein occurs on heating. The disassociation of only k-casein occurs during initial stages of heating at 120°C. The other caseins are disassociated after heating for 10 minutes where the disassociated protein is found to contain 70% k-casein, 20% β-casein and 10% α_s-casein.

It is necessary to adjust the pH of EM to increase the stability because pre-heating and evaporation lower the pH of EM which would be far below the optimum pH [24]; [25]; [26]. The

stabilizing salts such as sodium citrate, disodium phosphate and calcium chloride are used alone or in combination with a maximum concentration of 0.1% to adjust the pH of milk.

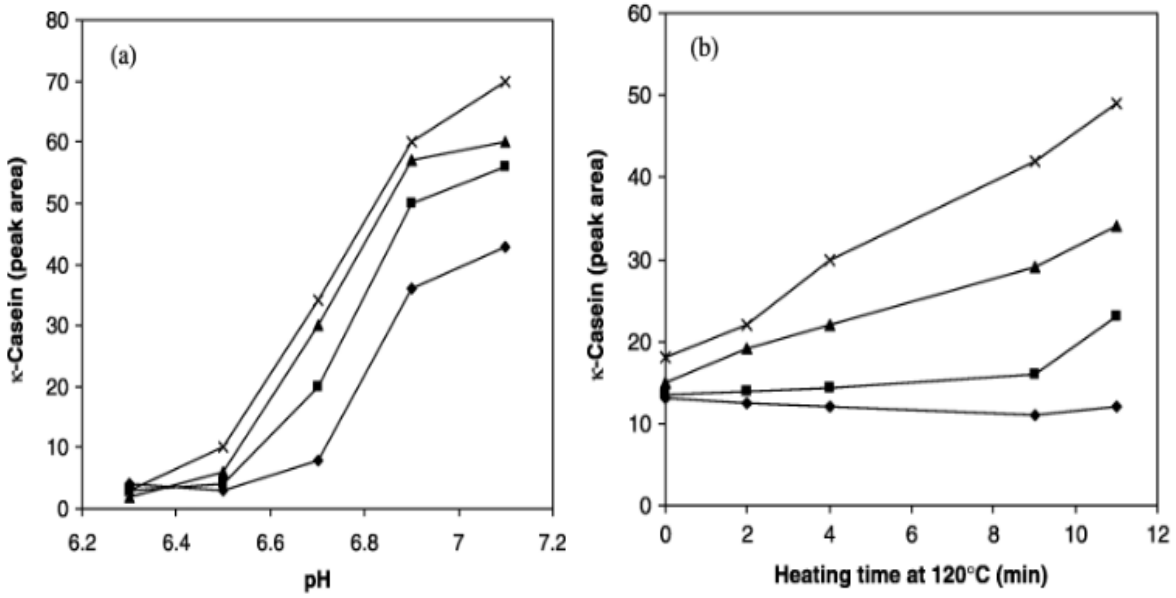


Figure 1.5. (a) influence of pH on the disassociation of k-casein micelles at 120ºC for 4 minutes, (b) Influence of heating time at 120ºC on the disassociation of k-casein micelles, x (25%)▲ (20%) ■ (15%) ♦ (10%) of total solids [10].

1.3.3. Effect of homogenization

The density factor is more troublesome in the fat separation of EM because of the lower density of the suspending medium which comes from high casein content of EM made from milk with high butterfat content. Homogenization must be optimum to disperse the fat commendable, however, destabilization effect on the protein occurs in the case of excessive homogenization.

The fat globule surface can be increased to 115 times by proper homogenization. About 2% of the casein is adsorbed at the fat globule surface before homogenization whereas, after the process, about 25% has been adsorbed [11]. The high temperature is required to reduce the size of the fat globules to prevent the coalescence of fat globules and the rate of creaming and for better heat stability. The fat separation does not occur if the milk is homogenized either before or after the evaporation. It also supports in decreasing the oiling off tendencies and to prevent the development of tallow flavor [27].

Hence the homogenization process can be carried out in 2 stage with pressures of 2500 and 500 psi on the first and second stages respectively. In the first stage, dispersion of fat globules by high pressure is done by the homogenizer and in the second stage, constant lower pressure

stabilized the newly homogenized globules and to stop subsequent cluster formation. However, the stability of protein has been decreased by high homogenization pressure resulting in the consequent risk of milk coagulation during the sterilization process but the stability in terms of cream separation has been improved greatly [28].

1.3.4. Effect of sterilization

During preheating of the milk, calcium is precipitated and thus it lowers the casein coagulation within the sterilizer [5]; [29] whereas calcium precipitation was not found in some work but there is a reduction in the soluble calcium when the milk is boiled for longer hours [30]. Sterilization can be done in the hermetically sealed can under pressure at 116 to 117ºC. By pre-heating the milk at about 95ºC, there is an attainment of higher viscosity but there is a chance of curdling of milk, hence pre-heating should be done at 100ºC. There is a gradual increase in the viscosity during the process of sterilization which tends to retard fat separation [31]. With the increase in the sterilization temperature and the time, there is a decrease in fat separation which could be expressed by an equation $VN = k$ where V is velocity, N is viscosity and k is a constant [11].

Age thickening and gelation can be delayed by the sterilization process under intense condition [32]. With the short fore-warming temperature and high sterilizing temperature, there will be a short gelation time. Heat treatment to ensure proper sterility is given by the equation as follows

$$F_0 = \frac{\text{Time(sec)}}{60} \times \frac{10(\text{Temp}(^\circ\text{F}) - 250)}{Z}$$

where F_0 is the amount of heat required which is a constant and Z is the number of degrees [33].

1.3.5. Effect of additives

Besides pre-heating, the addition of the salts such as phosphates and citrates control the heat stability of the milk but the choice is not forthright. The addition of sodium hydrogen phosphate and disodium hydrogen phosphate or trisodium citrate are done in the case of the natural pH of the milk higher than the pH of the maximal stability and natural pH of the milk lower than the pH of maximal stability respectively [20]. Stabilizers, when added to the milk during the course of the manufacturing process of EM either before or after pre-heating or concentration, influences the greater stability of EM while storage [21]. Though urea does act synergistically with aldehydes, it does not increase the heat stability of the EM (25% total solids) significantly. The addition of

formaldehyde increases the heat stability of milk by preventing heat-induced disassociation of k-casein [34].

Thickening agents

A macromolecular substance such as a protein or polysaccharide which swells by absorption of water, in some cases forming a stiff gel is known as hydrocolloids [35]. Rheological properties of the food systems are ultimately altered by hydrocolloids in the desired fashion [36]. A sugar backbone with protruding substituents is the classic structure of a food hydrocolloid [37]. Depending on the type, number and distribution of substituents protruding from the background determine the purpose of gum whether it is thickening or gelling agent [38].

The monosaccharide backbone, molecular weight, type of side chains and the distribution of side chains are the major factors influencing food gum properties [39]; [40]. In this, monosaccharide composition influences properties such as pH stability, ability to thicken or gel in food systems and thus affects the final behavior of the food hydrocolloid. The important concept in the hydrocolloids is hydration which varies uniquely from the other food ingredients. The side units on the backbone weaken the intermolecular association between layers and may provide space for water to slip between the layers and eventually making the hydrocolloid water soluble. Thickening depends on the concentration which generally occurs above the critical concentration known as overlap concentration (*C**) where fluid exhibits non-Newtonian behavior [41].

Carrageenan (marine polysaccharide)

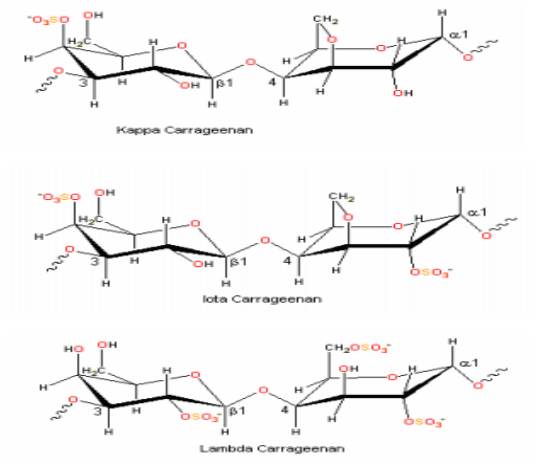


Figure 1.6. Structures of carrageenan [42].

Carrageenan gum is extracted from *Euchema cottonii* and *Euchema spinosum* species which come under the family *Rhodophycae* yield iota and kappa carrageenan gum respectively whereas *Chondrus crispus* produces both kappa and lambda carrageenan. The typical structure of

carrageenan consists of repeating units of galactose with different proportions and locations of ester sulfate groups and 3, 6 anhydrogalactose (anhydro bridges). The types of carrageenan can be distinguished depending on the number of ester sulfate groups and anhydro bridges on the backbone.

K - carrageenan is widely used in the dairy applications, the gel strength is influenced by the concentration of total solids present in the milk which can vary from 2.2% to 20% (w/w); k-carrageenan, from 0.1 to 0.4%; and cations [43]. K - carrageenan of about 0.0025% have been used in EM to prevent fat and protein separation [44] whereas about 0.01% around 100ppm which is very low usage levels is used in EM to prevent fat separation which occurs specifically due to carrageenan – protein interaction [41] and to avoid creaming, about 0.01 – 0.02% of either k or λ carrageenan can be used in EM.

The sulfate groups present in the carrageenan make it more water soluble whereas anhydro group inhibits its solubility as it possesses natural hydrophobic properties. Generally, carrageenan found to contain an ester sulfate content of about 20% or above and have an alpha (1,3) and beta (1,4) glycosidic linkages alternatively [45].

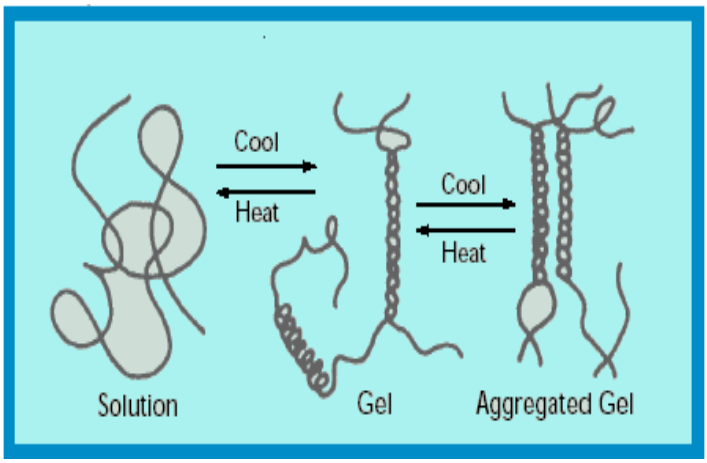


Figure 1.7. Double-helix formation upon cooling [42].

Carrageenan is more stable at neutral and alkaline pH and at elevated temperature. At pH below 4.4, due to autohydrolysis, carrageenan loses the gel strength and capacity to be more viscous [46]; [47]. Carrageenan gel is thermally reversible. Due to the thermal agitation where the temperature is above the melting point of the gel, random coils tend to form helices which upon cooling becomes a three-dimensional matrix [48]; [49]; [50]; [40] which is shown in Fig 1.7.

An important role is played by casein micelles by interacting casein micelles with k-carrageenan. Most of the studies suggested that there is an electrostatic interaction between k-casein micelles and k-carrageenan to stabilize the micelles against aggregation [51]. It has been

reported that minimum gelling concentration of k-carrageenan in milk is 0.003% where casein micelles are trapped within the three-dimensional network [52].

It is shown that carrageenan binds to the micelle surface initially and later it bridges and causes instability at a higher concentration shown in Fig 1.8. From this, it is understood that k-carrageenan chains have no effect on casein micelles at high protein concentration in terms of stability. It is found to possess gel-like properties for all protein concentrations above 0.05% (w/w) k-carrageenan concentration and behaves as a typical gel at 0.1% (w/w) concentration at 5ºC which is shown in the Fig. 1.9.

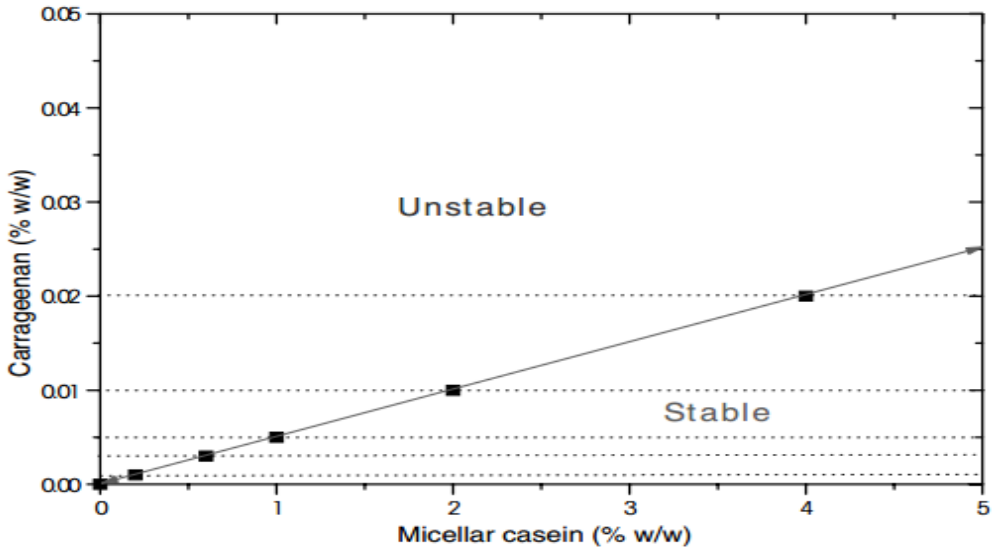


Figure 1.8. Stability diagram of micellar casein/k-carrageenan systems (>0.05% concentration) [52].

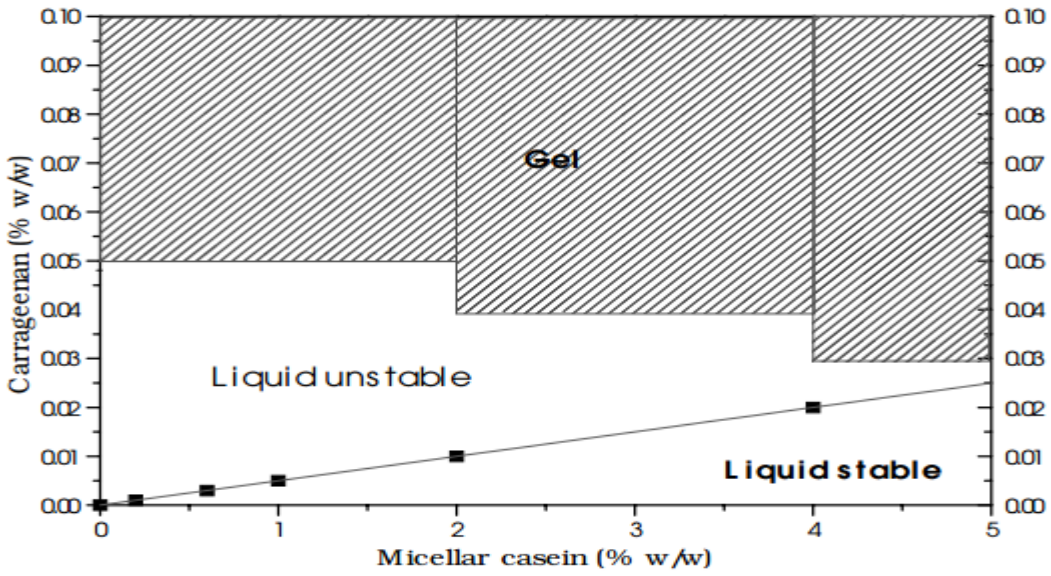


Figure 1.9. k-carrageenan system vs micellar casein phase state diagram at T=5ºC [52].

There is an electrostatic interaction between positive charge peptide chain of k-casein and negative charge sulfate groups of k-carrageenan endorsed to the synergistic effect at pH 6-7 although the protein is above its isoelectric point and carries a net negative charge. The formation of a mixed network via double helices occurs during the coil-helix transition where carrageenan in coil adsorbed to the casein micelles.

Schorsch also studied the behavior of carrageenan above and below the helix-coil transition temperature and reported which is schematically represented in the Fig. 1.10. K-carrageenan form helices and coils at below and above helix-coil transition temperature respectively. At below helix-coil transition temperature, the system behaves like a liquid tends to be stable or unstable depending on the protein concentration at low concentration of carrageenan about 0.03%. The instability occurs due to the formation of small agglomerates of carrageenan between casein micelles in the case of very low concentration of carrageenan. Nevertheless, it bridges and flocculates at high concentration.

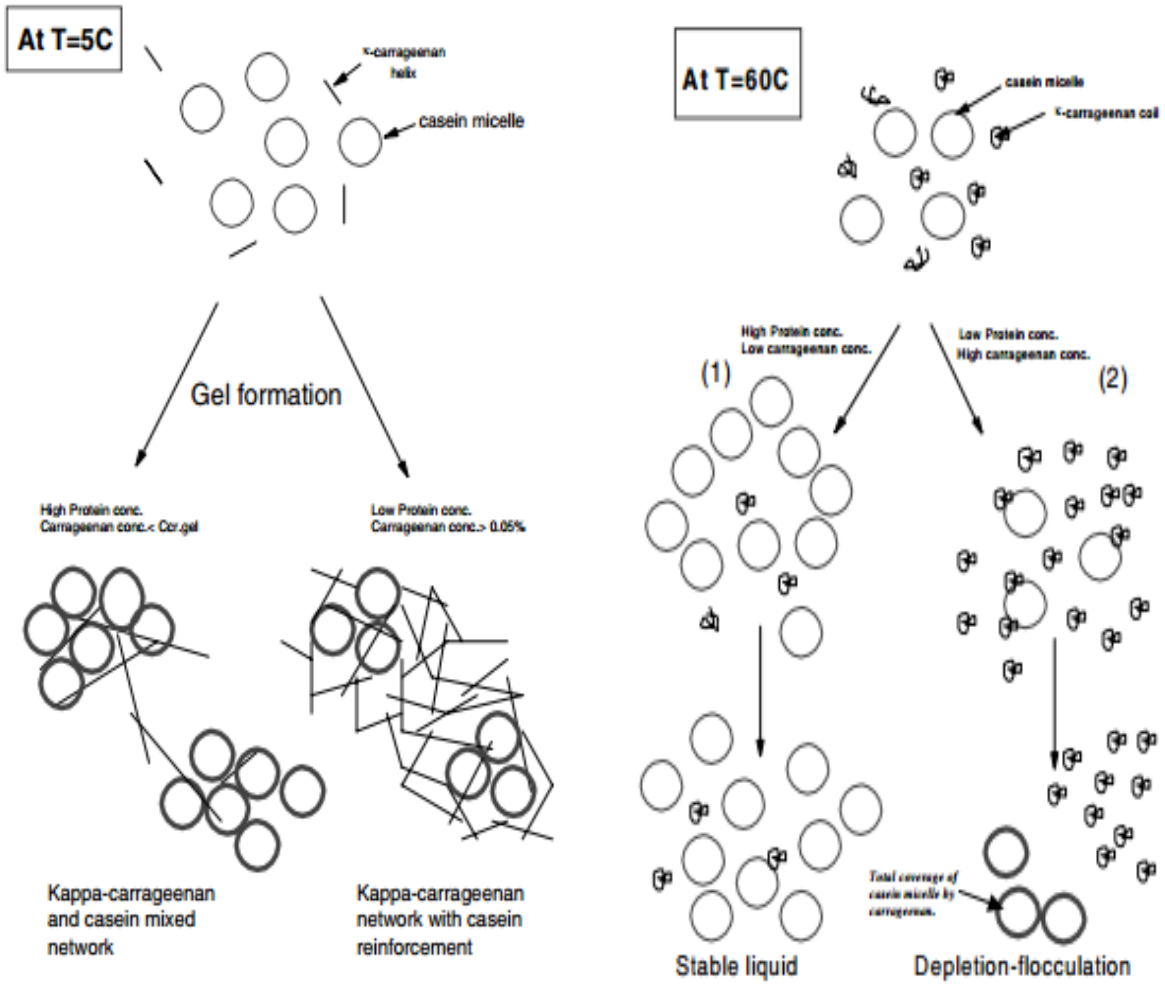


Figure 1.10. Schematic representation of the interaction between k-carrageenan/casein micelles at T=5ºC and T=60ºC [52].

The system remains stable if there is sufficient protein concentration for carrageenan to interact with casein micelles. Thus, there is no inadequate boundless carrageenan chains formation to induce flocculation that enables to create junction convergence neighborhood for the larger agglomerates between the casein micelles which intend to disturb the equilibria between casein micelles and carrageenan. This later resulted in complete disassociation of casein micelles at low protein concentration. There is an attainment of gel state at carrageenan concentration above 0.05% where the particles become restrained and stabilized against decantation resulted in polysaccharide gel due to excess of carrageenan in solution.

At above helix-coil transition temperature of about 60ºC, the system remains stable if the concentration of carrageenan is lower than the protein concentration since that concentration is not enough to cover the casein micelles due to reduced adsorption. Nonetheless, there is a disturbance in the phase interaction in the higher concentration and leads to depletion-flocculation. Therefore, polymer adsorbs at low concentration whereas depletes at high concentration [53]. This phase state was quite similar to iota-carrageenan-casein micelles interaction [54].

There is a formation of aggregates in the interaction between casein micelles and k-carrageenan under shear and the size of the aggregates depend on the concentration of k-carrageenan. The particle size distribution is directly dependent on the shear rate and carrageenan concentration whereas viscoelastic properties of the aggregates was directly dependent on the carrageenan concentration and not shear [55].

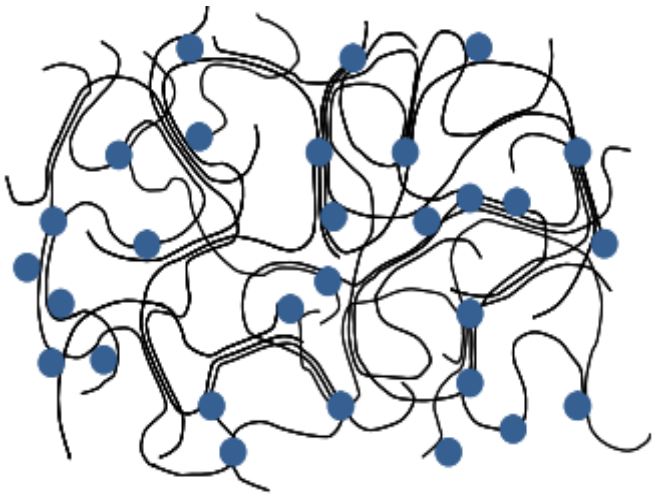


Figure 1.11. Gel formed in the mixtures of K-carrageenan and sodium caseinate [56].

There is a formation of mixed gels between sodium caseinate and k-carrageenan due to aggregation which is shown in the schematic diagram Fig. 1.11. The circles and lines represent the

small sodium caseinate aggregates and k-carrageenan chains in the helix conformation. Due to the parallel stacking of the k-carrageenan helices, the bridge is formed between their chains. The gels contain pure k-carrageenan bonds at a low content of sodium caseinate and sodium caseinate bonds are dominant at high concentration of sodium caseinate.

Carboxymethyl Cellulose (CMC)

CMC is obtained from the main polysaccharide cellulose which is the constituent of wood and all plant structures. It is commercially prepared from wood and then chemically modified. It is very soluble in water and can be fermented in the large intestine and lowers blood cholesterol level but consumption in large concentration leads to intestinal problems such as bloating, constipation and diarrhea. The recommended daily intake of CMC can be determined by none of the researchers until now.

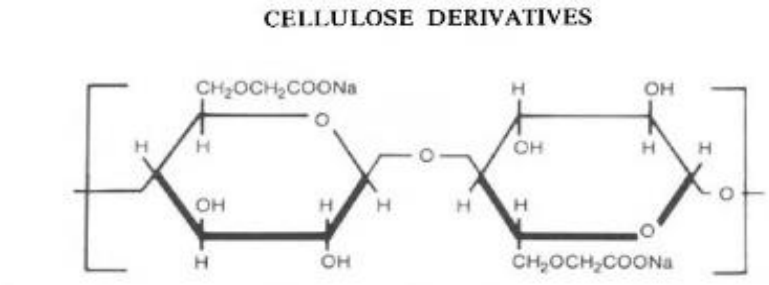


Figure 1.12. The idealized unit structure of CMC [57].

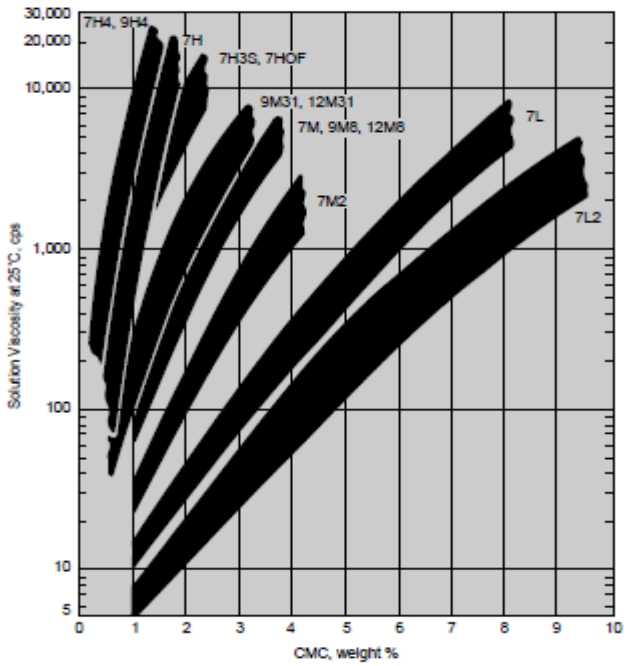


Figure 1.13. Effect of concentration of CMC (0.7 DS) on the viscosity of the aqueous solution [57].

The isoelectric point of casein and whey proteins are 4.6 and between 4.2-5.1 respectively. The ionic nature of CMC can interact with caseins in acidic milk systems at pH 3.5-5.5 to form stable and soluble complexes during heat treatment and storage shown in Fig 1.14. CMC solubilizes protein in the isoelectric range and thus shifts from isoelectric point to lower pH values. CMC possesses a unique behavior like acting as a protective colloid for protein and its solubilizing effect which are not found in other hydrocolloids. The stability of CMC is obtained from the gel formation, developing an internal network structure but their high performance reduced during intense heat treatment or when the concentration is diluted. Therefore, it is not suitable for high viscosity applications.

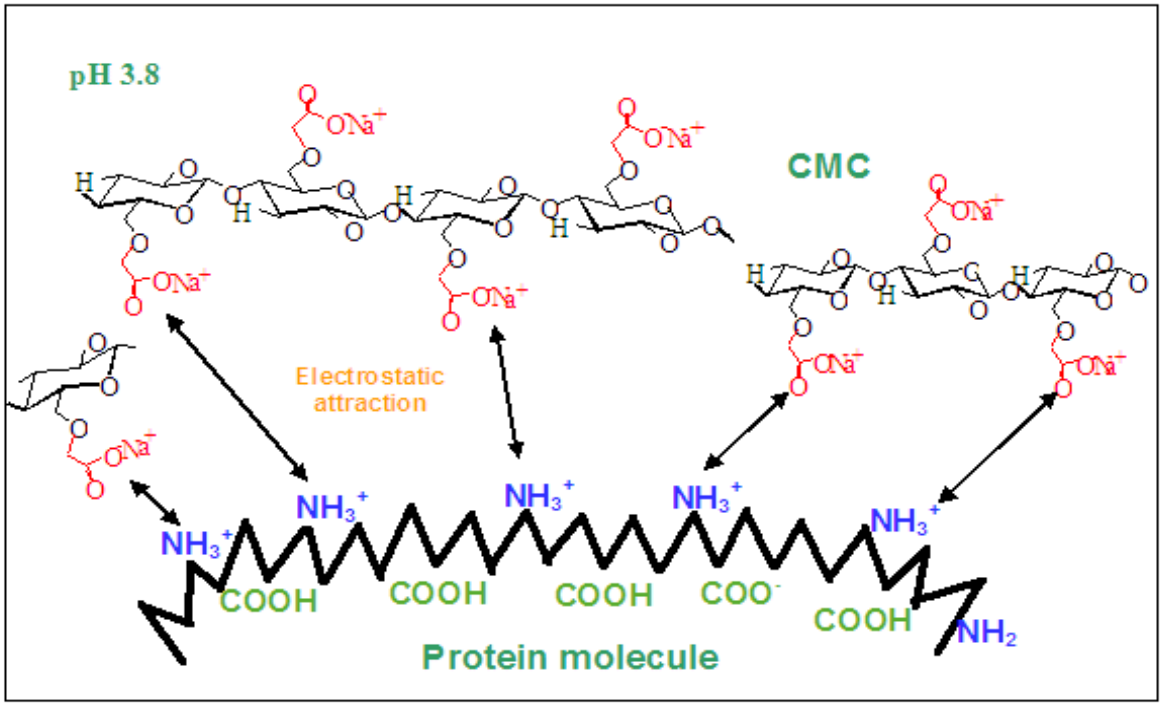


Figure 1.14. The performance of CMC at low pH (The Dow Chemical company) [58].

Enzyme Microbial transglutaminase (TG)

TG is an enzyme which comes under the class transferases which are widely known to modify protein functional properties in food systems. It is produced commercially through traditional fermentation by the microorganism *Streptoverticillium moboarense*. TG-modified protein differs from the native only in the number of bonds between glutamine and lysine residues [59].

Proteins are modified in TGs by incorporating amines, affecting intra and intermolecular cross-links or deamidation which causes insightful changes in the molecular structure [61]; [62] shown in the Fig. 1.15. The transfer of γ-carboxamide groups of peptides bound to glutamine

residues to a variety of primary amines including the ε- amino group of lysine residues occurs in the acyl transfer reaction [63]. The cross- linking of lysine and glutamine residues occur when ε-amino groups of residues of lysine molecules of proteins act as a primary amine. A stable protein network is formed by these iso- peptide bonds in the development of gels which produce changes in the hydrophobicity of the protein surface [64] resulting in the poor solubility and affect other functional properties such as gelation, emulsification, foaming formation and viscosity [65]. Stabilization of emulsions and foams are done by the deamidation action [66].

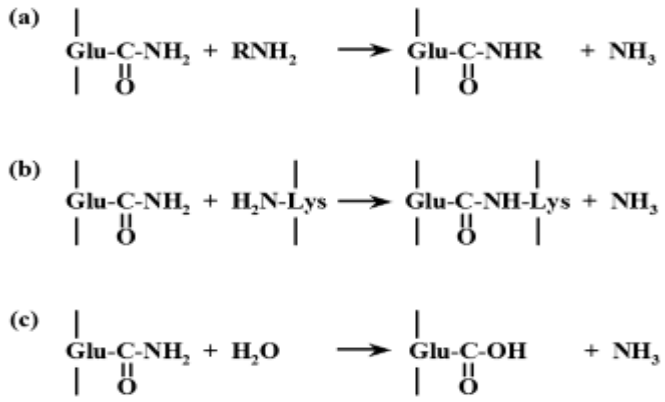


Figure 1.15. The reaction catalyzed by TG a. acyl-transfer b. cross-linking c. deamidation [60].

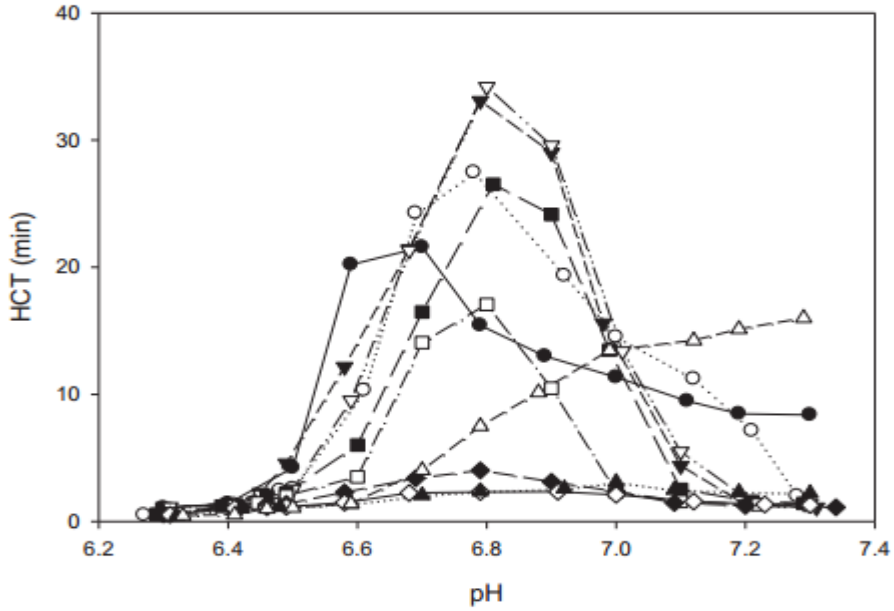


Figure 1.16. pH – heat coagulation time profile at 120ºC of concentrated skim milk (180gL^{-1}) transglutaminase at 30ºC. ●(0), ○(15), ▼(30), ■(60), □(90), ♦ (120), ◊ (240), ▲(480) [67].

The enzymatic cross-linking influences the stability of milk to heat induced coagulation [68]. With the increase in the incubation time with TG, there is a reduction in the levels of $\alpha_{s,}$ β and k-casein in both normal and concentrated milk [69]. Under longer incubation period for 90 –

480 minutes, there is a decrease in HCT at all pH values in the concentrated milk whereas after cross-linking for 1440 min, HCT of concentrated milk increased progressively with milk pH. TG treatment in the concentrated milk provides extensive prospects to increase heat stability [67] shown in the Fig. 1.16. After incubation with an enzyme TG, samples were heated at 70ºC for 10 min.

On treating milk proteins with transglutaminase, it tends to result in the formation of both intermolecular and intramolecular bonding. To achieve intermolecular bonding, skim or whole milk has been treated with TG and then followed by inactivation of the enzyme to attain higher viscosity. In order to decrease the viscosity, modification of the method to obtain more viscosity has been done where there was a formation of intramolecular bonds.

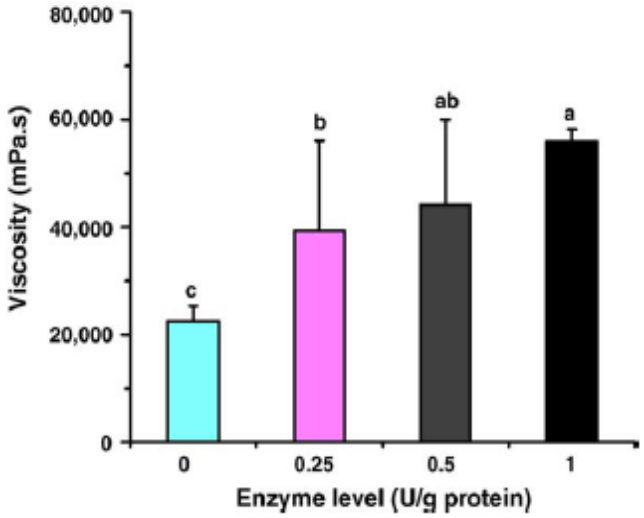


Figure 1.17. Effect of MTG on the viscosity of unstirred goat milk [70].

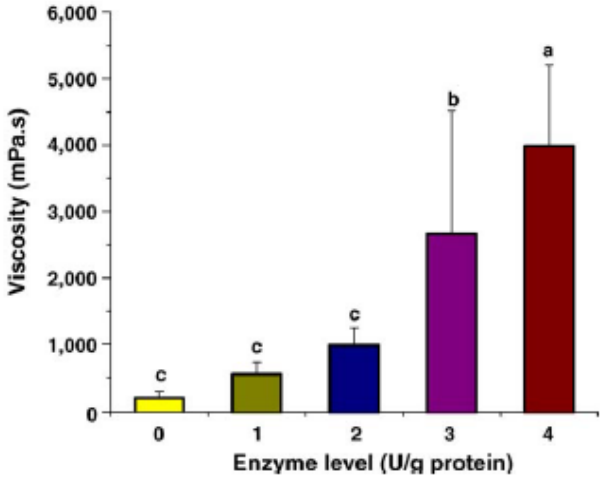


Figure 1.18. Effect of MTG on the viscosity of stirred goat milk [70].

In the method of preparation of goat milk yogurt, microbial enzyme Transglutaminase has been added at four different range up to 40U/EA and incubated at 50ºC for an hour then enzyme was inactivated at 75ºC for 5 minutes to make the product viscous. However, to prepare stirred goat yogurt, milk has been incubated with MTG at 43ºC for 5 hours, placed in a water-bath containing head stirrer for about 10 min at 50rpm and allowed to refrigerate for an overnight.

The comparative study was made between the effect of MTG on the viscosity of both unstirred and stirred goat milk yogurt. From the Fig. 1.17, it was understood that there was an increase in the gel breaking strength with the increase in an enzyme activity in the unstirred goat milk because of intermolecular cross-linking. Thus, the viscosity increases at about 75% whereas there was a negligible increase in viscosity with the increase in the total solids content and in the case of stirred goat milk. This is due to annihilation of a three-dimensional network. The similar response was reported for bovine treated milk [71].

2. MATERIALS AND METHODS

2.1. Experimental design

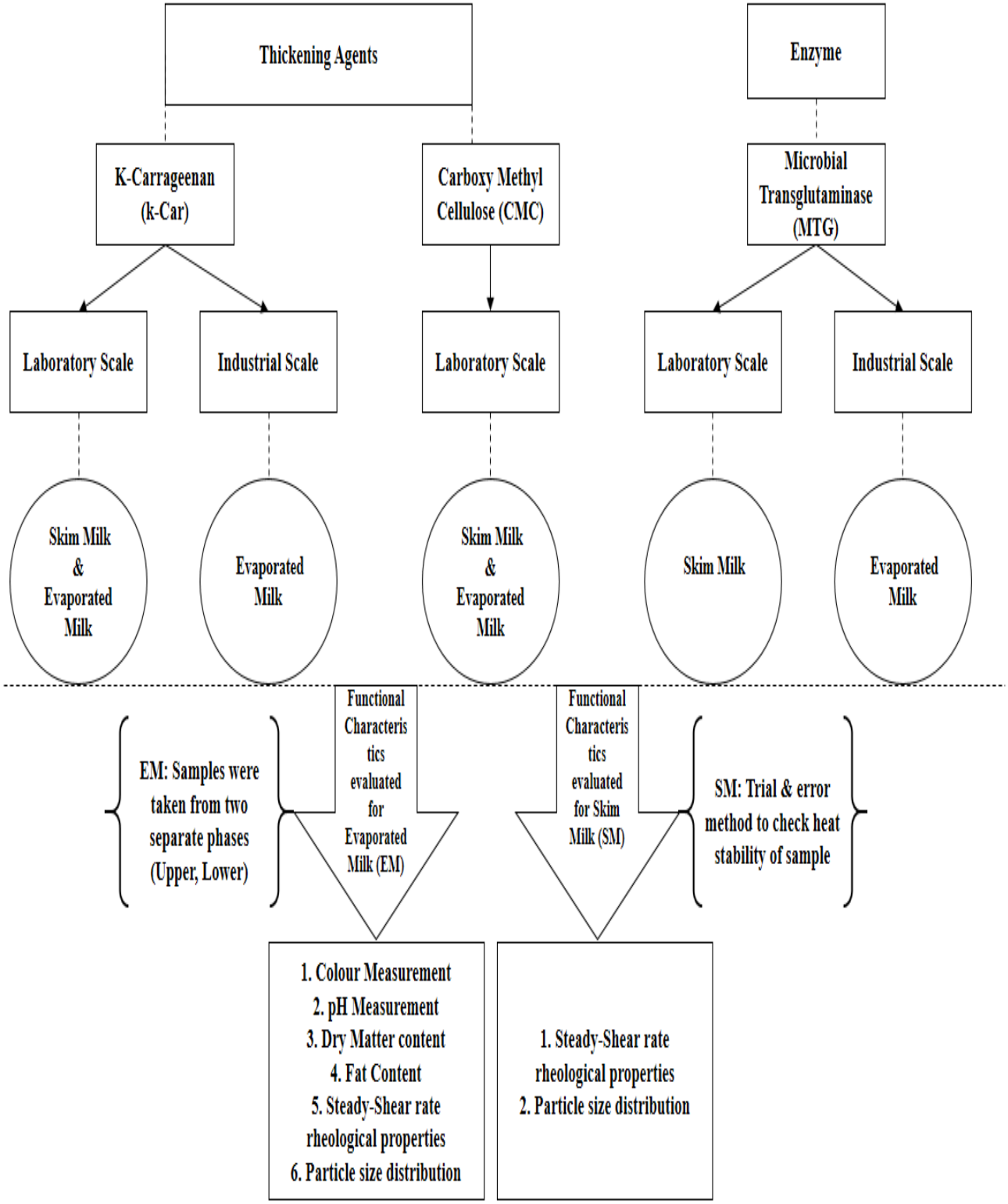


Figure 2.1. Experimental design.

The experimental design is presented in Fig. 2.1. Three independent experiments with different modifications of the composition of evaporated milk were carried out: evaporated milk, produced with the addition of different concentrations of k-Car; evaporated milk, produced with the addition of different concentrations of CMC; evaporated milk, produced from milk inoculated with TG at different pH. All experiments were carried out in two steps. Firstly, the laboratory scale experiment with the model system of EM with modified composition was conducted. The most promising modifications were selected and finally, the industrial scale experiments were conducted with EM with modified composition. In all experiments, the samples of EM were stored at 30^{o}C for several months. The stability of EM was evaluated by measuring creaming and sedimentation during the storage period using evaluation of pH, dry matter and fat content, rheological properties, oil droplet size distribution and color changes in EM. The measurements were made at room temperature in two fractions: top and bottom.

2.2. Materials

Evaporated milk

Evaporated milk (EM) was obtained from SC Marijampoles pieno konservai, in Lithuania. According to the manufacturer, the EM contained 25.5% total solids, 17.7% non-fat milk solids, 6.1% protein and 7.8% fat. It had a natural milk colour with creamy tone and possessed saltish and sweetish flavor. It got a shelf life of 12 months when stored between 2^{o}C and 25^{o}C or 18 months when stored between 2^{o}C and 15^{o}C. The product has been manufactured in accordance with LST 1940 certified by FSSC 22000 (HACCP), ISO 9001 and ISO 14001.

Skim milk powder

It was obtained from UAB "Mgl Baltija", Lithuania. The colour was white to cream white. It had fat content 1%, ash content 7.8%, moisture content 4%, protein 34%, lactose 50 to 54%, pH 6.5 to 6.7 and solubility index 1ml.

Carrageenan

Sima-Aldrich provided k-carrageenan (Grinsted ® of 99.8% purity, CL 110) given EC number 232-524-2. It was powder in form and white to light beige to light brown in colour. The moisture content was ≦ 12.5% and pH between 7.5 to 10.5 (1.5% in H_2O).

CMC

Carboxymethylcellulose known as the sodium salt of CMC was obtained from Danisco Ingredients which was 99.5% pure. It was odorless, tasteless and white in colour. The major ingredients of CMC were cellulose, mono chloroacetic acid and sodium hydroxide.

Microbial transglutaminase

Microbial transglutaminase (MTG) was obtained from Ajinomoto Foods Deutschland GmbH (Hamburg, Germany). Activa ® MP (E.C.2.3.2.13) composed of Transglutaminase (1%), lactose and maltodextrin. Microbial transglutaminase has a specific enzyme activity of 1000 U g^{-1} of powder.

2.3. Laboratory scale sample preparation

Samples of EM with two different thickening agents added were prepared from rehydrated (to 25%) skimmed milk powders in the bottles and sterilized in an autoclave at 120°C for 3 minutes to sterilize the samples and checked for heat stability. The trial and error were done and the optimum concentration was chosen for EM to enhance the stability of EM at the storage at high temperature of about 30°C. The samples without any hydrocolloids were stored at 6°C and 30°C denoted as P1 and P2, taken as the control samples.

Samples with Carrageenan

Table 2.1. Preparation of EM fortified with carrageenan (k-Car)

Sample name	concentration of k- car (%) in EM	k-Car (g) in 100 ml of EM
K1	0.001	0.1
K2	0.002	0.2
K3	0.003	0.3
K4	0.004	0.4
K5	0.005	0.5

The stock solution of carrageenan was prepared by dissolving 1 g of k-carrageenan and allowed to agitate by a magnetic stirrer for few minutes in 100 ml of distilled water to obtain 1% of carrageenan solution. Then this stock solution was used to prepare the EM samples with carrageenan as a thickening agent. The laboratory preparation of the samples employed with five different concentrations (0.001%, 0.002%, 0.003%, 0.004% and 0.005%) of carrageenan is shown in the Table 2.1.

Samples with Carboxymethylcellulose (CMC)

CMC was employed in three concentrations (0.001%, 0.00050% and 0.00025%) in EM to prepare the samples. The stock solution of CMC was prepared by dissolving 1 g of CMC in 100ml of distilled water to make 1% of CMC solution. Then CMC was taken from a stock solution at different concentrations and added in EM which is shown in Table 2.2.

Table 2.2. Preparation of EM fortified with Carboxymethylcellulose (CMC)

Sample name	concentration of CMC (%) in EM	CMC (g) in 100 ml of EM
C1	0.001	0.1
C2	0.0005	0.05
C3	0.00025	0.025

Samples with MTG

Table 2.3. Preparation of TG incorporated skim milk solution.

Sample	Skim milk powder (g)	Water (g)	TG (U/EA)	TG (g/l)
1	115	385	40	0.276
2	115	385	80	0.552
4	115	385	120	0.828

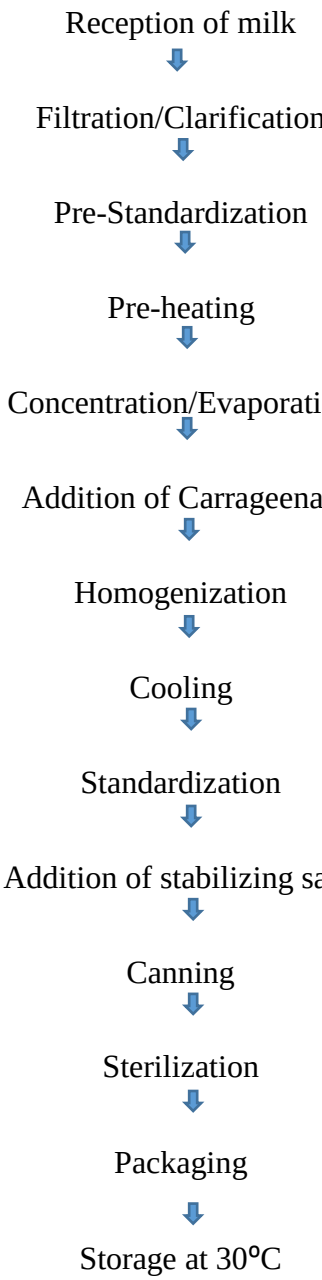
Skim milk powder was dissolved in water at 50ºC for 30 minutes and then cooled down till 30ºC or 40ºC. Now, the milk solution has been inoculated with 40, 80 and 120 U g^{-1} of protein. The preparation of TG incorporated skim milk samples were summarized in Table 2.3. The pH of the milk was adjusted by the addition of either 1M NaOH or 1M HCl to bring it in the following range (6.3, 6.4, 6.5, 6.6, 6.7). TG incorporated skim milk was being incubated for 30, 60, 120, 180 minutes at 30ºC or 40ºC and then heated at 80ºC for 2 min to inactive the enzyme. Pre-treated skim milk was cooled down to 8±2ºC and tests were carried out.

2.4. Industrial scale sample preparation

At first, heat-treated milk was pumped into an evaporator. Evaporation was taken place under vacuum at about $65^0$to 70^0C, which was an ideal temperature to control the growth of spores and heat-resistant bacteria. The samples were incorporated with five different concentration of carrageenan and 120U/g of protein TG inoculated at different pH in EM.

Samples with Carrageenan

Carrageenan was employed in five different concentrations (0.001, 0.002, 0.003, 0.004, 0.005) and homogenized. It was further cooled and standardized with the addition of some stabilizing salts such as E332 (potassium citrate) and E339 (sodium phosphates). The evaporated milk of different concentrations was canned and sterilized at 115^0C.



Flow chart 2.1. Industrial scale production of EM.

Samples with MTG

In the case of EM production incorporated with TG, the production method was quite similar to that of the flow chart 2.1. but EM after the addition of 120U/EA TG at five different pH

(6.3, 6.4,6.5, 6.6, 6.7) has been incubated for a day at 10ºC and then preheated at 80ºC for 2 min to deactivate the enzyme.

2.5. Methods of analyses

2.5.1. Colour measurement by CIElAB colour space

Principle

It is a three-dimensional (tristimulus) colour models developed by International Commission on Illumination (CIE). There were three axes L*, a*, and b* where L* represents lightness which is neutral ranging from 0 (black) to 100 (white); a* value ranges from −60 (greenness) to +60 (redness) and the b* value ranges from −60 (blueness) to +60 (yellowness). It was based on the principle that colour could be either red or green and yellow or blue but not together. The degree of colour saturation at the center of the axis was 0 where the colour was neutral and increases as the distance from the center increases [72]. Colour space was widely used in many industries because it comprehends the entire scale besides human vision.

Procedure

The colour values of different samples were measured by Minolta Chromometer CR-410 (Konica Minolta, Osaka, Japan) which had D65 illuminant and a 10º observer. The calibration of the instrument was done before the analysis using a white standard plate. About 20ml of the sample was taken in a petri dish without any void space at the bottom and chromometer was placed upon the sample then the values L*a*b* were generated. All the samples were measured thrice.

2.5.2. pH measurement

Principle

A pH meter determines whether the milk is acidic or alkaline. It is defined as a negative logarithm of the hydrogen ion activity. The basic principle of the pH meter is to measure the concentration of hydrogen ions or hydroxyl ions present in the solution [73]. Acids dissolve in water forming positively charged hydrogen ions (H+) whereas bases dissolve in water forming negatively charged hydrogen ions (OH-).

Procedure

The pH of different samples was measured by a pH-sensitive electrode attached to a temperature element which provides a signal to the pH analyzer. A pH electrode was placed in contact with a sample and pH was noted down till it attains a constant value at ambient temperature.

2.5.3. Analysis of fat content by Gerber's method

Principle

Fat content was estimated according to Gerber's method named after Dr. N. Gerber. To separate milk plasma from fat globules, sulphuric acid is added to increase the specific gravity of milk plasma and dissolves all solids-not-fat and thus prevent adhesiveness of milk. Amyl alcohol is added to the milk that aids in separation of fat from the milk-acid mixture and avoids accusing of fat and sugar by sulphuric acid. An exothermic reaction occurs between acid and milk that dissolves the fat and thus fat globules rise to the surface by centrifugation.

Procedure

To prepare the milk sample, about 10 grams of the sample was taken and add about 15 ml of diluted water (60ºC) make it up in the standard measuring flask. Put the butyrometer in the stand and 10 ml of H_2SO_4 (1.84 specific gravity) was poured into the butyrometer. Then shake well the milk sample before pipetting out of about 10.77 ml and put along the sides of the butyrometer and add 1 ml of amyl alcohol to it so that three discrete layers must be formed. Then use the rubber stopper to lock the butyrometer and shake well till it attains mahogany red or purple colour and to avoid any curdy white particle kept undissolved. They are then kept in a water bath at 65ºC for 5 minutes and placed in a centrifuge allowed to rotate at 1100 rpm for 5 minutes. Butyrometers were taken and kept in a water bath again for few minutes and fat was found to be separated in the butyrometer and readings are taken in the lower meniscus of the scale.

Fat content of the milk can be calculated by,

fat content (%) = fat content in ml x 2.57

2.5.4. Determination of dry matter content

Principle

The difference in weight from the original weight of the sample was the dry matter content of the sample. The loss in weight was due to moisture removal from the sample at high temperature. The dry matter content was determined by the gravimetric method.

Procedure

About a gram of sand was taken in the container with a lid and weigh the container before placing it in a hot air oven at 102± 2ºC and note down the weight. Then allowed to dry for an hour and transfer into a desiccator containing silica gel for 30 minutes to bring down to ambient

temperature and measure the weight of the container containing sand. Now add 2 grams of the sample into the container and weigh it followed by addition of 4ml of water in order to avoid the film formation and weigh again. Placed the samples in a hot air oven and final weight of the samples was noted down till it attains a constant value.

Dry matter content of the sample was calculated by,

Dry matter content (%) = ((m2-m0) / (m1-m0)) x 100

where m0 = weight of the container containing sand after drying, grams

m1 = weight of the container containing dry sand and sample before drying, grams

m2 = final weight of the sample after drying, grams

2.5.5. Viscosity measurements by rheometer

Principle

A rheometer is a device used to understand the rheology of the fluid in response to applied forces. There are more parameters involved to study the flow behavior whereas the only viscosity of the fluid is studied in the viscometer. There are two studies done in the rheometer – steady state and viscoelastic properties. The electrically commutated motor of the Physica MCR 301 guarantees accurateness from low viscosity polymer solution to high viscosity magnetorheological fluids at high shear rate. The permanent magnet in the rotor and coils provided with magnetic poles of opposite polarity in the stator attract each other gives a frictionless identical movement of rotor produced by rotating flux of coil in the windings. EC motor also provides a direct relationship between the torque and input current to the stator coil which favors accurate torque control and rheological measurements. It is widely used in industries in process design and product quality evaluation.

Procedure

Anton Par Physica MCR 301 rheometer model (Messtechnik, Stuttgart, Germany) was used to measure rheological properties of EM at 20ºC. It was equipped with a temperature controller maintained by cryo-compact circulator and measuring system (Julabo GmbH, Germany). The sample was loaded in the measuring cylinder and flow curves were recorded for different samples by increasing shear rate from 0 to $100s^{-1}$. Shear stress and viscosity were obtained as a function of the shear rate. The flow behavior of EM samples well fitted with an Ostwald model since $R^2 > 0.98$ which is represented by the equation

$$\boldsymbol{\tau = K.\gamma \text{^} n}$$

where τ = shear stress

K = consistency coefficient

γ = shear rate

A hysteresis loop was also obtained between the upward and downward apparent viscosity-shear rate flow curves to evaluate a thixotropic behavior for all the samples.

2.5.6. Particle size determination by laser diffraction technique

Principle

The particle size analysis is based on the theory of Fraunhofer diffraction. The particle size is based on the intensity of light scattered by a particle, however, there is an inverse relationship between the angle of the laser beam and particle size. He-Ne laser made up of the laser tube, a high-voltage power supply, and structural packaging was used for the experiments. A beam of light where detection of the light energy produced by the laser endure an interruption and then onto a sensor. Between the object being analyzed and the detector's focal point, there is a lens. This equipment is generally connected with a computer to detect the fluid's particle sizes from the light energy produced and also data collected on the partial frequencies and wavelengths.

It is used in the dairy emulsion to evaluate the particle size of the fat droplets in order to define sensory and physical properties such as flavor release, mouthfeel and emulsion stability. There is an increase in the surface area due to a decrease in the particle size and the emulsion remains stable; nevertheless, it causes flocculation in the case of larger particle size because of creaming. It also measures larger protein micelles such as casein and thus interaction between the protein and emulsified fat phase are understood. The particle size was considered as one of the main factors in the study of the shelf life of the emulsion systems.

Procedure

The particle size distribution and average particle size of the samples were analyzed by Malvern Mastersizer 2000 (Malvern Instrument Ltd.) laser diffraction analyzer. To avoid multiple scattering effects, the samples were diluted in water and then thus the sample was added until obscuration was in range and thus dispersed in diluted water at 2100 rpm. A dispersed phase refractive index was 1.5, droplet absorbance was 0.00, the refractive index of water was 1.330 and

obscuration was between 10 to 40% and the threshold was 65%. These are the optical parameters that are in consideration.

2.5.7. Statistical analysis

All the experiments were statistically analyzed in Microsoft Excel 2013. The results are reported as the mean and standard deviation.

3. RESULTS AND DISCUSSION

3.1. The effect of k-carrageenan on the stability of evaporated milk

3.1.1. Results from laboratory scale experiment

The variation in physio-chemical characteristics of the samples with carrageenan (water in the emulsion) prepared in the laboratory was reported. The samples were prepared in the month of March 2015 and the analysis was carried out from the month of preparation (1st month, 2nd month, 3rd month, 5th month, 7th month, 9th month) till November to study the effect of k-carrageenan on the stability of evaporated milk during the storage of the samples at 30^{0}C.

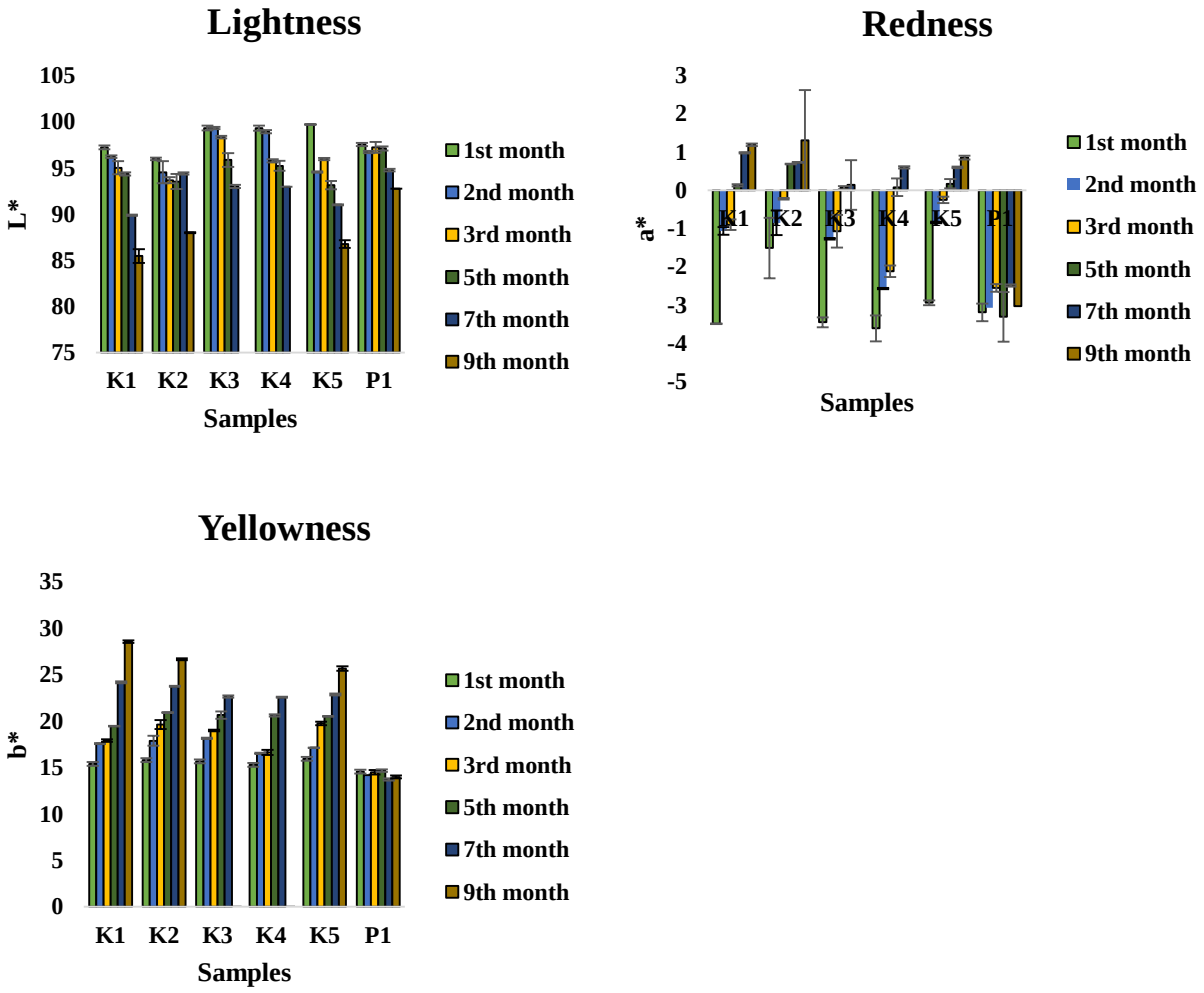


Figure 3.1. Changes in L*a*b* values of EM made with the different amount of k-Car during storage at 30^o and the control sample stored at 6^{o}C.

The average of L, a, b values was taken for every month and presented in Fig. 3.1. It was found that lightness value decreased in the last month with a great variation when compared to values measured initially. The lightness value was found to be higher for the samples K3, K4 and

K5 during the initial month of storage. This could be due to the increase in the concentration of carrageenan in the samples. The redness values were negative until three months of storage and then found to increase with the storage period.

There was a persistent increase in the values of yellowness conversely with the decrease in the value of lightness. It was vivid that browning reaction i.e the reaction between lactose and amino groups occurred in the samples because of the storage at a higher temperature. The samples K3 and K4 got spoiled with complete gelation after 7 months. These factors were observed in the control samples P1 stored at 6^0C and P2 stored at 30^0C. There was only quite variation in the sample P1 since, at low temperature, EM found to be stable for 12 to 18 months without any additives. The redness values were negative throughout the duration of storage. Whereas, sample P2 got spoiled after the four months of storage because no hydrocolloid was added to stabilize the intermolecular interaction. The standard deviation of the measurement varied from 0.01 to 0.87 for the samples.

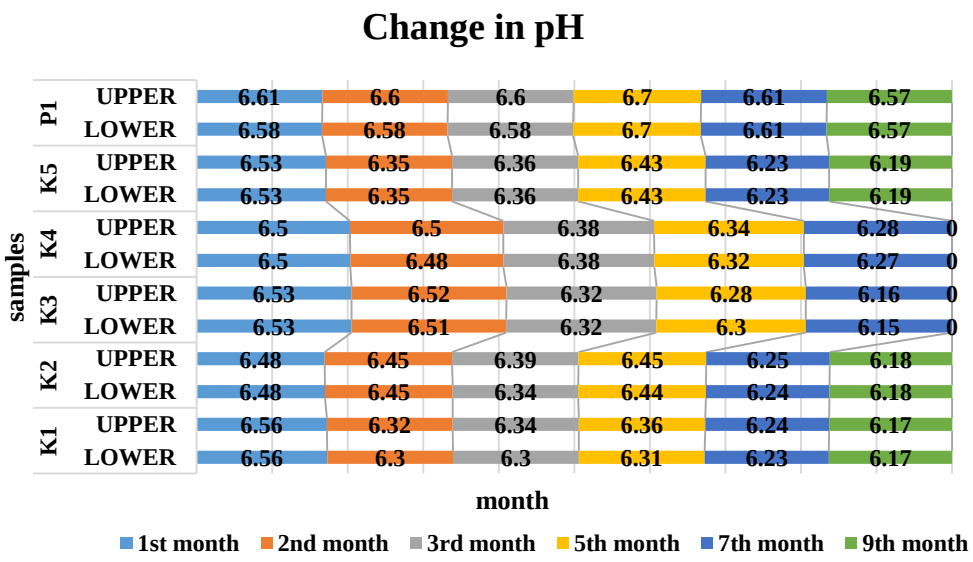


Figure 3.2. Changes of pH made with the different amount of k-Car during storage at 30° and the control sample stored at 6°C.

The pH of all the samples decreased with the storage duration at a higher temperature [74]; [75]. The pH of the samples varied from 6.53 to 6.17. Also, the upper phase of the samples had high pH when compared to the lower phase. It was apparent that high temperature had an effect on the pH of the EM with the addition of carrageenan stored at 30^0C. However, P1 samples were stable stored at 6°C and pH ranged between 6.57 to 6.62 even without the incorporation of hydrocolloid. On contrary, pH reached 6.14 at the 3rd month of storage for P2 samples stored at

30°C and spoiled after that month. From the Fig. 3.2, the pH of the milk found to be decreasing. This could be due to the hydrolysis of a peptide bond.

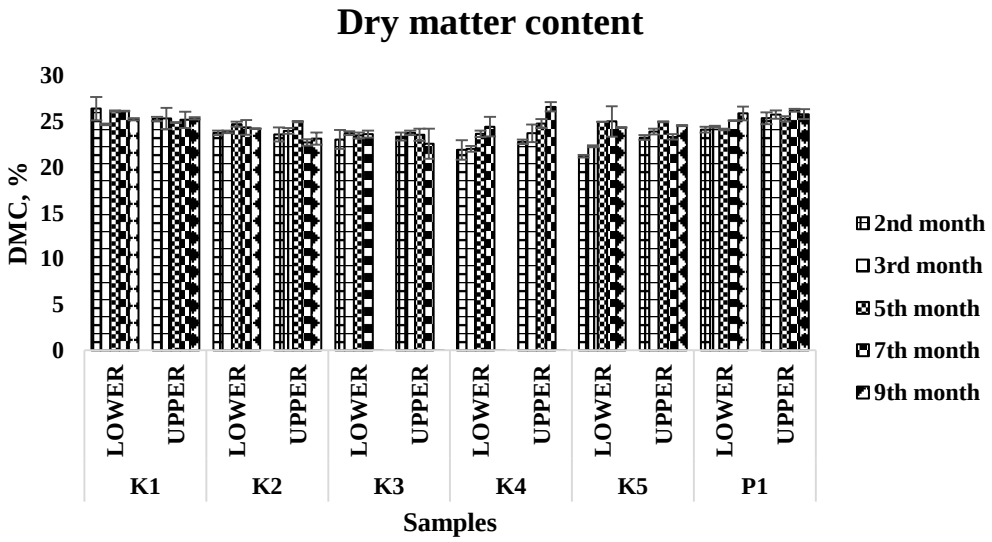


Figure 3.3. Changes in dry matter content made with the different amount of k-Car during storage at 30° and the control sample stored at 6°C.

The dry matter content (DMC) of the samples varied from 21.20 to 26.59 during the storage shown below in the Fig. 3.3. It was 25.32 before sterilization without the addition of polysaccharide. But after sterilization, DMC of the samples got reduced and then increased to 26.59 with the storage at 30^0C. Nonetheless, the P1 samples were found to be quite similar in their dry matter content. The fat content (FC) of the samples before sterilization without polysaccharide was 7.84. After sterilization, the FC of the samples ranged from 6.11 to 7.71 with the storage duration at 30^0C. Initially, the FC of the samples got reduced for the samples K1 and K2 whereas FC ranged between 6.5 to 7.7 for the K5 samples. The samples without polysaccharide stored at 6^0C were also quite stable and reached even 8. There were no significant changes in the DMC and FC. In all the k-Car samples with the increase in storage duration, upper phase found to have quite high DMC and FC compared to lower phases. This could be due to the reason that fat globule rose to the upper phase and thus the upper phase of the samples possessed more DMC and FC. As the P2 samples were spoiled, measurement was not taken after the 3rd month of storage.

The flow behavior of the samples was recorded for both upper and lower phases of all the samples. The samples were found to exhibit non-Newtonian pseudoplastic behavior which was greatly dependent on the shear rate is shown in the Fig. 3.4. Since R^2 was not great for Herschel-Bulkley equation, yield stress was not reported for the samples. And Ostwald equation was well

fitted with the flow curves and thus consistency coefficient and flow index of the samples was taken.

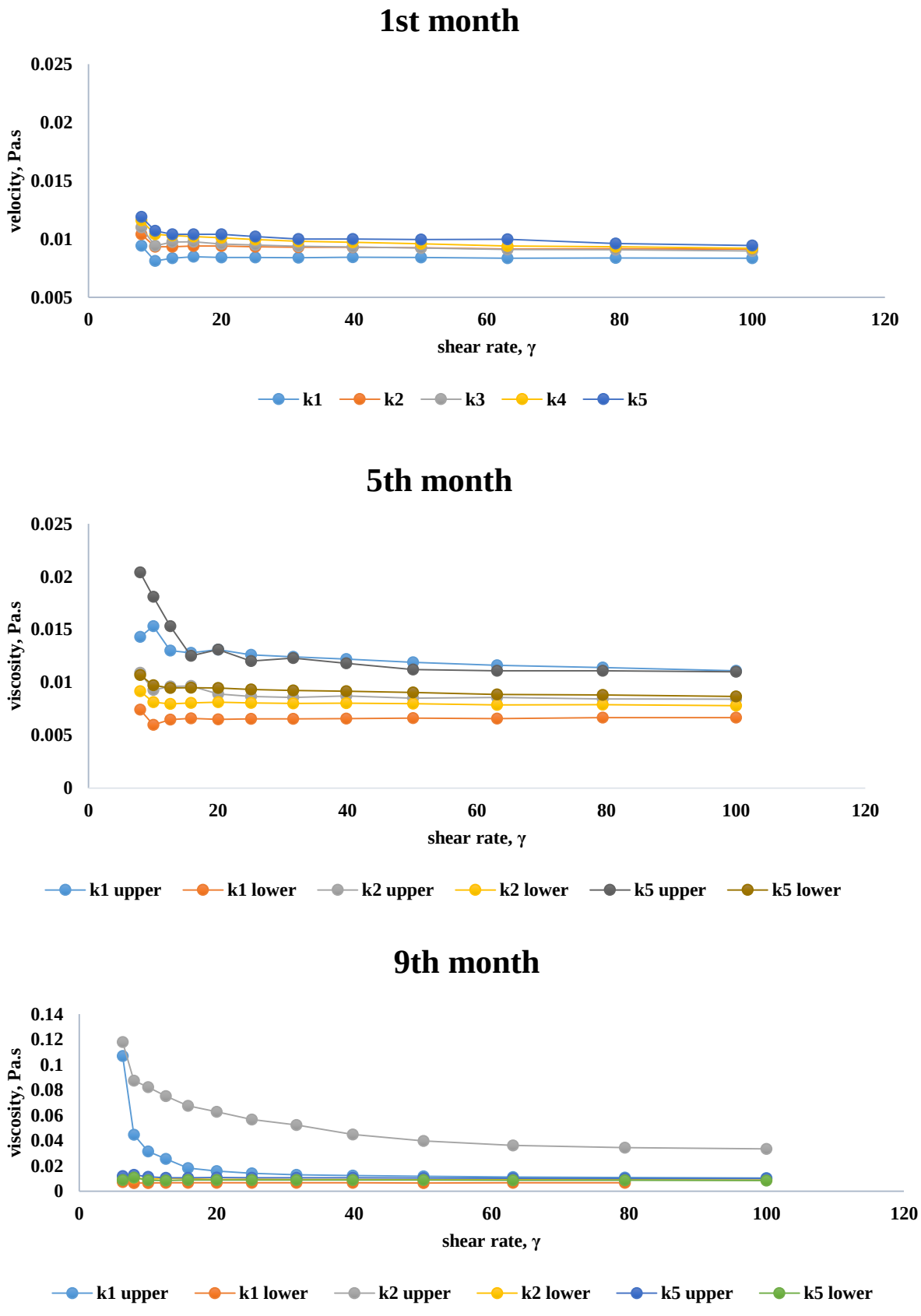


Figure 3.4. Viscosity curves of EM made with the different amount of k-Car during storage at 30°.

The viscosity curves of the upper phase of K1 and K2 samples recorded in the last month of the storage was greater. There was no much variation in the other months for the same samples. The upper layer found to be more viscous in the final month of measurement because fat got separated completely and raised to the top phase. The flow curves were recorded approximately between -0.2 to 0.2 Pa.s for both K1 and K2 samples throughout the storage duration.

The viscosity of the sample K3 reached 95 Pa.s for the upper phase in the 4th and later the samples were not stable. Hence the measurements were not carried for the further months. The viscosity curves were recorded around 0.1 to 0.2 Pa.s for the remaining months and the lower phases of all the samples for K3 and K4 samples. They got spoiled after the 7th month of storage. The viscosity was between -0.2 to 1.2 for the highest concentration (0.005%)of carrageenan and the samples were quite stable and there was no cream separation as well. The upper phase of the K5 samples was more viscous only in the 2nd and 3rd months. It could be the reason that those particular samples were not properly homogenized or that might be the optimum period for the intermolecular interaction between casein micelles and k-carrageenan of K5 concentration.

Table 3.1. Consistency coefficient of EM made with the different amount of k-Car during storage at 30°.

Upper phase	**2nd month**	**3rd month**	**5th month**	**7th month**	**9th month**
K1 (0.001%)	0.1538	0.1534	0.0339	0.0157	0.414
K2 (0.002%)	0.0139	0.0628	0.019	0.0351	0.8901
K3 (0.003%)	8.8605	11.997	0.3319	spoiled	spoiled
K4 (0.004%)	0.0414	0.0254	0.0584	spoiled	spoiled
K5 (0.005%)	0.1694	0.3625	0.0356	0.0277	0.0207
Lower phase	**1st month**	**2nd month**	**5th month**	**7th month**	**9th month**
K1 (0.001%)	0.0132	0.0418	0.0126	0.0145	0.0142
K2 (0.002%)	0.0118	0.0113	0.0158	0.0351	0.0101
K3 (0.003%)	0.015	0.4089	0.0559	spoiled	spoiled
K4 (0.004%)	0.0149	0.014	0.0144	spoiled	spoiled
K5 (0.005%)	0.0142	0.0133	0.0151	0.0133	0.0181

The consistency coefficient, K reflects the viscosity of the samples and it varied from 0.101 to 13.997 depending on the concentration of k-Car of the samples. It was found to be higher for the upper phases of the K3 (0.003%) samples ranged between 8.8605 to 11.997 summarized in the Table 3.1. The flow index (n) was inversely proportional to consistency coefficient (K). As the consistency coefficient increased, the flow index of the samples decreased. This is because, if the samples were more viscous, there would me be some resistant to the flow of the samples which

was explained by the factor flow index. The R^2 was above 0.8456 for the samples measured from laboratory scale experiment. The measurements were not taken for the unstable samples.

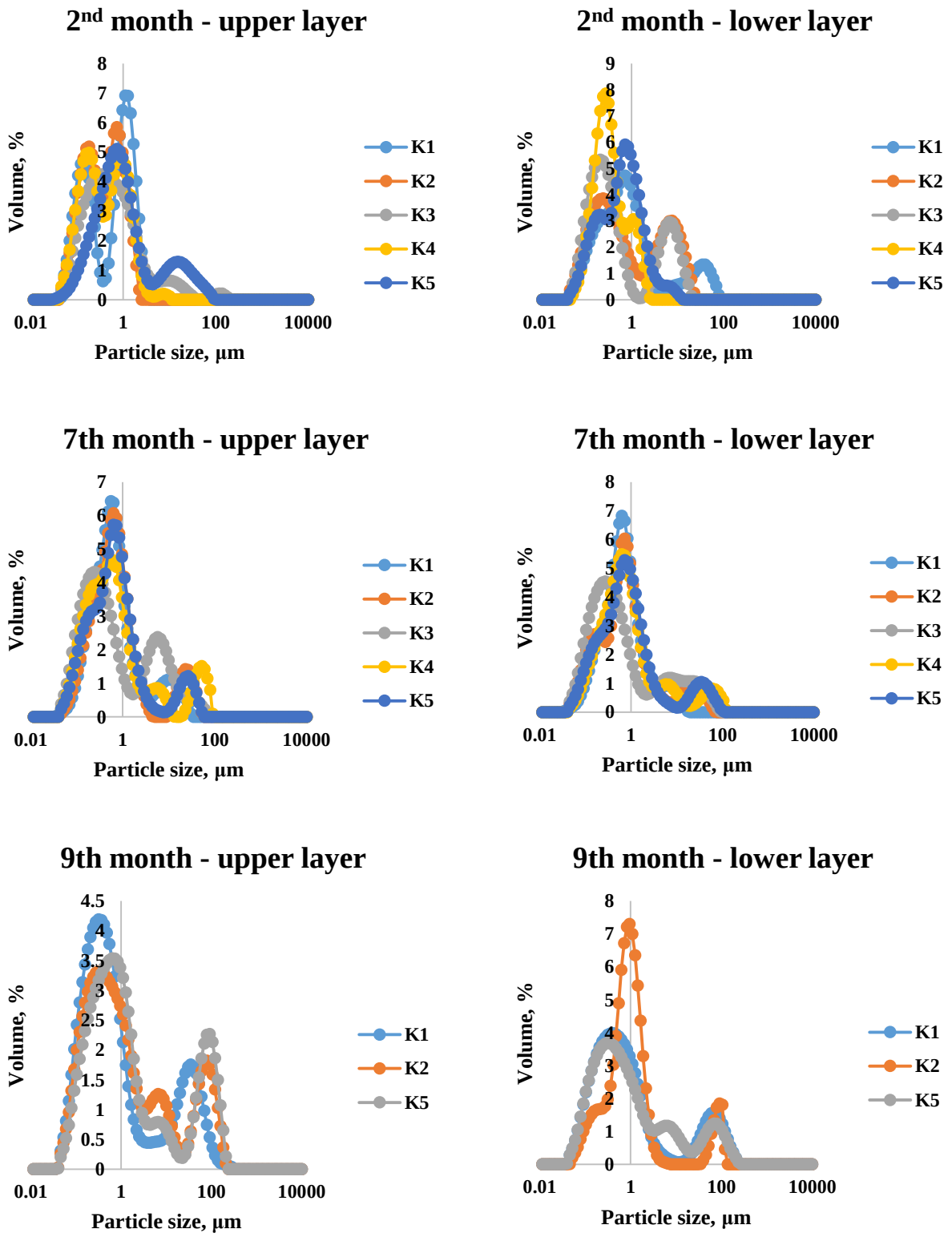


Figure 3.5. Distribution of particles (by volume) in the samples of EM made with the different amount of k-Car during storage at 30°.

The particle size distribution, volume and average particle size during the storage at 30°C were studied by laser diffraction technique to determine the variation in the above factors of evaporated milk. The logarithmic distribution (by volume) of the samples of both the phases taken in the 2nd, 7th and 9th month was shown in the Fig. 3.5. All the samples were distributed between 0.01 to 100 μm. Since the upper phase was more than the lower phase, the particle sizes of the upper phase of the samples were distributed widely by volume in the range above 10μm. From the Fig. 3.5, it was clearly seen that particles sizes seem to be increased slightly with the storage duration especially in the upper phase of the samples. There were only significant changes until the 7th month of storage. The size of the particles was greater in the upper phase and in both the phases in the 7th and 9th month of storage.

3.1.2. Results from industrial-scale experiment

Evaporated milk fortified with carrageenan of different concentration was prepared in SC Marijampoles Pieno Konsevai in the month of December 2016 and packed in a tin. From the literature, it was understood that for the first 3 months, there will not be any significant functional changes in the EM. Hence, measurements were taken from the month of March 2016 (3rd month) and done for consecutive months till 8th (4th month to 8th month) and the last measurement (12th month) was taken in the month of December 2017 where two separate phases were formed. All the samples were stored at 30°C. The effect of different concentration of carrageenan on physical-chemical characteristics, rheological properties and particle size distribution of the samples of EM was reported.

Due to high-temperature processing and storage at 30°C, change in the colour of the samples happened during the storage of Evaporated milk. There was a decrease in the values of L (lightness) and increase in the values of a (redness) and b (yellowness) summarized in Table 3.2. The increase in yellowness values also could be acidification of the milk during the storage. Hence, the discolouration occurs due to the formation of some brown colour products called pyrazines and melanoidin, polymerized molecules such as lactulose lysine and fructuloselysine and some acid molecules by enzymatic browning reaction [76]. It was seen that the browning increases suddenly during the last month of storage with the trait of a decrease in lightness (L*) and increase in yellowness (b*) in k-Car added EM stored at 30°C. However, according to the results reported by other researchers, in the case of sodium bicarbonate added as a neutralizer to EM, the effect of temperature was vivid and browning occurs rapidly in the initial stages of the storage [77].

Table 3.2. Variation in L*a* b* values in EM made with the different amount of k-Car during storage at 30°.

Lightness	K1 (0.001%)	K2 (0.002%)	K3 (0.003%)	K4 (0.004%)	K5 (0.005%)
3rd month	90.645±0.05	92.01±0.011	93.78±0.000	93.45±0.150	91.65±0.000
5th month	90.06±0.186	91.79±0.076	91.615±0.03	91.615±0.03	90.78±0.030
6th month	90.22±0.157	90.40±0.065	90.73±0.010	91.40±0.000	90.50±0.020
7th month	89.63±0.037	90.20±0.035	90.355±0.20	90.36±0.010	89.48±0.000
8th month	89.33±0.029	89.73±0.132	90.36±0.010	90.25±0.000	88.24±0.000
12th month	85.86±0.084	87.83±0.049	86.21±0.014	83.65±0.021	84.68±0.007
Redness	**K1 (0.001%)**	**K2 (0.002%)**	**K3 (0.003%)**	**K4 (0.004%)**	**K5 (0.005%)**
3rd month	1.525±0.000	1.540±0.014	1.315±0.000	1.435±0.000	1.535±0.000
5th month	1.750±0.010	1.815±0.007	1.950±0.010	1.895±0.000	2.130±0.000
6th month	1.910±0.000	2.105±0.000	2.070±0.000	2.320±0.000	2.305±0.007
7th month	1.970±0.000	2.090±0.000	2.120±0.000	2.415±0.020	2.415±0.020
8th month	2.250±0.007	2.370±0.010	2.640±0.000	2.620±0.000	2.620±0.010
12th month	3.725±0.000	3.735±0.007	3.780±0.155	4.120±0.028	3.355±0.007
Yellowness	**K1 (0.001%)**	**K2 (0.002%)**	**K3 (0.003%)**	**K4 (0.004%)**	**K5 (0.005%)**
3rd month	22.77±0.000	22.18±0.014	21.81±0.021	21.71±0.000	22.81±0.021
5th month	25.31±0.028	26.30±0.035	25.83±0.000	26.37±0.014	27.86±0.084
6th month	27.68±0.014	27.83±0.000	27.63±0.014	27.65±0.056	30.51±0.106
7th month	27.04±0.040	27.87±0.000	27.65±0.056	29.14±0.020	30.75±0.030
8th month	29.23±0.000	27.04±0.045	29.10±0.000	29.73±0.000	30.98±0.000
12th month	31.34±0.035	31.71±0.070	31.92±0.021	33.46±0.007	34.62±0.000

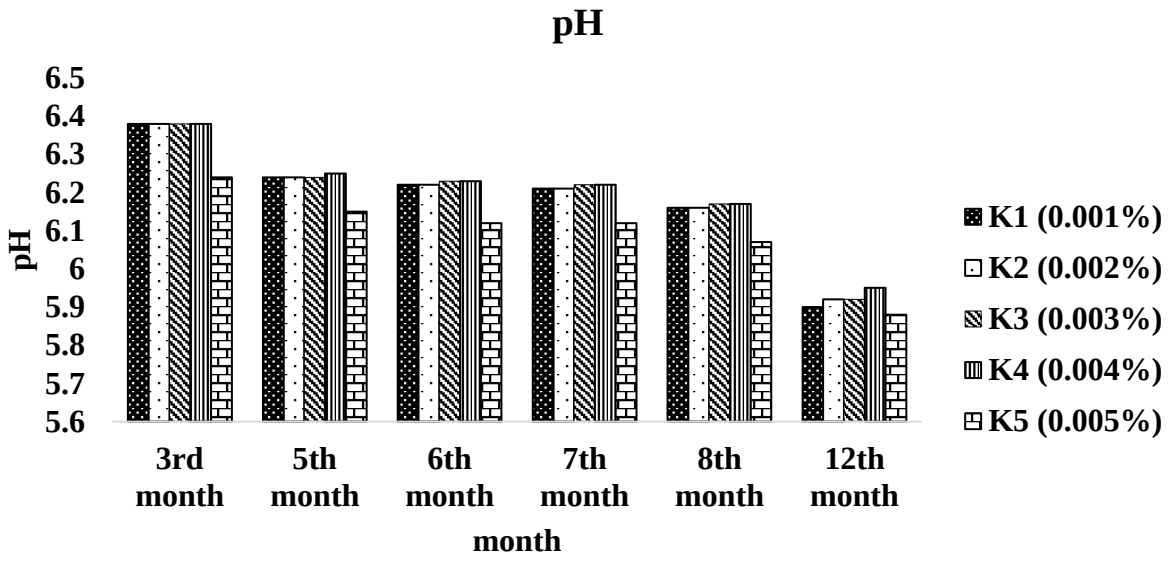


Figure 3.6. Variation in pH of EM made with the different amount of k-Car during storage at 30°.

There was a decrease in pH of EM during the storage at 30ºC shown in the Fig. 3.6. The pH of K5 sample was less when compared to other samples whereas pH of the other samples was quite similar. It was reported in the literature that pH of EM ranges from 6.0 to 6.5 and become unacceptable if pH goes below 5.9 [4]. In this study, pH was below 6 after the month of September (8 months later) where there was cream separation but there was no such phenomenon in K5 sample. The decrease in pH could be attributed to many reasons: 1. Maillard reaction, 2. The increase in non-casein nitrogen and non-protein nitrogen, 3. The formation of formic acid due to the degradation of lactose [78].

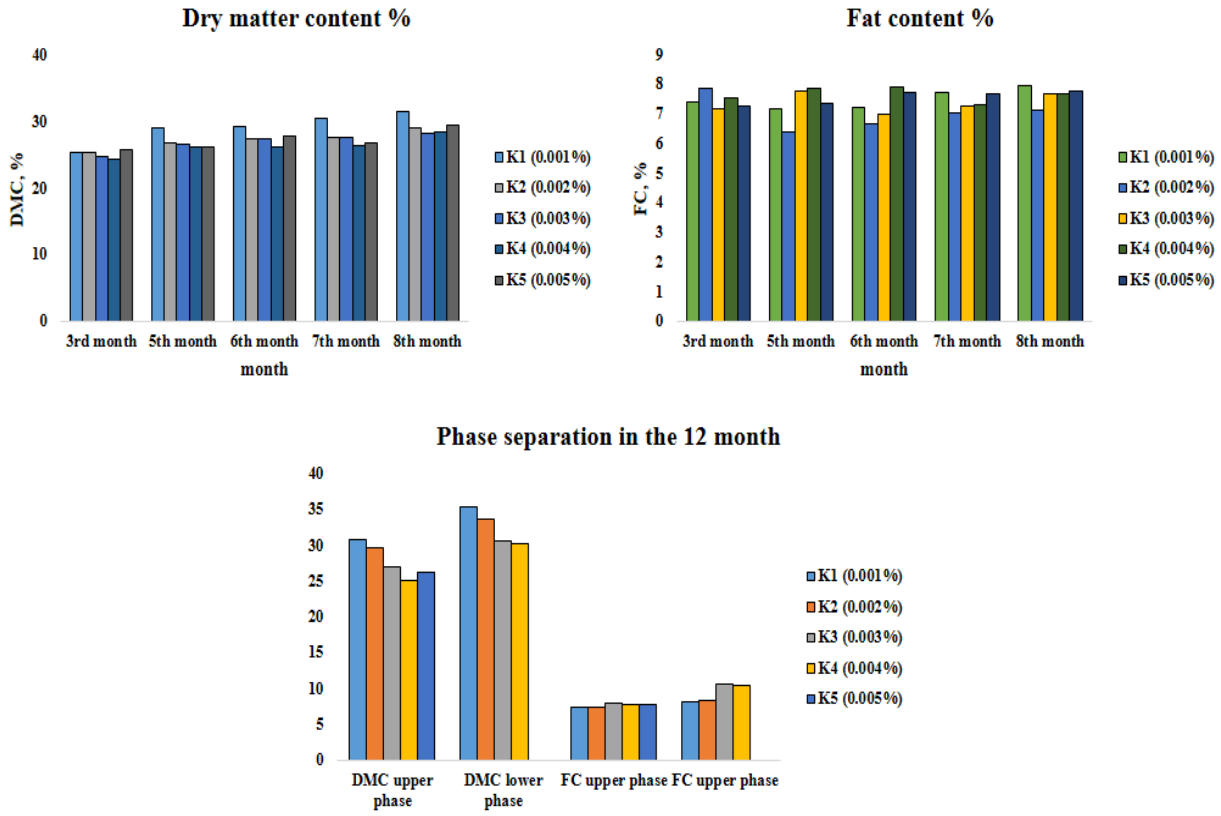


Figure 3.7. Variation in dry matter content, fat content and phase separation in the 12th month of EM made with the different amount of k-Car during storage at 30ºC.

The dry matter content of the samples ranged between 25.435 and 29.456 and the fat content of the samples ranged between 6.41 and 8.738. There were no significant changes during the storage at a higher temperature since the evaporated milk incorporated with the polysaccharide hydrocolloid, k-carrageenan. In the final measurement of two phases, upper phase found to have more dry matter content and fat content than the lower phase. It was due to the reason that fat globules moved towards the upper phase surrounded by the high amount of protein and thus the upper phase of the samples have higher dry matter and fat content. According to Schmidt, after 6

months of storage, dry matter and fat content would be more in the cream layer. The last sample (0.0005%) was found to be quite stable in physio-chemical changes and there were no uncertainties like fat separation, age thinning etc. It was clear that K5 concentration was more stable when compared to other samples since casein micelles interaction with k-carrageenan optimum and there is no gelation as well.

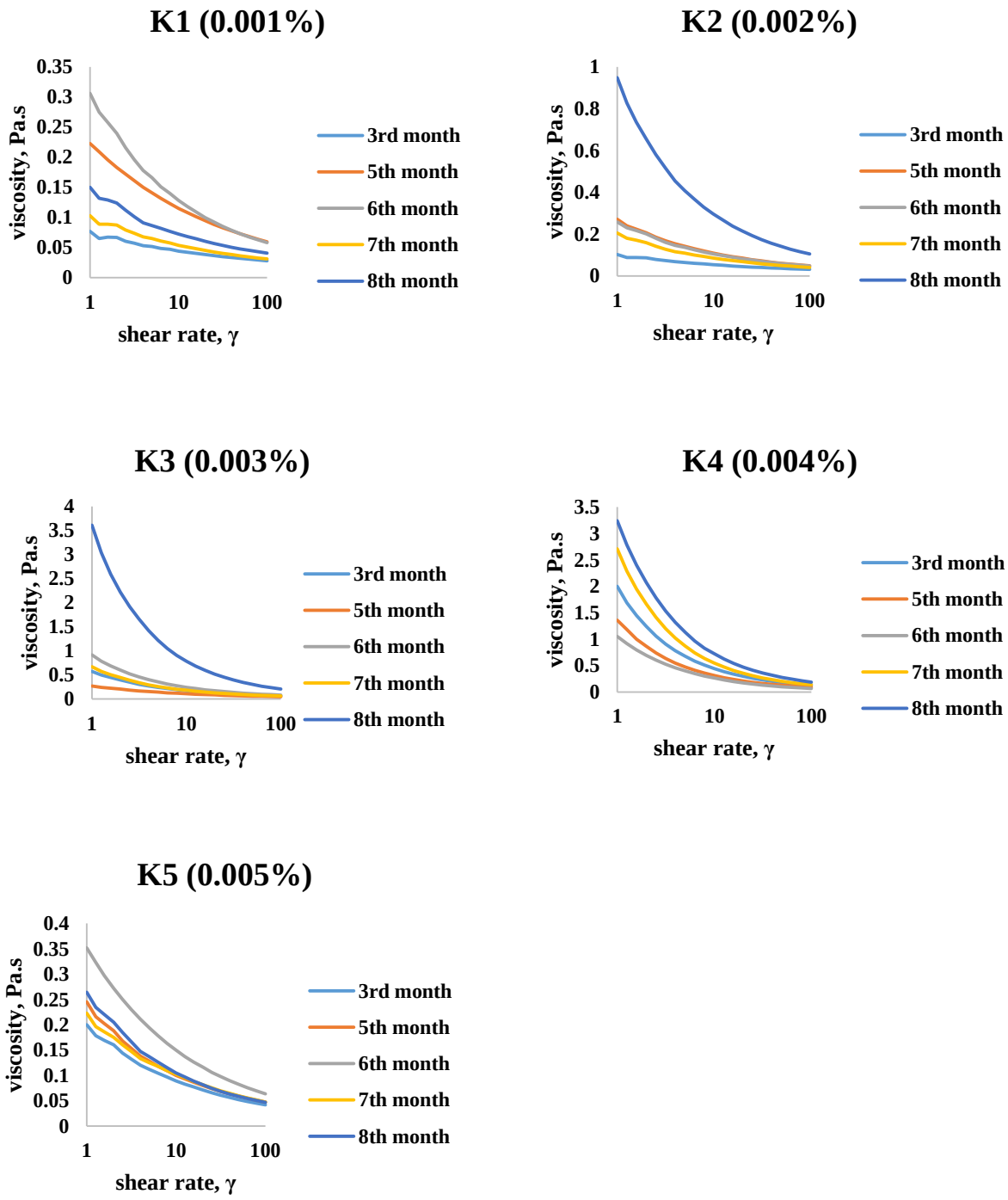


Figure 3.8. Effect of different concentration of carrageenan on the viscosity of evaporated milk at a storage temperature of 30°C.

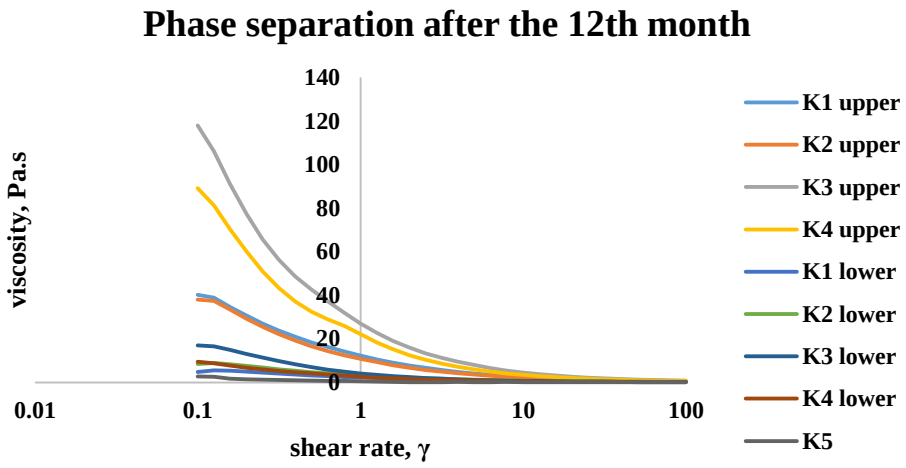


Figure 3.9. The viscosity curves of upper and lower phases of EM made with different concentration of k-Car after the 12th month of storage at 30°C.

The flow curves were recorded from 0.1 to 100s^{-1} shear rate for EM with different concentration of carrageenan for every month to study the stability of evaporated milk on the addition of k-carrageenan as a stabilizing or thickening agent. All the samples were found to exhibit pseudoplastic behavior because of the fact that above 22.3% solids-not-fat, fluid behaves as a non-Newtonian liquid [79]; [80]. The viscosity curves of the EM samples dependent to concentration and storage time shown in the Fig. 3.8. From the Fig. 3.9, it was clear that viscosity was greater for the upper layer than the lower layer greater in the 12th month of storage. The viscosity increases with the increase in the concentration of carrageenan [81]. This trait has been followed only till the month of July (7 months) until the concentration of 0.004% and for further months, K3 sample was found more viscous with the comparison of the other samples shown in Table 3.3.

The consistency coefficient and flow index were summarized in Table 3.3. The flow curves were well fitted with an Ostwald equation since R^2 was >0.98845 where consistency coefficient was denoted by K and flow index by n. Since it did not follow any trait, it was difficult to conclude the phenomenon. The consistency coefficient was directly proportional to the viscosity of the samples. It increased with the increase in viscosity. The flow index varied from 0.2645 to 0.8434 which complied with consistency coefficient. The flow index decreases with increases in the consistency coefficient.

The viscosity of the samples did not follow any trend conditional on the different concentration of k-Car. In the 12th month of measurement, the viscosity of the EM samples was more when compared to the viscosity of the other months and the viscosity of the upper phase of

all the samples was higher than the lower phase of the samples. However, samples K5 were found to be quite similar in viscosity throughout the storage period. Therefore, it could be the better concentration for the optimal interaction between casein micelles and k-carrageenan and the emulsion was more stable in terms of viscosity since the viscosity ranged between 0.217 to 1.410 Pa.s. Besides, there was no bridging flocculation that tends to provoke age gelation and no fat separation of milk.

Table 3.3. The rheological parameters and viscosity of the EM made with different concentration of k-Car at the $0.251s^{-1}$s shear rate during storage at 30°C.

Consistency coefficient, K	**K1 (0.001%)**	**K2 (0.002%)**	**K3 (0.003%)**	**K4 (0.004%)**	**K5 (0.005%)**
3rd month	0.0545±0.06	0.1278±0.16	0.6125±0.07	1.6550±0.88	0.1545±0.01
5th month	0.2234±0.45	0.2824±0.05	2.0267±0.03	1.2961±0.14	0.2058±0.03
6th month	0.2951±0.03	0.2390±0.01	0.8416±0.09	1.0603±0.014	0.3238±0.04
7th month	0.0762±0.01	0.2238±0.06	0.6648±0.00	2.6793±0.019	0.1854±0.02
8th month	0.1793±0.06	0.5430±0.00	2.4641±1.05	3.5681±0.053	0.7558±0.74
12th month (LP)	2.0996±0.06	3.0721±0.00	4.0136±1.05	2.397±0.0530	0.4820±0.74
12th month (UP)	11.35±1.694	10.251±0.57	24.862±1.05	10.251 ±0.45	
Flow index, n	**K1 (0.001%)**	**K2 (0.002%)**	**K3 (0.003%)**	**K4 (0.004%)**	**K5 (0.005%)**
3rd month	0.8434±0.02	0.3349±0.01	0.5749±0.12	0.3918±0.02	0.7266±0.03
5th month	0.7132±0.34	0.6406±0.02	0.3739±0.00	0.3905±0.02	0.6765±0.02
6th month	0.6706±0.01	0.6632±0.01	0.4623±0.01	0.3932±0.01	0.6331±0.01
7th month	0.8197±0.04	0.6731±0.02	0.4619±0.00	0.3290±0.01	0.7099±0.01
8th month	0.7155±0.04	0.5729±0.00	0.3872±0.01	0.3755±0.02	0.6087±0.07
12th month (LP)	0.4849±0.04	0.4393±0.00	0.3032±0.01	0.3379±0.02	0.3693±0.07
12th month (UP)	0.3693±0.65	0.3524±0.07	0.2645±0.87	0.3524±0.66	
viscosity, Pa.s at 0.0251/s	**K1 (0.001%)**	**K2 (0.002%)**	**K3 (0.003%)**	**K4 (0.004%)**	**K5 (0.005%)**
3rd month	0.110±0.06	11.00±0.99	1.390±0.18	5.420±2.62	0.264±0.78
5th month	0.226±0.15	0.543±0.11	5.375±0.06	3.360±0.44	0.387±0.05
6th month	0.537±0.06	0.452±0.03	1.855±0.05	2.595±0.13	0.576±0.02
7th month	0.135±0.02	0.424±0.11	1.57±0.028	7.375±0.14	0.217±0.20
8th month	0.340±0.12	1.560±0.65	6.090±2.75	9.06±0.021	1.410±1.36
12th month (LP)	4.500±0.12	6.800±0.65	11.40±2.75	5.96±0.021	1.320±1.36
12th month (UP)	27.00±0.24	25.50±0.76	65.70±1.59	51.0±0.720	

There was no great variation in the flow curves and the viscosity of the samples of K1 and K2 till the storage period of 8 months. Whereas for the samples K3 and K4, there was a great deviation in the viscosity of the samples throughout the storage duration and found to be more viscous when compared to the other concentrations because a large number of aggregates formed because of casein micelles and k-carrageenan interaction. The viscosity of the samples of K4 was

more than K3 samples in all months of storage except 5th and 12th month of storage. This could be due to improper homogenization during the processing step in the industry and liquid was quite unstable at 0.003% and 0.004%.

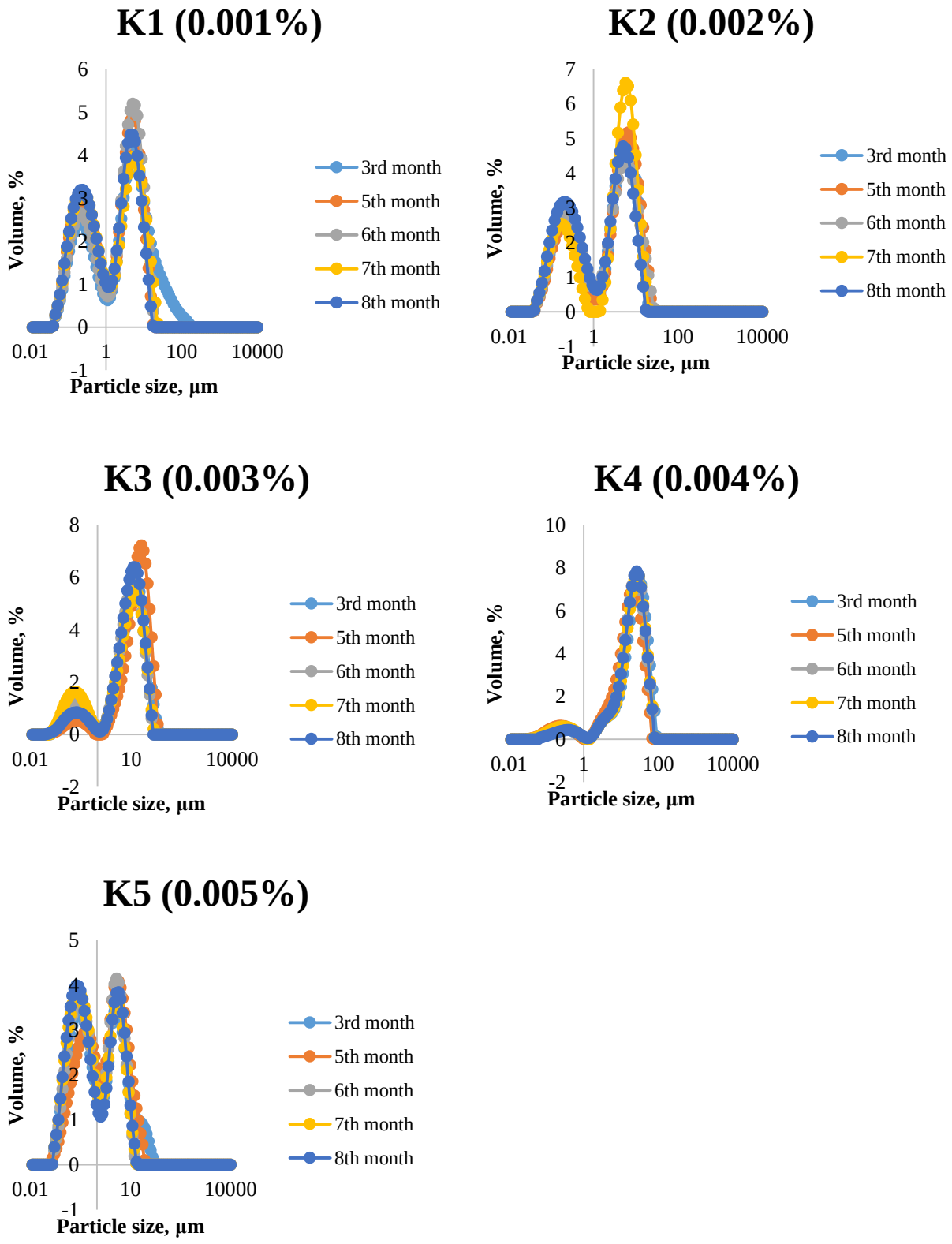


Figure 3.10. Particle size distribution of EM made with different concentration of k-Car during storage 30°C.

The particle size distribution, volume and average particle size diameter were dependent on the fat globule size, dry matter content, fat content and casein micelle interaction. In general, evaporated milk are concentrated by the evaporation process which intends to increase the ionic strength and decrease the pH of milk and therefore there is an increase in voluminosity and solubility of β-casein [82].

The distribution of particles varied widely among the concentration of carrageenan. For K1 and K2 samples, some of the particles ranged between 0.01 and 1µm and most of the particles ranged between 1 and 70µm approximate. K1 sample found to have a high percentage of volume in the 7th month whereas volume was greater in the second peak measured in the 8th month for the K2 sample. K3 and K4 samples were quite similar in their distribution and volume were greater around 6.5 and 7.5 respectively throughout the duration of storage.

The particle size fall before 100µm for the K3 samples and particles appeared till 100µm for K4 samples. Thus, the particle size increased with the increase in concentration. The sample K4 exhibited the uniform distribution from the first month till the end. Among all the concentration, K5 provided a unique trait that there were particles distributed equally from 0.01 to 1nm and from 1 to 75nm approximate. The K5 samples were stable and from the Fig. 3.10, it was clear that there was no formation of larger aggregates during the storage at 30°C. It was clearly understood that particle size distribution decreases with the increase in shear rate [83].

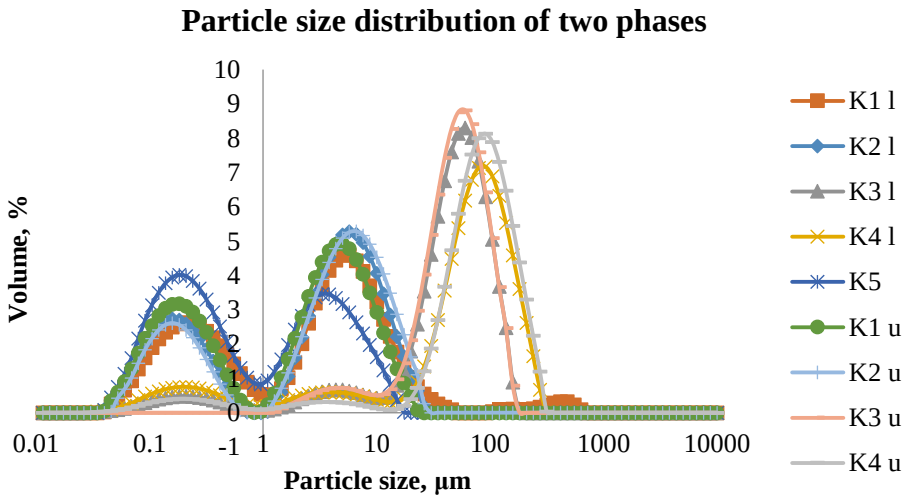


Figure 3.11. Particle size distribution of upper and lower phases of EM made with different concentration of k-Car after the 12th month of storage at 30°C.

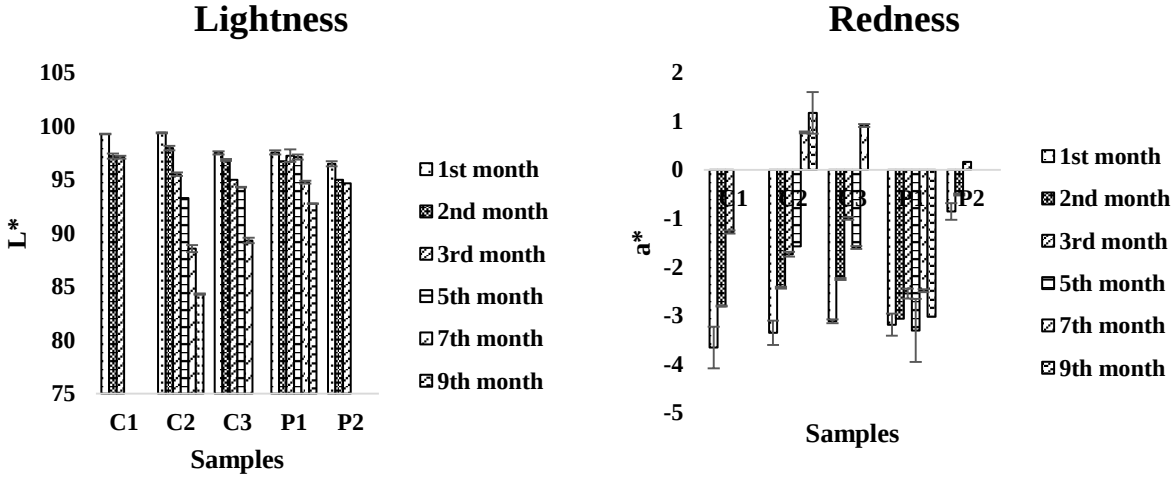
Though there was a cream separation of milk, samples followed the same trend in distributing the particles. The samples K3 and K4 exceeded the particle size of 100 nm. This could

be due to the accumulation of fat particles on the surface and caused bridging between milk protein and fat content. The sample K1 and K5 also possessed uniform distribution with the variation in the volume. The average particle diameter D [3,2] of the sample varied from 0.29 to 15.97. The sample K4 found to possess larger particle diameter followed by K3 sample. During the last month of storage, the samples K1, K2 and K5 were quite similar in diameter. The aggregates formed rely upon the concentration where K3 and K4 got more number of aggregates which also implied that possesses high-fat content [84].

The storage temperature at 30^0C and carrageenan concentration had a significant effect on the particle size and viscosity of the samples. This is because of rearrangement of the casein micelles interaction with the carrageenan lead to increase in the strength and number of hydrophobic or ionic bonds formation and occurrence of the tertiary and quaternary structure of casein micelles. Above 0.003% concentration of carrageenan, k-carrageenan interacts with carrageenan and forms a network. Therefore, sample exhibited a gel-like behavior. Whereas, the samples with 0.005% concentration of k-Car was stable without the formation of the larger aggregates [83].

3.2. The effect of carboxymethyl cellulose on the stability of evaporated milk

The three different concentrations of carboxymethylcellulose (CMC) were employed in the laboratory scale to the evaporated milk and the stability was studied during the storage at a higher temperature of about 30°C. C1 (0.001% of CMC), C2 (0.0005% of CMC) and C3 (0.00025% of CMC) were the three concentrations chosen to check out the stability. The milk seemed to be unstable for the concentration above 0.001%. The samples were prepared in the month of March of 2015 and the analyses were done for 6 times in 9 months. They have been compared with the control samples P1 and P1 without CMC stored at 6°C and 30°C respectively.



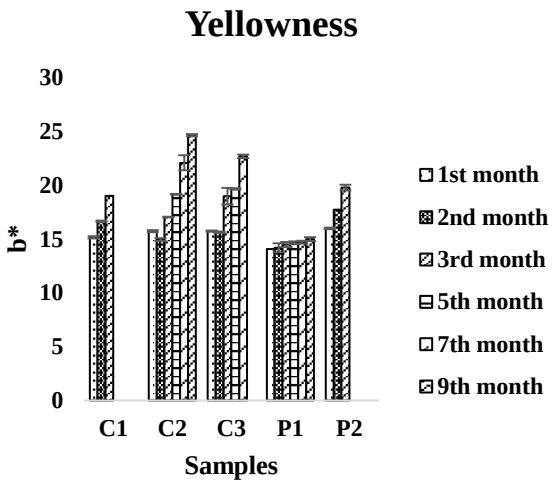


Figure 3.12. Changes in L*a*b* values of EM made with different concentration of CMC during storage at 30°C and the control samples stored at 6°C and 30°C.

The C1, C2 and C3 samples were stable until 3rd month, 9th month, 7th month of storage period respectively. The measurements were taken until the samples were stable and not taken for two separate phases since there were no significant differences between the phases of the samples. Due to the influence of sterilization and storage, the lightness values got decreased, redness and yellowness were increased shown in the Fig. 3.12. This is due to a phenomenon called Maillard reaction otherwise known as non-enzymatic browning. The other reason could be due to acidification of the milk. The C1 concentration samples and the samples without any stabilizing or thickening agent stored at 30°C followed the same trait and became unstable after the 3rd month of storage duration. From this, it was clear that concentration 0.001% of CMC and above were not feasible for the stability of evaporated milk. The redness values were negative until the 5th month of storage and then moved to the positive integer for C2 and C3 samples.

The pH of the EM with the addition of CMC ranged between 6.17 to 6.56. There was a slight decrease in the pH of the milk because of high-temperature storage. The samples with the concentration 0.001% of CMC attained pH below 5.9 after the 5th month of storage. This could be due to protein denaturation at 30°C which also resulted in gelation of milk. Both the phases of all the samples found to have similar pH with less that 0.01 standard deviation. The pH of the control samples stored at 30°C also attained pH below 5.9 after the 5th month of storage. From this, it was concluded that 0.001% of CMC effect on the stability of EM was similar to that of the P2 samples stored at 30°C.

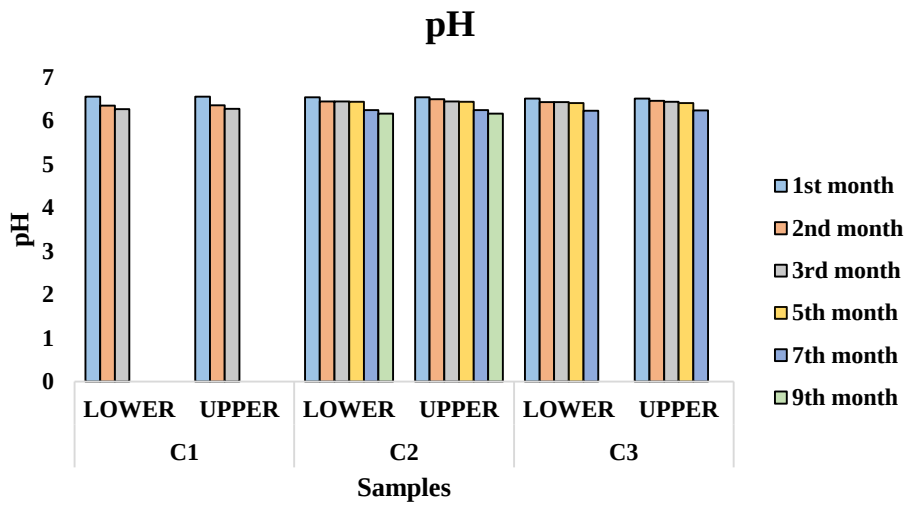
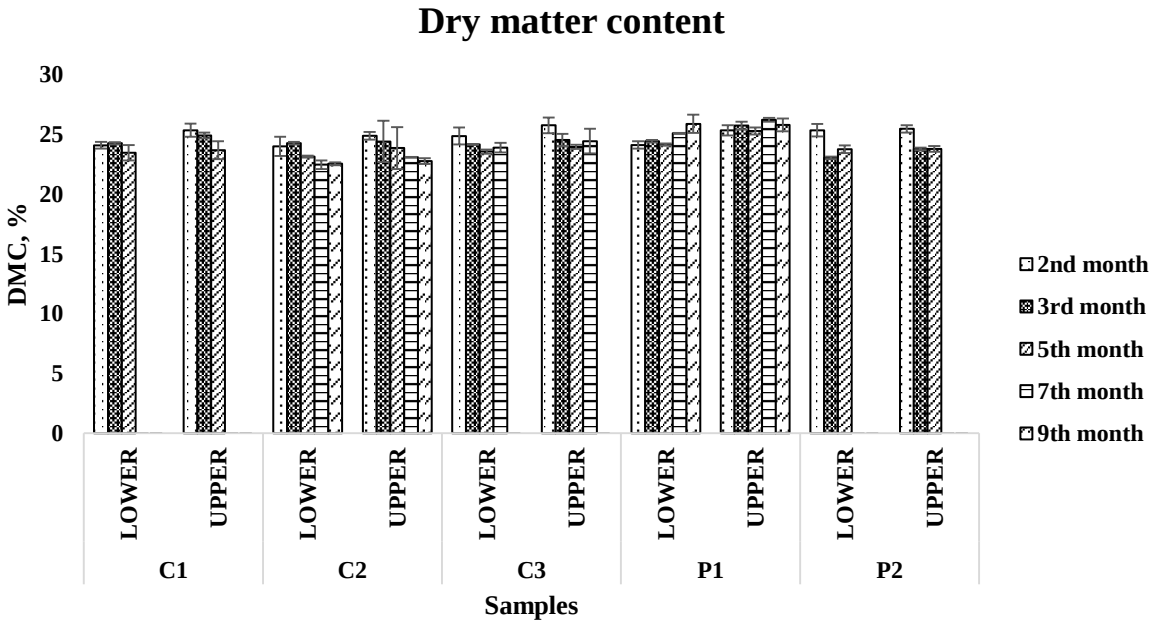


Figure 3.13. Change in pH of EM made with different concentration of CMC during storage at 30°C.

The dry matter content of the milk ranged between 23.09 to 25.75 for the samples incorporated with CMC at the different concentration shown in the Fig. 3.14. No significant changes. There was no particular trend followed during the storage. The upper phases found to be slightly high that the lower phase in %. The fat content of the samples ranged between 6.4 to 7.87 in %. The upper layer found to have more fat and dry matter content that the lower layer. Since the fat globules and protein move to the top of the storage container during the storage. There was no much standard deviation.



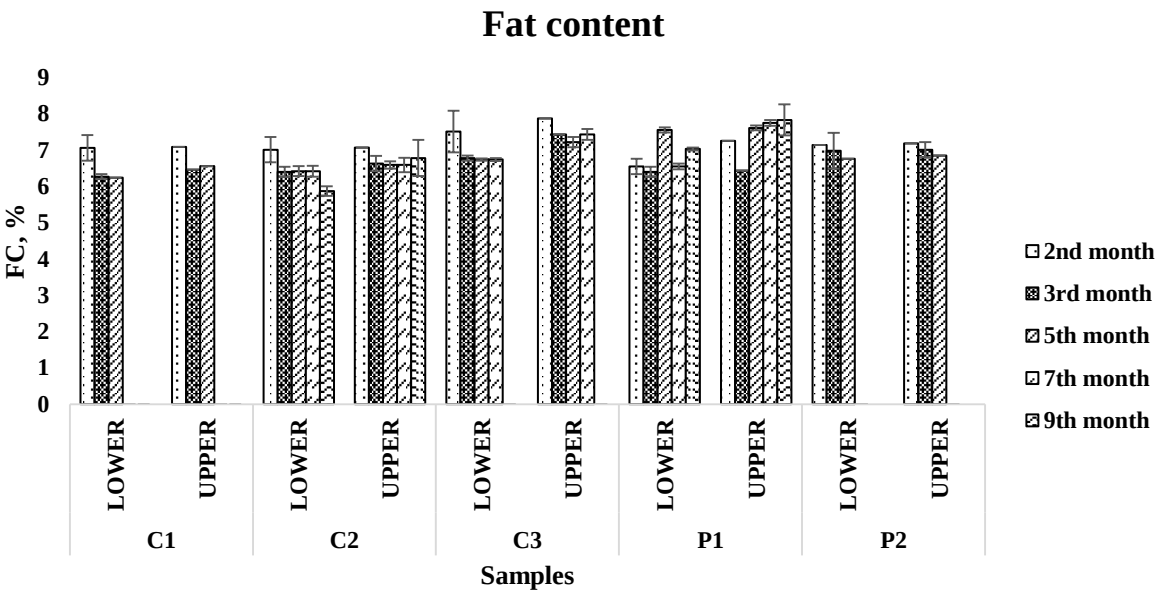


Figure 3.14. Change in dry matter and fat content of EM made with different concentration of CMC stored at 30°C and the control samples stored at 6°C and 30°C.

The variation in viscosity values related to the shear rate was determined at temperature 30°C. The viscosity curves of different concentration of CMC were shown in the Fig. 3.15. All the samples exhibited pseudoplastic behavior with some yield stress. In the first month of measurement, all the samples were seen similar. The C1 samples were increased in viscosity with the storage duration and found to be more viscous in the 3rd month especially the upper phase of the sample and became unstable after the same month. The viscosity curves of the C2 samples were same until the 5th month of storage and then increased slightly after the 7th month. The C3 concentration was unique that the viscosity of the sample increased till the 3rd month of storage followed by the decline in the viscosity. Among all the concentration, C3 found to more viscous and C2 samples were quite similar in their flow properties throughout the duration.

An Ostwald equation was fitted well for CMC employed samples as well. The viscosity was dependent on the shear rate. With the increase in shear rate, there was a decline in the viscosity of the samples. As the viscosity increases, there was a decrease in the flow index. The coefficients and viscosities at 0.251/s of shear rate were summarized in Table 3.4.

There was great variation among the coefficients of the samples. The consistency coefficient varied from 0.0115 to 23.547. The coefficient factors and viscosity completely dependent on the concentration of the hydrocolloid added to the samples. The highest viscosity was 74 at 0.251/s of shear rate obtained in the sample C3 in the 3rd month of storage and in the further storage months, the viscosity was decreased. This could be due to the shear thinning

behavior of C3 sample with the increase in duration. The samples were not homogenized by special equipment in a laboratory scale for uniform dispersion of hydrocolloid in the sample. Some of the samples were destroyed after a day period of storage. It was difficult to provide a proper homogenizing effect to the samples and those samples could not withstand the sterilization temperature.

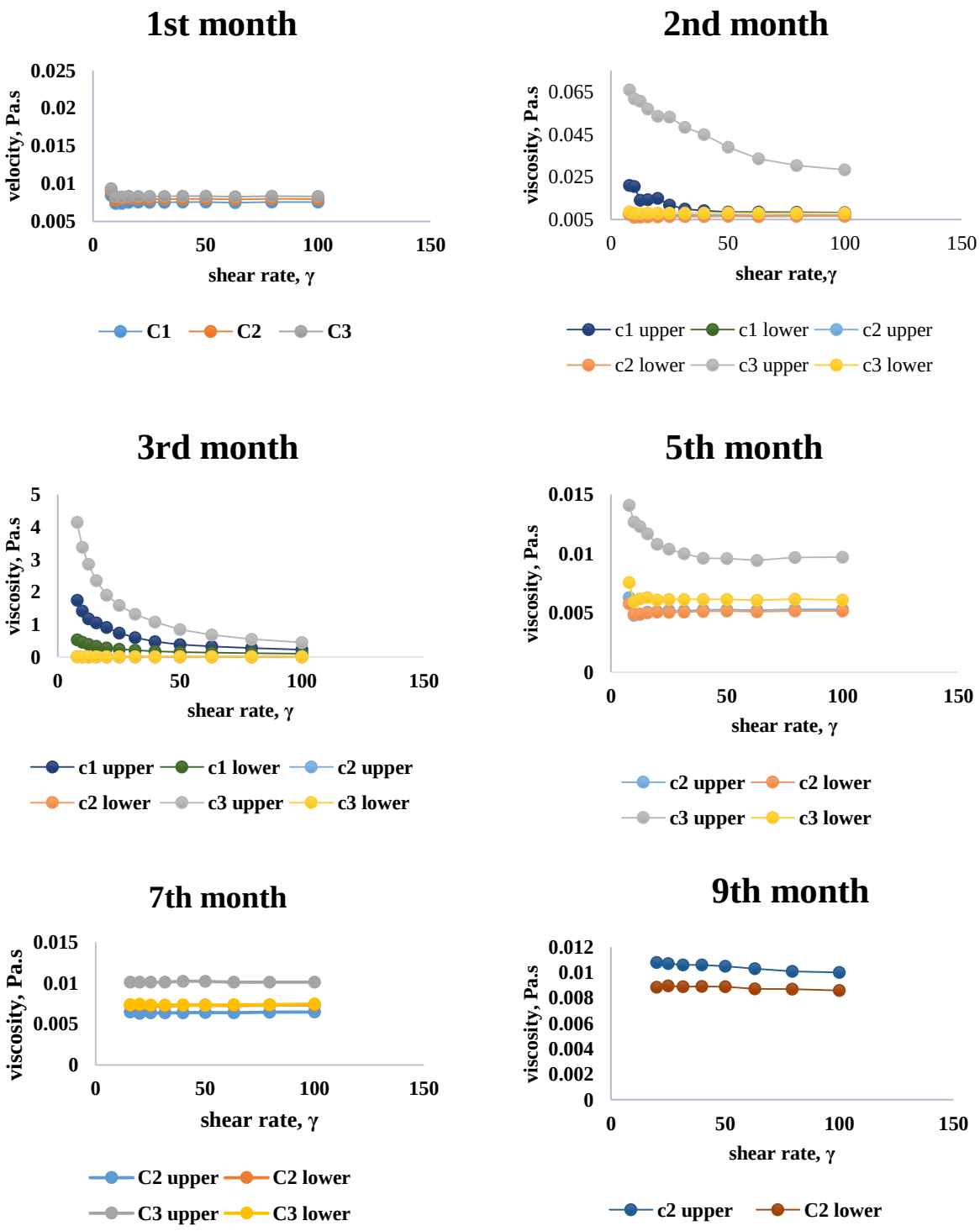


Figure 3.15. The viscosity curves of EM made with different concentration of CMC stored at 30°C.

Table 3.4. The rheological coefficients and viscosity at 0.251/s of shear rate of EM made with different concentration of CMC stored at 30°C.

Consistency coefficient, K					
Upper phase	**2nd month**	**3rd month**	**5th month**	**7th month**	**9th month**
C1 (0.001%)	0.1694	10.849		spoiled	
C2 (0.0005%)	0.1520	0.0358	0.0115	0.0126	0.3948
C3 (0.00025%)	0.0124	23.547	0.0295	0.0153	spoiled
Lower phase	**1st month**	**2nd month**	**5th month**	**7th month**	**9th month**
C1 (0.001%)	0.0142	2.1450		spoiled	
C2 (0.0005%)	0.0822	0.0117	0.0088	0.0119	0.0146
C3 (0.00025%)	0.0127	0.0169	0.0176	0.0153	spoiled
Flow index, n					
Lower phase	**1st month**	**2nd month**	**5th month**	**7th month**	**9th month**
C1 (0.001%)	0.7279	0.1431		spoiled	
C2 (0.0005%)	0.7868	0.8028	0.7455	0.8021	0.2850
C3 (0.00025%)	0.4730	0.1528	0.6701	0.7321	spoiled
Lower phase	**1st month**	**2nd month**	**5th month**	**7th month**	**9th month**
C1 (0.001%)	0.4150	0.3325		spoiled	
C2 (0.0005%)	0.7988	0.1523	0.8209	0.8209	0.7458
C3 (0.00025%)	0.7935	0.8478	0.6698	0.8691	spoiled
Viscosity at 0.251/s of shear rate, Pa.s					
Upper phase	**2nd month**	**3rd month**	**5th month**	**7th month**	**9th month**
C1 (0.001%)	0.2520	36.20		spoiled	
C2 (0.0005%)	0.0374	0.136	0.0381	0.0244	0.9360
C3 (0.00025%)	0.6000	74.00	0.1450	0.0683	spoiled
Lower phase	**1st month**	**2nd month**	**5th month**	**7th month**	**9th month**
C1 (0.001%)	0.0524	5.4800		spoiled	
C2 (0.0005%)	0.0459	0.0341	0.0373	0.0191	0.0465
C3 (0.00025%)	0.0434	0.0450	0.0579	0.0374	spoiled

There was a decrease in the size of the particle of the samples with the increase in the shear rate. From the literature, it was also understood that the concentration of hydrocolloids added to stabilize the emulsion, processing and storage temperature influenced greatly on the particle sizes of the samples. In the Fig. 3.16, it was seen that there were no significant differences among two phases of the samples. In a 3rd month, the C2 samples found to be quite different from the other

samples and the particle size was greater for the other samples in the range between 1 and 75µm approximate.

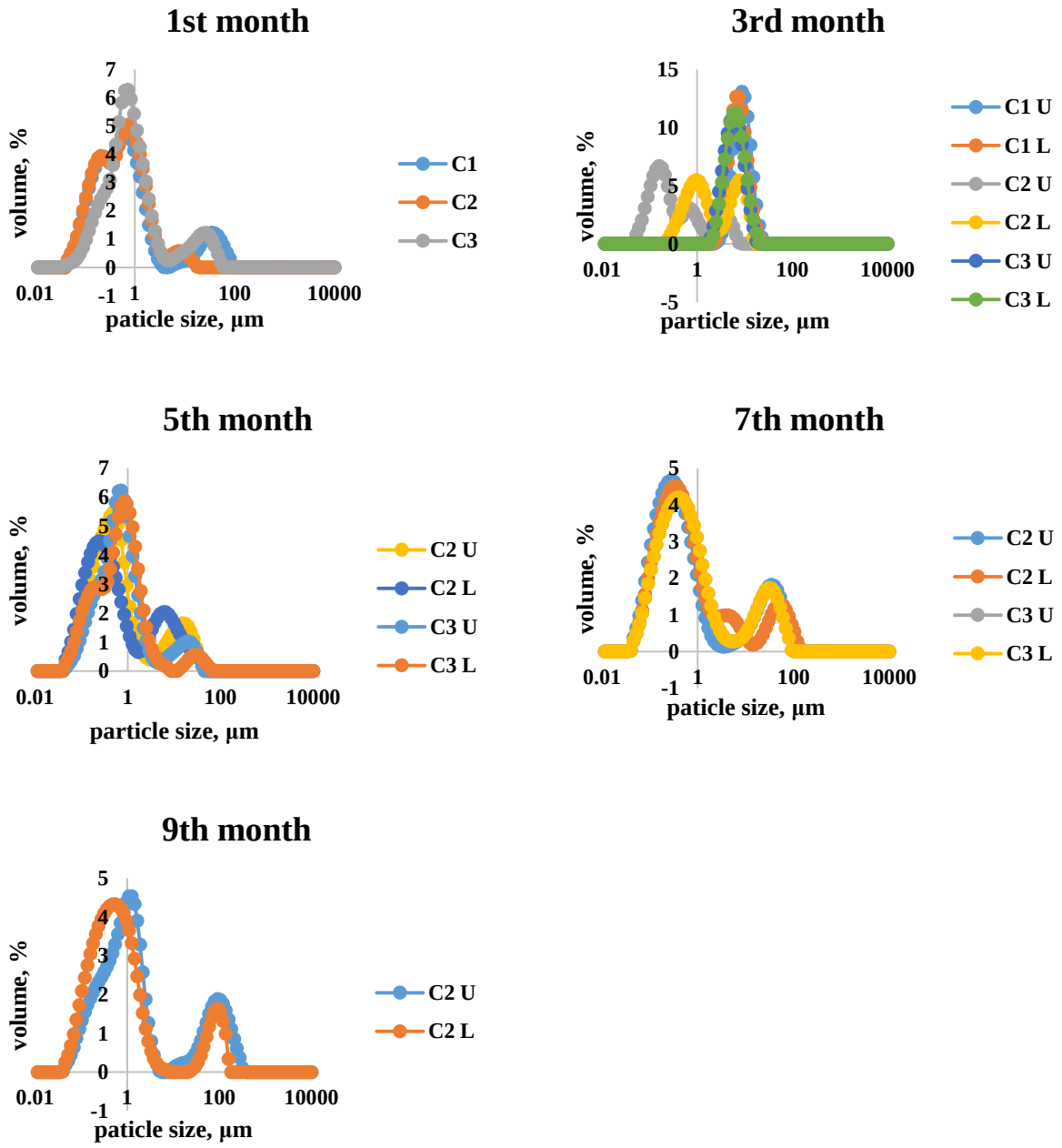


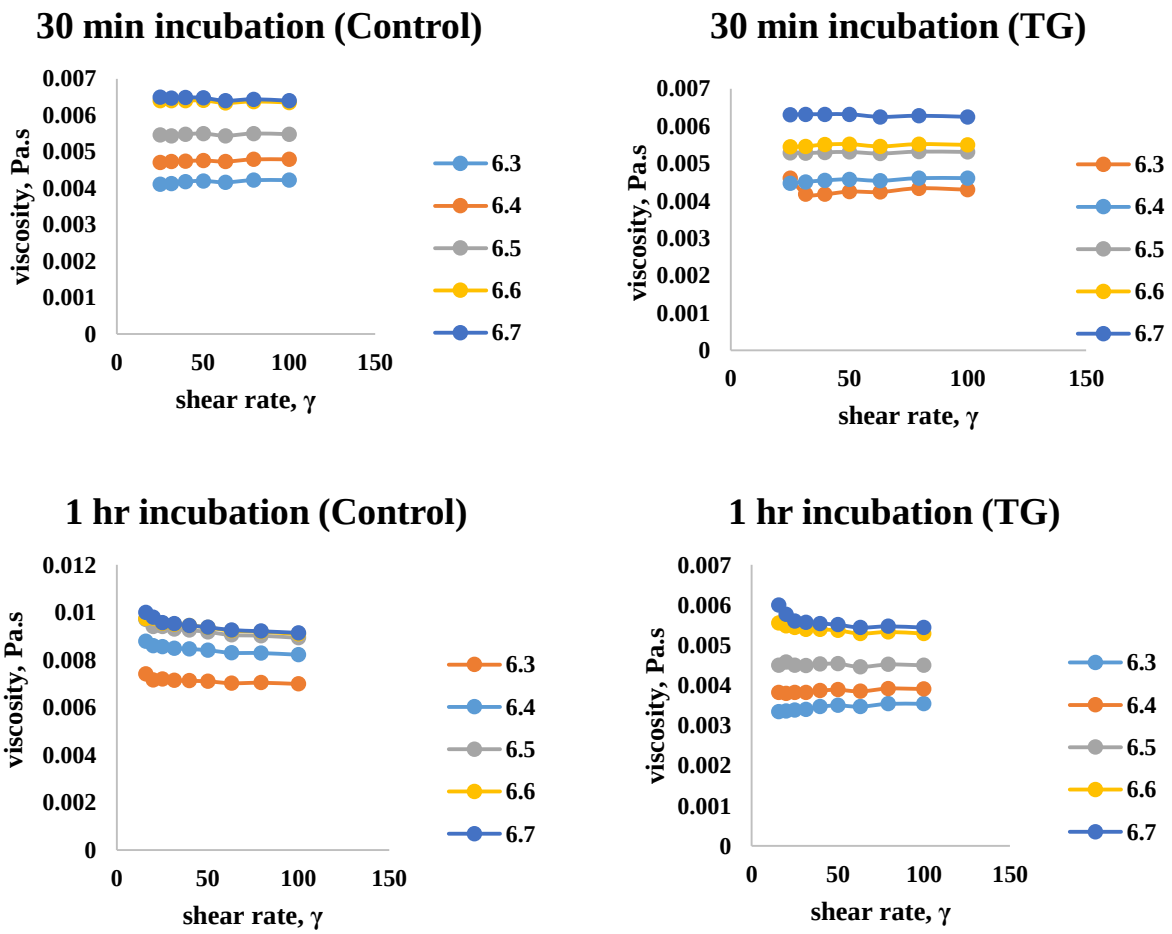
Figure 3.16. Particle size distribution of EM made with different concentration of CMC during storage at 30°C.

In the 5th month of storage, C3 sample of both the phases was dominant in the range between 0.01 and 50µm. The same trend was followed in the next month as well. Among all the samples, the C2 sample was stable until 9th month and there was no increase in the particle of the samples with the increase in the storage duration. Both the flow curves and the size distribution of the particle found to be similar throughout the storage duration.

3.3. The effect of microbial transglutaminase on the stability of evaporated milk

3.3.1. The effect of microbial transglutaminase on the physical properties of skimmed milk

The two different experiments were conducted with TG employed in the skimmed milk. In the first experiment, the samples of skimmed milk were incubated with TG at 50°C for 30, 60, 120 and 180 minutes. The second experiment was done by incubating the samples at 10°C for about 24 hours. The pH of the samples ranged between 6.3 and 6.7. The experiments were carried out for different enzyme activity (40, 80 and 120 U g^{-1} of protein) as well. The aim of these experiments was to find the conditions for TG inoculation in evaporated milk causing the most pronounced effect on the viscosity of the milk. It is known that the TG action on the casein can be due to the: introducing intra-molecular bonds to micelle cores, rending the micelles more stable under different treatment; by cross-linking the caseins at the surface of the micelle, the strength of gels formed by cross-linked micelles was increased.



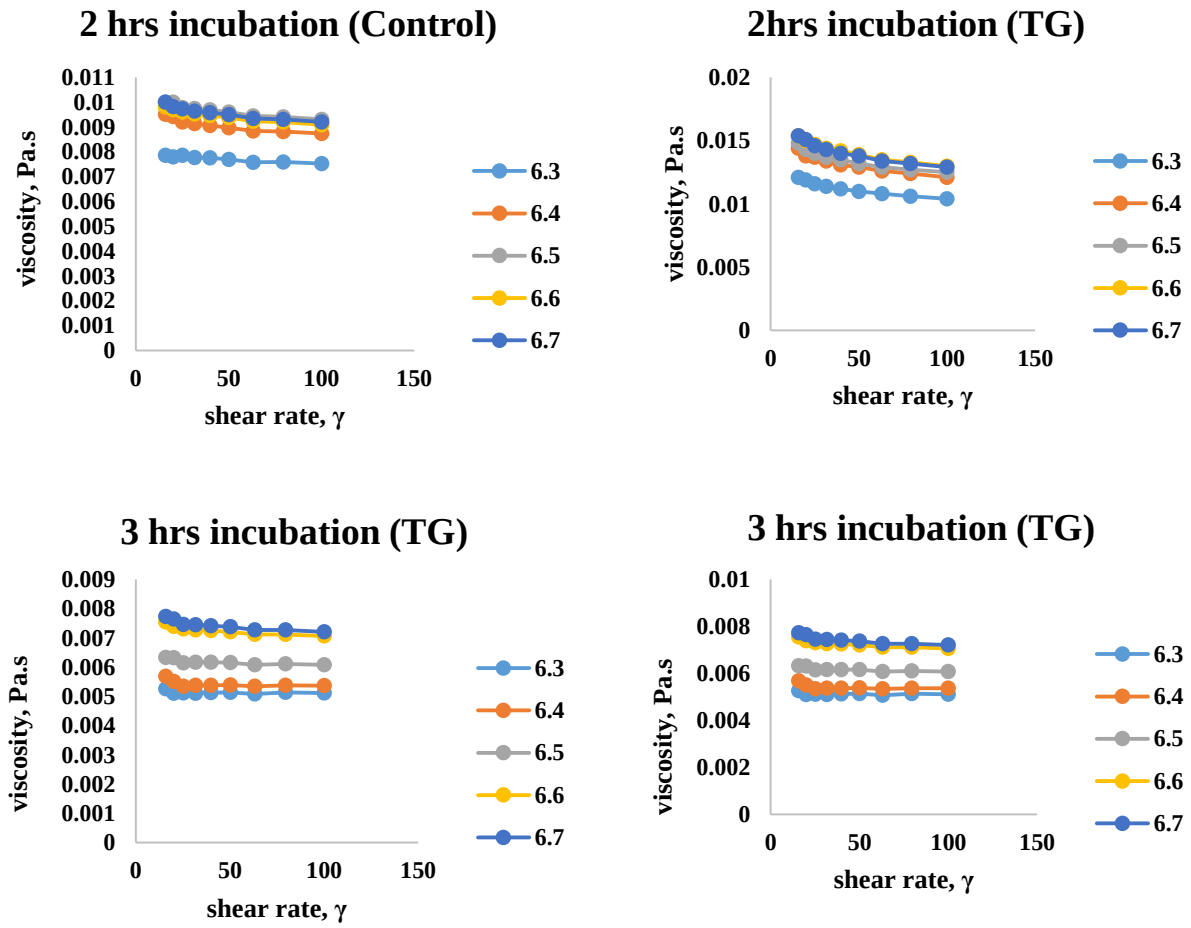


Figure 3.17. The effect of 40U g^{-1} of the protein of TG at different incubation period on the viscosity curves of skim milk at pH 6.3 to 6.7 (control samples-skim milk without TG).

The flow curves, consistency coefficient, flow index and viscosity of all the samples were obtained. They were compared with the control samples (skim milk samples without TG inoculation) to check out the influence of 40U g^{-1} of protein TG on the viscosity curves of the samples shown in the Fig. 3.17. The variable chosen here was incubation period and pH, adjusted by the addition of either 1M NaOH or 1M HCl. All the samples were incubated at 50°C. The flow curves were recorded at a shear rate from 0.1 to 100s^{-1}.

All the samples have shown similar kind of flow behavior. It was also seen that 6.7 pH samples are little more viscous when compared to another pH. There was no significant difference between control and TG treated samples on incubation period for 30 minutes and 1 hour. Whereas, the viscosity was higher for the samples with TG at 2hours incubation period. At 3hours incubation period, the viscosity of the samples was little higher than the control samples. There was a trait that viscosity increased with increase in pH of the milk. From this, it was vivid that, more the alkaline nature, higher the viscosity of the samples. On the other hand, the viscosity was also increased with the increase in the incubation period of the enzyme treatment.

Table 3.5. The effect of different TG activity and pH on the rheological properties of skim milk incubated with the enzyme for 1-hour at 50°C (C-skim milk without TG).

Consistency coefficient				
pH	**K(C)**	**K (40U)**	**K (80U)**	**K(120U)**
6.3	0.00672	0.01073	0.00887	0.01047
6.4	0.00831	0.01102	0.00908	0.00965
6.5	0.01049	0.01104	0.01212	0.01152
6.6	0.01009	0.01126	0.01273	0.00811
6.7	0.00285	0.01013	0.01233	0.01015
Flow index				
pH	**n (C)**	**n (40U)**	**n (80U)**	**n (120U)**
6.3	0.84816	0.67462	0.76841	0.68735
6.4	0.82762	0.70226	0.79453	0.74190
6.5	0.79253	0.75112	0.73413	0.71830
6.6	0.80822	0.76339	0.74241	0.87439
6.7	0.54470	0.80508	0.75620	0.78912
viscosity at 1/s of shear rate, Pa.s				
pH	**γ (C)**	**γ (40U)**	**γ (80U)**	**γ (120U)**
6.3	0.00857	0.01340	0.01240	0.01340
6.4	0.00860	0.01390	0.00998	0.01250
6.5	0.01230	0.01290	0.01460	0.01050
6.6	0.00949	0.00982	0.01530	0.01410
6.7	0.00520	0.00946	0.01350	0.00923

The shear state properties were obtained by increasing the enzyme activity of transglutaminase up to 120U g^{-1} of the protein of TG and incubated for an hour at 50°C. The flow curve coefficients and the viscosities were summarized in the Table 3.5. No particular trait was followed. The consistency coefficient ranged between 0.00672 to 0.02285, flow index ranged between 0.54470 to 0.87439 and the viscosity was between 0.00857 to 0.1530 Pa.s. All the samples were heat stable. The effect of different enzyme activity and incubation period on the viscosity of the skim milk was not great. Hence the experiment was carried out at low temperature for the long span to increase the viscosity to the greater extent.

The second experiment was conducted for different enzyme activity ranged from 6.3 to 6.7 at a low incubation period of 10°C for 24 hours. The rheological properties and particle size distribution were obtained for the samples. There was a decrease in the viscosity of the sample with the increase in the shear rate. The flow curves were well adapted to an Ostwald equation and R^2 was above 0.9876. There was no great difference between viscosity curves of the control (skim milk without TG) and the enzyme treated samples shown in the Fig. 3.18. But the slight increase

in the viscosity of the samples with the increase in pH was recorded. With the viscosity curves, it was difficult to obtain the conclusion regarding the effect of the enzyme on the viscosity of the skimmed milk.

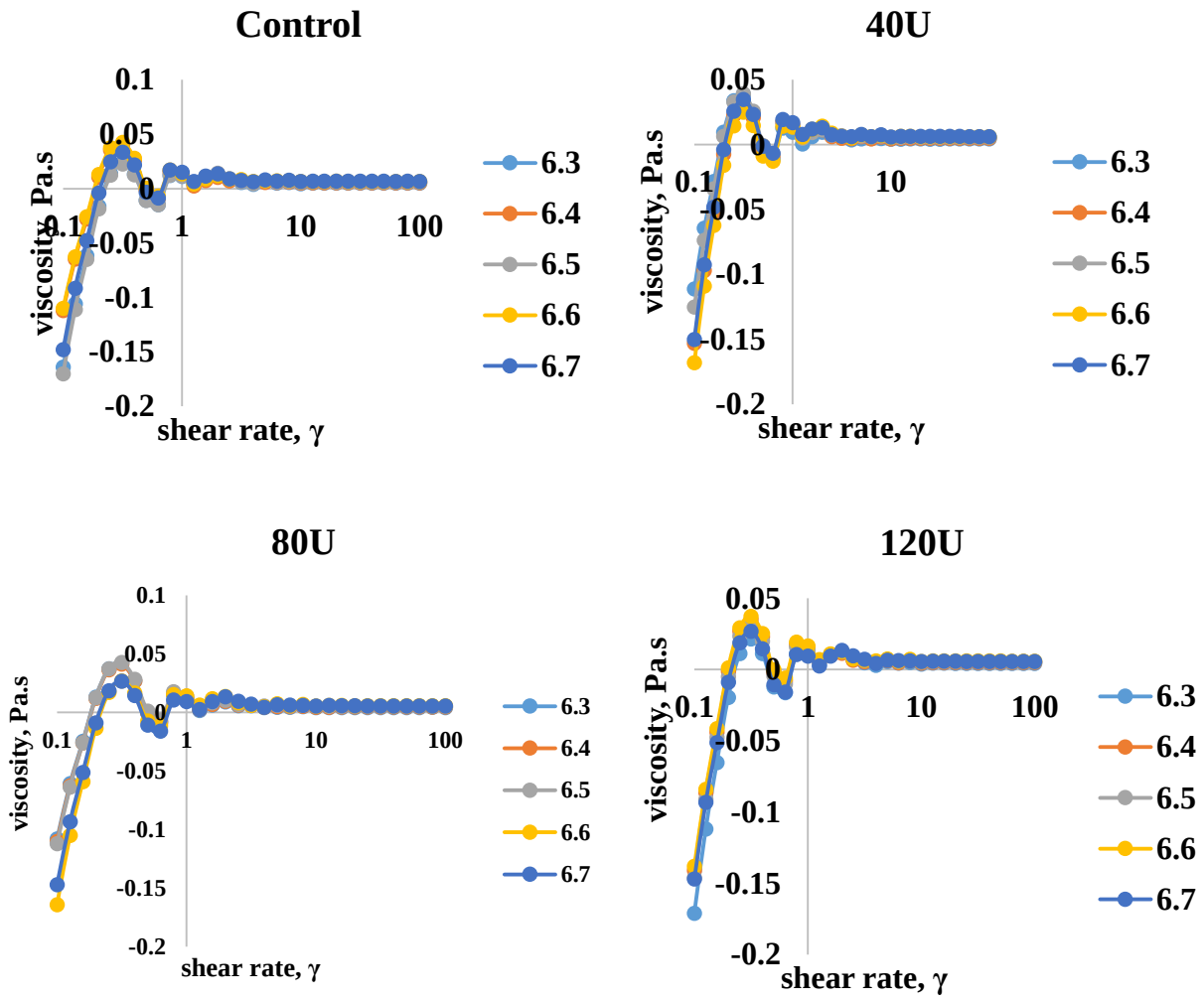


Figure 3.18. The effect of different TG activity and pH on the viscosity curves of skim milk samples incubated with TG at 10°C (Control-skim milk without TG).

Hence the particle size distribution of all the samples obtained for different pH and enzyme activity shown in the Fig. 3.19. The particles were distributed similarly for both control and all enzyme treated samples till pH 6.5 and the size of the particles fallen below 10µm. The size of the particles has been increased for pH 6.6 and 6.7 and ranged above 10µm. The particle sizes increased by volume with the increase in the TG activity of the samples. The cross-linkage between casein micelles and the enzyme transglutaminase was prominent with the increase in the enzyme activity and incubation duration. The low-temperature incubation might inhibit the growth of microorganisms and thus increase the stability of the samples. Among the two different protocol,

the experiment conducted at low-temperature incubation for long span has got an advantage in terms of both viscosity and stability of the milk.

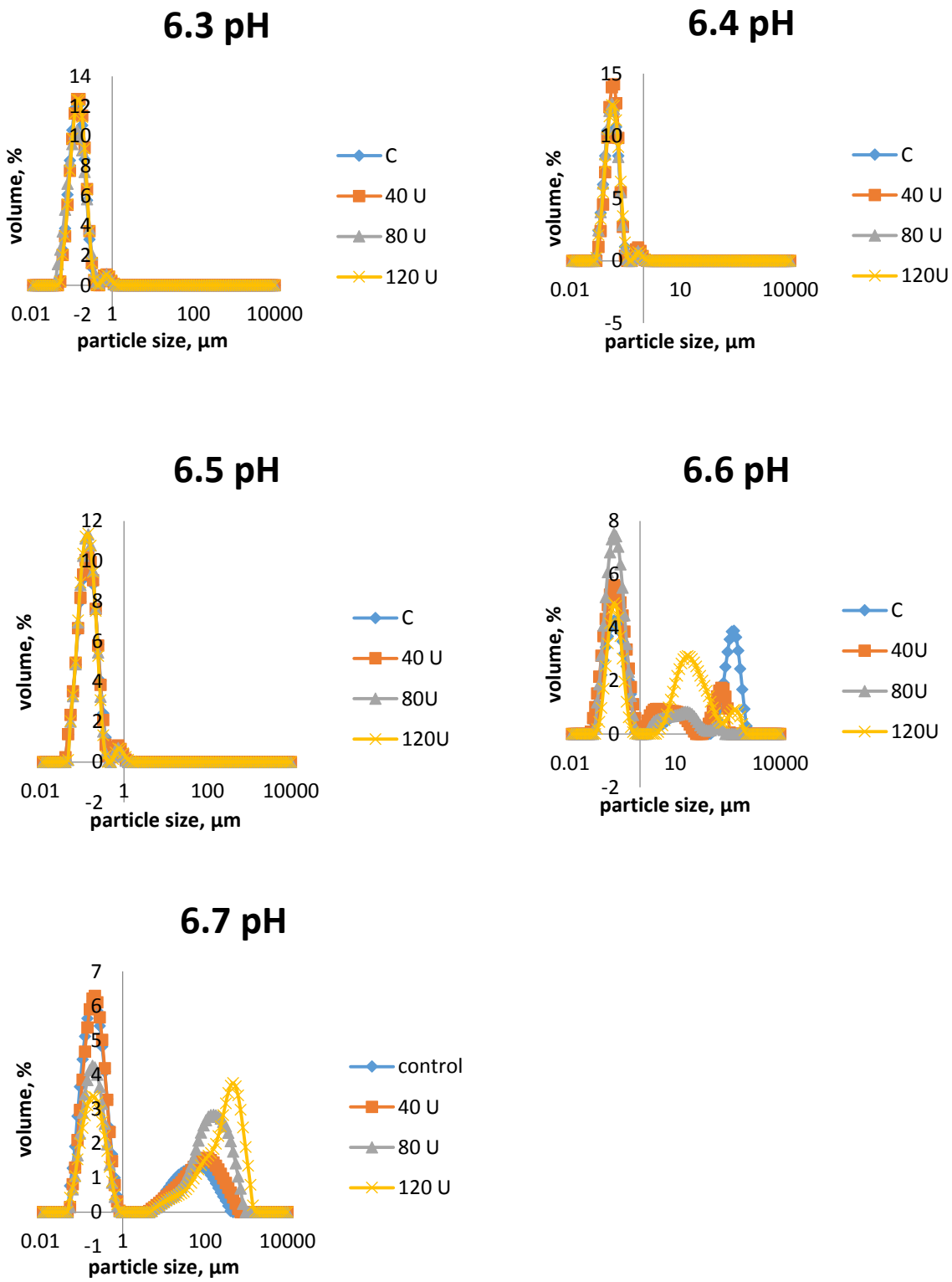


Figure 3.19. The effect of different TG activity and pH on the particle size distribution of skim milk samples incubated with TG at 10°C (Control-skim milk without TG).

3.3.2. Effect of microbial transglutaminase on the stability of evaporated milk

The samples of evaporated milk incubated with 120U g^{-1} of the protein of TG at 10°C for 24 hours at pH ranging between 6.3 and 6.7 were prepared in the industrial scale. The analyses of the samples were done from the 5th till 9th month of storage at 30°C at 2 months interval. There was no phase separation of the samples till the 5th month of storage. After 7th month, the cream layer has been separated and thus analyses were done for upper and lower phases. The stability of EM was evaluated by measuring color, dry matter content, fat content, viscosity and particle size distribution.

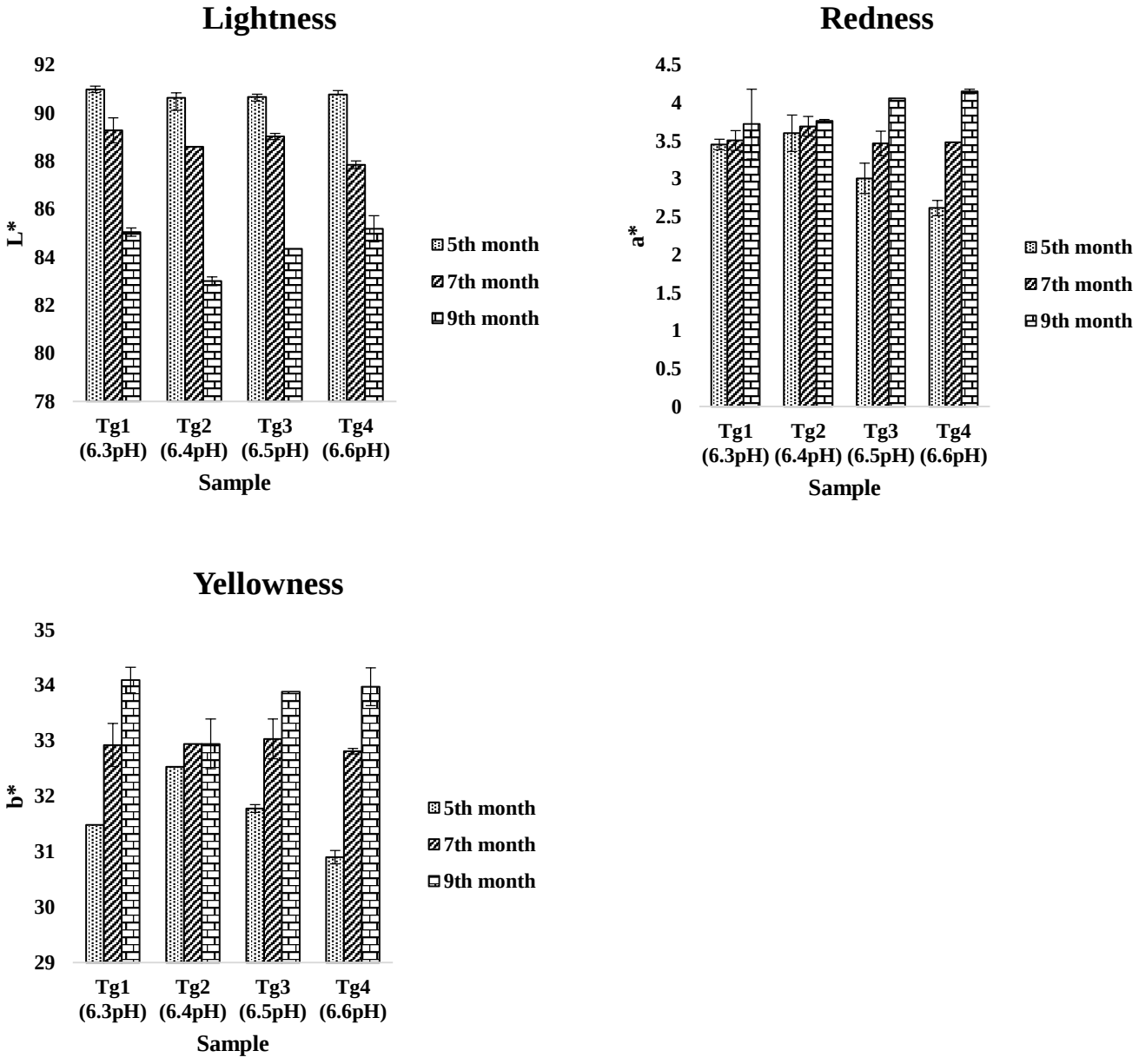


Figure 3.20. Changes in the colour values L*a*b* of EM made with 120U g^{-1} of protein with pH ranging from 6.3 to 6.6 during storage at 30°C.

The colour Lab values were recorded for all the TG treated samples shown in the Fig. 3.20. Like in the samples made with the addition of other hydrocolloids, the lightness was decreased, redness and yellowness of the samples were increased with the storage duration stored at 30°C. This was due to the Maillard reaction where free amino acids from the milk protein reacted with the lactose to form brown colour products called melanoidin. There was no great variation in the redness values for pH 6.3 and 6.4 during the storage.

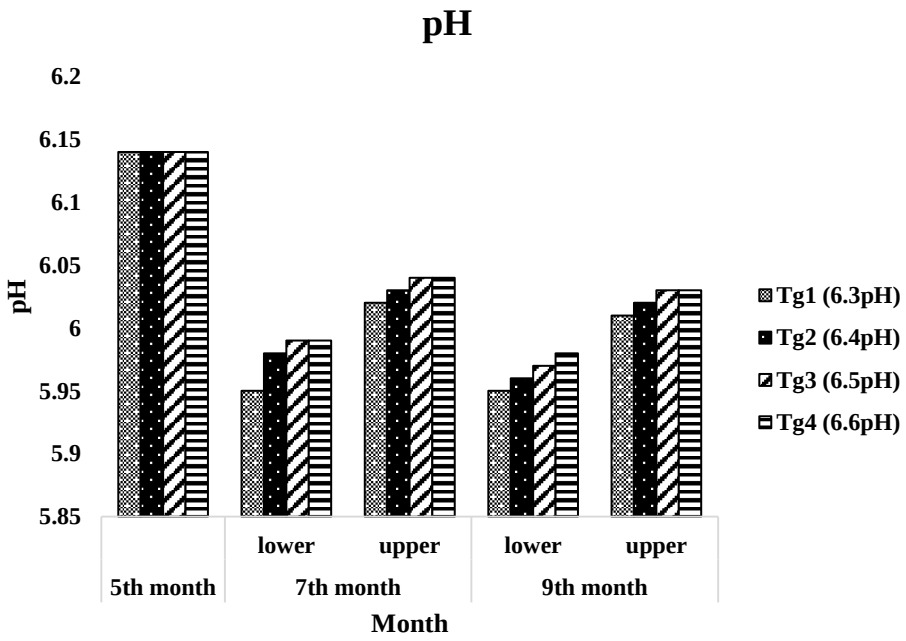


Figure 3.21. Change in pH of EM made with 120U g^{-1} of protein with pH ranging from 6.3 to 6.6 during storage at 30°C.

The samples were prepared with 5 different pH from 6.3 to 6.7. The samples with pH 6.7 were completely spoiled within 5 months. Hence, no analyses were conducted for the samples with pH 6.7. In the 5th month of storage, all the samples were in same pH 6.14 shown in the Fig. 3.21. Then pH was decreased greatly in the 7th month of storage and reached below 6 in the lower phase whereas, upper phases of the samples were slightly higher in pH on the comparison with the lower phase. The decrease in pH in the storage duration at 30°C was clearly due to hydrolysis of protein. Since upper phase was always more viscous than the lower phase, pH was slightly greater for the upper phase of the samples.

The dry matter of the samples was between 25.443 and 27.834 for the lower phase of the samples and reached 31.986 for the upper phase of the samples. The fat content of the samples ranged from 6.27 to 10.50 in %. The higher fat content was found for the upper phase of the samples. Before the phase separation, the samples were within 27.834 and 7.04 in % of dry matter

and fat content respectively shown in the Fig. 3.22. This was due to the accumulation of fat surrounded by protein in the upper phase of the samples during the phase separation. The standard deviation was not significantly greater for the analyses.

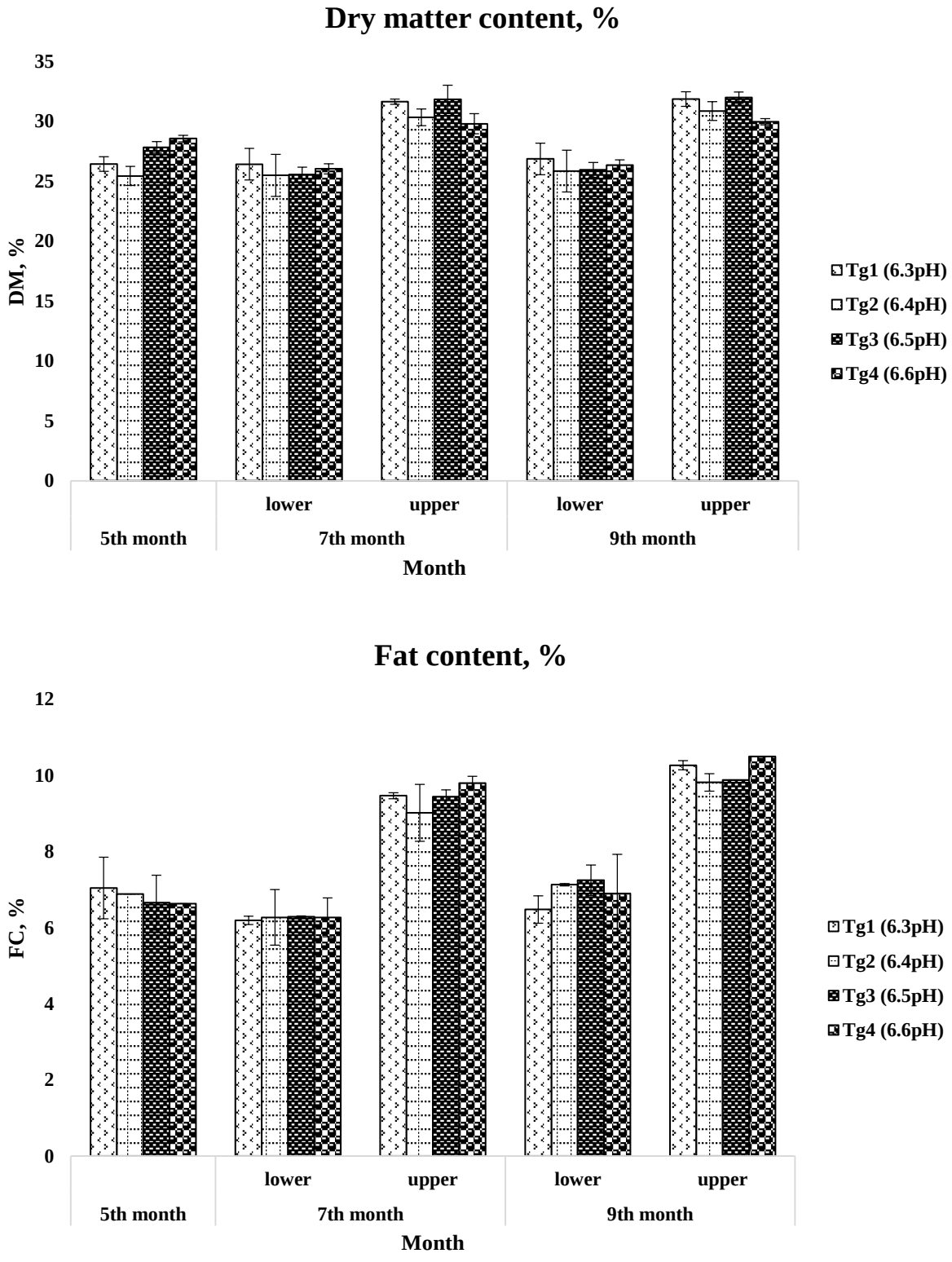


Figure 3.22. Variation in the dry matter and fat content of EM made with 120U g^{-1} of protein with pH ranging from 6.3 to 6.6 during storage at 30°.

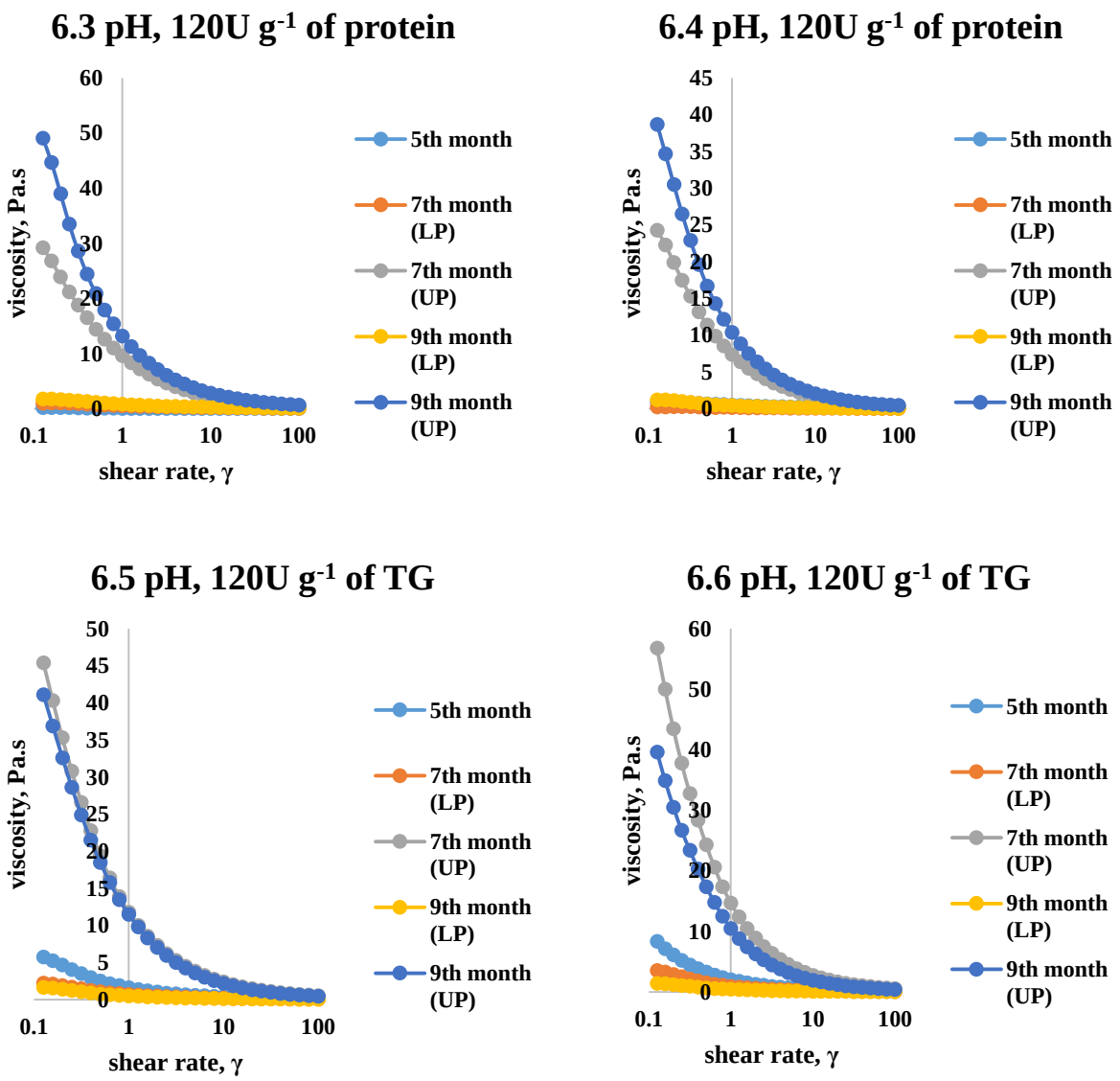


Figure 2.23. The viscosity curves of EM made with 120U g^{-1} of protein with pH ranging from 6.3 to 6.6 during storage at 30°.

The flow curves and their coefficients were recorded for the EM samples made from milk treated with TG. All the samples were found to exhibit pseudoplastic behavior which was clearly understood by the flow curves reported in the Fig. 2.23. The flow curves samples with pH 6.3 and 6.4 were quite similar and the samples with pH 6.3 were little more viscous than the samples with pH 6.4. The samples measured in the 5^{th} month and lower phase of the samples measured in the other months was similar. With the increase in storage period, the viscosity of the cream layer increased for both the samples with pH 6.3 and 6.4. The viscosity of the samples decreased with the increase in the storage month for both the upper and lower phase of the samples with pH 6.5 and 6.6. From the results, it was understood that age thickening occurred followed by age thinning of the samples for the samples with pH 6.5 and 6.6. The samples with pH 6.3 and 6.4 contradicted with the samples with pH 6.5 and 6.6.

The flow curves got fit with Ostwald equation and thus consistency coefficient and flow index were obtained for the samples. The consistency coefficient ranged between 0.0841 to 12.915. It was always associated with the viscosity of the samples. The consistency coefficient was higher in value if the viscosity of the samples was high. However, flow index decreased with the increase in the viscosity of the samples. The high viscosity and hence high consistency coefficient was found in the upper phase of the sample with pH 6.6 at the 7th month of storage. The standard deviation was not much greater.

Table 3.6. The rheological coefficients and viscosity at a 0.251/s shear rate of EM made with 120U g^{-1} of protein with pH ranging from 6.3 to 6.6 during storage at 30°.

Consistency coefficient, K					
Samples	**5th month**	**7th month**		**9th month**	
		lower	**upper**	**lower**	**upper**
Tg1 (6.3pH)	0.0841±0.65	0.5141±1.45	9.0280±0.43	0.7724±1.23	12.915±0.65
Tg2 (6.4pH)	0.4674±0.75	0.1483±1.67	7.0992±0.56	0.4300±0.98	9.9810±0.36
Tg3 (6.5pH)	1.6416±0.54	0.7193±0.76	11.593±1.24	0.5129±0.76	10.830±0.87
Tg4 (6.6pH)	2.0471±0.23	0.9677±0.56	14.104±0.78	0.4451±0.56	9.9897±0.54
Flow index, n					
Samples	**5th month**	**7th month**		**9th month**	
		lower	**upper**	**lower**	**upper**
Tg1 (6.3pH)	0.6433±0.61	0.3961±1.31	0.6166±0.22	0.5580±1.31	0.3514±0.61
Tg2 (6.4pH)	0.6172±0.79	0.3797±1.75	0.7014±0.70	0.4959±1.75	0.3161±0.79
Tg3 (6.5pH)	0.4221±0.47	0.3206±0.62	0.4722±1.18	0.4581±0.62	0.3102±0.47
Tg4 (6.6pH)	0.3206±0.27	0.2981±0.42	0.4050±0.83	0.4620±0.42	0.3005±0.27
Viscosity at 0.251/s of shear rate, Pa.s					
Samples	**5th month**	**7th month**		**9th month**	
		lower	**upper**	**lower**	**upper**
Tg1 (6.3pH)	0.205±0.61	0.978±0.79	21.20±0.47	1.570±0.27	33.50±0.33
Tg2 (6.4pH)	0.891±0.60	0.259±0.03	17.50±0.04	0.993±1.45	26.50±0.60
Tg3 (6.5pH)	4.070±0.14	2.120±0.17	30.80±0.18	1.250±0.71	28.60±0.90
Tg4 (6.6pH)	5.230±1.19	3.270±0.60	37.80±0.35	1.080±1.04	26.70±0.44

The particle size distribution (by volume) were recorded for the samples shown in the Fig. 3.24. The peak was extended to 120μm approximate for the samples with pH 6.3. That was the 3rd peak and the volume increased with the storage duration. Whereas the peak ended in 100μm for the other pH samples, only two major peaks were seen for them. The first peak was inversely proportional to the second peak. If the volume decreased in the first peak, then volume increased in the second peak. The particle size was distributed in the above-said fashion for the samples with pH 6.4 and 6.5 and the volume has been increased with the increase in storage period. The samples with pH 6.6 were stable in terms of particle size, it was seen that particle size distributed similarly

throughout the storage duration. Thought there was a cream separation in all the samples, we can conclude that samples were quite stable in terms of particle size distribution where there was no great variation. The particle size of the samples with pH 6.6 appeared in the same peak without change.

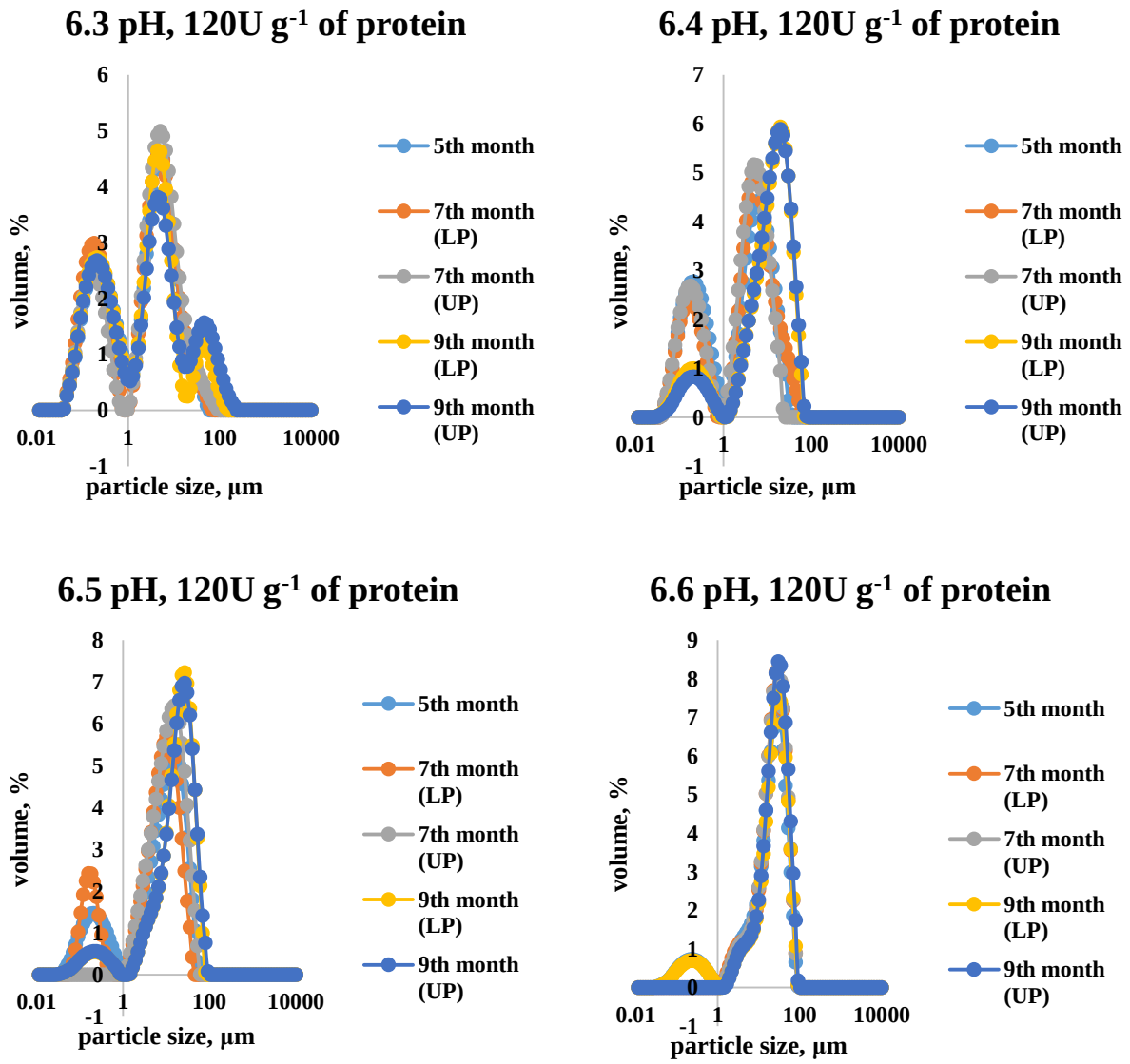


Figure 3.24. Particle size distribution of EM made with 120U g^{-1} of protein with pH ranging from 6.3 to 6.6 during storage at 30°.

CONCLUSIONS

1. The storage of evaporated milk at elevated temperature (30^{o}C) accelerated both chemical and physical changes over the storage period of 12 months. Investigation of color changes, as well as the decrease in pH, revealed the formation of Maillard reaction products during storage. The creaming separation was established as the main reason for the instability of evaporated milk during storage at 30^{o}C. After the phase separation, the upper phase had higher dry matter and fat content than the lower phase because of accumulation of fat globules in the upper phase.
2. The addition of a different amount of carrageenan (in the range 0.001 – 0.005%) as a thickening agent to the continuous phase of evaporated milk had an effect on the physical properties of evaporated milk. All the samples were found to exhibit pseudoplastic behavior and poly-dispersal distribution of particles size. There was no phase separation in the samples with 0.005% k-Car and the viscosity was ranged between 0.217 and 1.410 Pa.s throughout the storage period.
3. The addition of a different amount of carboxymethylcellulose (in the range 0.00025 – 0.001%) as a thickening agent to the continuous phase of evaporated milk had no positive effect on the stability of evaporated milk during storage at 30^{o}C.
4. Skim milk treatment with transglutaminase had an impact on the dispersity and viscosity of skim milk; incubation time (30 – 180 min and 24 h) and temperature (10 and 50^{o}C) with this enzyme as well as the amount of enzyme (40 - 120 U/g protein) had an impact on these properties. As a result, higher molecular weight macromolecules were composed due to the crosslinking of milk protein at the pH 6.6-6.7.
5. The phase separation was observed during storage of evaporated milk, produced from the milk treated with 120U g^{-1} of protein at different pH (6.3 – 6.7) for 24 hours at 10 ^{0}C, after 5 months. The crosslinking of proteins did not prevent the separation of two phases of the evaporated milk due to the accelerated chemical and physical changes of the system during storage at 30 ^{0}C.

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